

**Enhancing the Efficacy of *Azotobacter chroococcum*
and *Bacillus subtilis* by Dose Optimization and
Immobilization within Organic Carrier for High
Wheat (*Triticum aestivum* L.) Productivity**

THESIS

**SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
LUCKNOW, INDIA**

**BABASAHEB
B H I M R A O
AMBEDKAR
UNIVERSITY**



FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL SCIENCE

BY

ROSE PRATIMA MINJ

**DEPARTMENT OF ENVIRONMENTAL SCIENCE
SCHOOL FOR ENVIRONMENTAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
VIDYAVIHAR, RAIBARELI ROAD, LUCKNOW - 226025, U.P., INDIA**

ENROLEMENT NUMBER -1127/07

2016



**THIS THESIS IS
DEDICATED TO THE
MEMORY
OF
MY FATHER...**

DECLARATION

This is to certify that the material embodies in the present work entitled “**Enhancing the Efficacy of *Azotobacter chroococcum* and *Bacillus subtilis* by Dose Optimization and Immobilization within Organic Carrier for High Wheat (*Triticum aestivum* L.) Productivity**” is based on candidate’s original research work. It has not been submitted in part or full for any other diploma or degree of any University. The indebtedness of the candidate to others has been duly acknowledged at relevant places.

Name and Signature

(Candidate)

Department of Environmental Science
Babasaheb Bhimrao Ambedkar University
(A Central University)
Lucknow

Countersigned

(Supervisor)

Department of Environmental Science
Babasaheb Bhimrao Ambedkar University
(A Central University)
Lucknow

Countersigned

Head of the Department

Department of Environmental Science
Babasaheb Bhimrao Ambedkar University
(A Central University)
Lucknow

ACKNOWLEDGEMENTS

In this great venture of research there are several persons who have contributed, I would like to acknowledge their guidance, support and concerns.

Above all it is to the Almighty, for giving me strength and courage to complete this work peacefully.

*I express my deep sense of gratitude and deep regards to my guide **Prof. (Dr.) Rana Pratap Singh**, who has supervised my work, even after holding many academic and administrative responsibilities in the **Babasaheb Bhimrao Ambedkar University, Lucknow**. The blessing, help and valuable suggestions given by him time to time for the improvement of my work and endless encouragement to complete this study is deeply acknowledged.*

*I owe my sincere thanks to the Head of Department, **Prof. (Dr.) D. P. Singh** and the former Head **Prof. (Dr.) M. Yunus**, (presently Vice Chancellor, **Jauhar University Rampur, India**) for providing the necessary facilities in the department during my research tenure. I will always be grateful for their kind and helping attitude.*

*I gratefully acknowledge and extend my heartfelt gratitude to research Advisory committee members **Dr. S. K. Dwivedi, Mr. N.K. S. More, Dr. Shikha, Dr. Narendra Kumar, Dr. Venketash Dutta and Dr. Richa K. Tyagi** for their valuable guidance and generous advice.*

*I have special thanks to Archbishop of the Agra Most **Rev. Dr. Albert D'Souza** and Bishop of Lucknow **Rev. Dr. Gerald John Mathias** for their blessings and prayer.*

*I am grateful to the technical and administrative staff of the department especially **Mr. A. K. Jain, Mr. Aviral, Mr. Rahul, Mr. Saluddin** and lab attendant especially, **Mr. Ranjeet** without whose support this study would have been not possible within the stimulated time.*

*My sincere thanks to my very energetic seniors **Dr. Vikram Ghavri, Dr. Shiv Shanker, Dr. Satish Rawat, Dr. Shobha, Dr. Veena, Dr. Sonal Dixit, Dr. Neha Vishnoi, Dr. Kamini Narain, Dr. Ritu, Dr. Sangeeta, Dr. Ramgopal Verma, Dr. Preeti Tripathi, Dr. Amit, Dr. Swadesh** and*

Mr. Vivek for their ungrudging assistance, whenever I needed as their help.

I am forever thankful and in debt to *Dr. Kuldeep Bauddh, Dr. Sanjeev Kumer, Dr. Vishalakchhi Ashok, Mr. Anurag Singh, Mr. Ambuj Mishra, Ms. Swati Sachdev, Mr. Mahesh* and *Mr. Pradeep* who have stood beside me as and when, I took on the challenges and obstacles of this research. Their encouragement, helpfulness and support have helped me not only to overcome but also to persevere and excel.

I lovingly appreciate cheerful support of my friends *Ms. Madhulika Yadav, Ms. Savita Yadav, Ms. Priyanka Yadav, Mr. Basil, Dr. Praveen Verma (BHMS) Mr. Aman, Mr. Nadeem* and my school friends *Dr. Satyapal Singh (MBBS), Ms. Gunjan Sharma, Mrs. Shilpi Awasthi, Dr. Shekhar Azad (MBBS)* and *Dr. Chandrpal (MBBS)*. Their friendship was a source of joy and inspiration for me.

My grateful thanks goes to all my lab mates *Dr. Virendra Kumar, Mr. Abdul Shah Barey, Mr. Shamshad, Mr. Vinayak, Mr. Dhananjay, Mr. Sushil, Ms. Neelam, Ms. Poonam, Mrs. Beenu, Ms. Neha, Mr. Atin, Mr. Harmohan, Ms. Urvashi, Ms. Sangeeta, Mrs. Neha, Ms. Jyoti* and *Ms. Namita* for their cheerful company and zestful enthusiasm.

I am thankful to *youth of ICYM*, India for their prayer and support.

My sincere appreciation and deepest gratitude to my parents *late Policarp Minj* and *Mrs. Anna Minj* have been the greatest strength and inspiration in my life for their love, faith and prayers. All your sacrifices and dedication which made me what I am today. Words cannot begin to describe how grateful I am to both of you.

I would like to thanks my cute sister *Ms. Sunanda Minj* and my brother *Mr. Paul Prakash Minj* for their love and support.

Last, but not least, I would like to thank to my parish priest *Rev. (Dr.) Peter Castelino* for his constant encouragement, which kept me afloat amidst the storm.

Last, I would like to thank to one and all, whosoever wished and helped me to reach the pinnacle of my life. Thank you all for your support in the completion of this degree.

Rose Pratima Minj

PREFACE

India is known as an agriculture country. The Indian agriculture is however, highly vulnerable to the climate change, and getting down consistently due to the drought, altered rainfall patterns and other biotic and abiotic factors. In the process of solving these problems and to enhance the crop productivity per unit area the conventional green revolution practices are falling into another pit of environmental, economic and health related problems. The excessive and indiscriminate use of chemical fertilizers e.g. urea and DAP etc. has increased the crop productivity per unit area, but also get disposed in soil, water, and atmosphere through runoff, leaching, volatilization and gaseous emissions. The use of these fertilizers on a large scale emits significant amount of the greenhouse gases into the atmosphere. The chemical fertilizers have influenced the soil physical, chemical and biological properties in irrigated agro-ecosystems. Animal manures and urea release methane, nitrous oxide, ammonia and CO₂ in varying quantities, depending on their physical form i.e. solid and liquid. The production and application of chemical fertilizers, which consist of nitric oxides and ammonium bicarbonate results in emission of nitrogen oxides, nitrous oxide and ammonia etc. into the atmosphere. Nitrogen containing inorganic and organic fertilizers cause soil acidification. When it goes down, it contaminates the ground water. When the organic matrix decays, it yields N₂O. This N₂O leads to destruction of ozone layer and help in forming harmful acid rain.

To reduce the load of these kinds of pollutants in the environment and to enhance the crop productivity, use of organic matrix immobilized biofertilizers based granules prepared under this study have shown promising results. The Organic matrix contains vermicompost or cow dung or farm yard manure with clay soil and neem leaves. It maintains the soil quality and enriches the soil fertility. It provides food, aerated and moist shelter to the microbes and makes nutrient available for the better plant growth in a sustainable manner. It allows reuse of organic waste and fulfils fertilizer scarcity by providing an efficient organic slow release fertilizer, prepared from

the local materials in place of harmful synthetic fertilizers or highly expensive commercial slow release fertilizers.

The co-immobilized form of biofertilizers (*Azotobacter chroococcum* and *Bacillus subtilis*) prepared by us with organic carrier such as vermicompost, clay soil, and neem leaves and nutritive organic binders such as *acacia* gum, jaggery and molasses. The application of vermicompost, clay soil and neem leaves used jaggery as binder enhanced wheat yield by 21.71%, followed by 15.99% enhancement by co-immobilized form of biofertilizers in vermicompost, clay soil and neem leaves with binder molasses, and 13.89% with the application of vermicompost, clay soil and neem leaves with binder *acacia* gum over the recommended dose of urea and DAP. It also improved soil fertility in terms of nutrient availability, soil organic matter (SOM), soil organic carbon (SOC) and microbial enrichment in the experimental field during the cultivation of wheat using these biofertilizers as soil nutrient.

This study reveals that a cost effective, ecofriendly and efficient organic fertilizer can be prepared using organic matrix and the consortium of biofertilizers (*A. chroococcum* and *B. subtilis*) in optimized dose. The newly developed biofertilizer granules may provide the essential nutrients to the plants in a more ecological and economical way. It enriches the soil fertility and enhances wheat productivity over the conventional chemical fertilisers e.g. urea and DAP for wheat cultivation, a major cereal crop of the world including North Indian States, suffering from adopting green revolution package .

CONTENTS

DECLARATION
CERTIFICATE
ACKNOWLEDGE
MENTS
PREFACE

CHAPTER	DESCRIPTION	PAGE NO.
I.	Introduction	1-9
II.	Review of Literature	10-33
2.2.	Climatic factors	10
2.2.1	Drought	10
2.2.2	Salinity	12
2.3.	Anthropogenic aspects	12
2.3.1	Use of chemical fertilizers and losses	12
2.3.2	Cadmium toxicity	16
2.4.	Application of biofertilizers as an ecofriendly Alternative	17
2.4.1	Drought tolerant microbes	23
2.4.2	Salinity tolerant microbes	24
2.5.	Organic carrier	26
2.6.	Integrated nutrient management	28
2.7.	Organic binders	32
III.	Materials and Methods	34 -54
3.1.1	Experimental site	31
3.1.2	Seed source	31
3.1.3	Immobilized of organic matrix based granular slow release microbial biofertilizers	34
3.1.4	Preparation of experimental plots and application of organic matrix based granular slow release fertilizers granules in the plots and experimental design	34
3.2.	Method of biofertilizers application	39
3.2.1	Seed inoculation	39
3.2.2	Soil application	39
3.3.	Maintenance of crop growth conditions	39
3.4.	Measurement of the growth parameters	40
3.4.1	Root length and shoot length	40
3.4.2	Leaf number and number of roots	40
3.4.3	Fresh weight	40

3.4.4	Dry weight	40
3.5.	Measurement of the yield parameters	40
3.5.1	Number of tillers per plant	40
3.5.2	Grain and straw yield (t ha ⁻¹)	40
3.6.	Enumeration of bacteria by plate count or serial dilution agar plate	40
3.7.	Estimation of ammonium content	42
3.8.	Estimation of nitrite content	42
3.9.	Estimation of nitrate content	44
3.10.	Estimation of phosphate content	44
3.11.	Estimation of nitrate reductase activity	45
3.12.	Protein estimation	46
3.13.	Scanning electron microscope energy dispersive x-ray (SEM EDX) based analysis	47
3.14.	Fourier transform spectroscopy (FTIR) based analysis	47
3.15.	Soil analysis	48
3.15.1	pH (pouvoir hydrogen or hydrogen power)	48
3.15.2	Electrical conductivity	48
3.15.3	Water holding capacity	48
3.15.4	Organic carbon	48
3.15.5	Organic matter	50
3.15.6	Total nitrogen	50
3.15.7	Available nitrogen	50
3.15.8	Available phosphorous (P ₂ O ₅)	51
3.15.9	Available potassium (K)	51
3.15.10	Alkaline phosphate Activity	52
3.15.11	Urease activity	53
3.15.12	Dehydrogenase activity	54
3.16.	Cost analysis	54
3.17.	Statistical analysis	54

IV.	Results	55-145
------------	----------------	---------------

4.1.	Growth and productivity of wheat (<i>Triticum aestivum</i> L. cv. PBW-502) as well as soil enrichment applied with organic matrix immobilized biofertilizers (<i>Azotobacter chroococum</i> and <i>Bacillus subtilis</i>)	55
4.1.1	Growth and productivity of wheat (<i>T. aestivum</i> L.) applied with enhance dose and free form of charcoal based biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>), parameters recorded on 30, 60, 90 and 120 days after sowing (DAS)	55
4.1.2	Level of nitrate, nitrite, ammonium and phosphate content in rhizospheric soil, of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of free form of biofertilizers	58
4.1.3	Level of nitrate, nitrite, ammonium and phosphate content in roots of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of free form of biofertilizers	59

4.1.4	Level of nitrate, nitrite, ammonium and phosphate content in leaves of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of free form of biofertilizers	60
4.1.5	Yield and productivity of wheat (<i>T. aestivum</i> L.) plant applied with free form of biofertilizers	62
4.2.	Growth and productivity of wheat (<i>T. aestivum</i> L.) applied with charcoal based biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) in enhanced doses and seed inoculated form on 30, 60, 90 and 120 days after sowing	63
4.2.1	Growth parameters of wheat applied with biofertilizers in enhanced doses and seed coated form, recorded on 30, 60, 90 and 120 days after sowing	63
4.2.2	Level of nitrate content in rhizospheric soil, roots and leaves of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of biofertilizers in seed coated form	66
4.2.3	Level of nitrite content in rhizospheric soil, roots and leaves of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of biofertilizers in seed coated form	67
4.2.4	Level of ammonium content in rhizospheric soil, roots and leaves of wheat (<i>T. aestivum</i> L.) Plant applied with enhance doses of biofertilizers in seed coated form	68
4.2.5	Level of phosphate content in rhizospheric soil, roots and leaves of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of biofertilizers in seed coated form	69
4.2.6	Yield and productivity of wheat (<i>T. aestivum</i> L.) plant applied with free form of biofertilizers in seed coated form	71
4.3.	Growth and productivity of wheat (<i>T. aestivum</i> L. cv. PBW-502) applied with charcoal based biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) in enhanced dose and seed coated with biofertilizers and organic matrix form on 30, 60, 90 and 120 days after sowing (DAS)	72
4.3.1	Growth parameters of wheat applied with seed coated biofertilizers and organic matrix, recorded on 30, 60, 90 and 120 (DAS)	72
4.3.2	Level of nitrate, nitrite, ammonium and phosphate content in soil of wheat (<i>T. aestivum</i> L.) plant applied with seed coated with biofertilizers and organic matrix	76
4.3.3	Level of nitrate, nitrite, ammonium and phosphate content in roots of wheat (<i>T. aestivum</i> L.) plant applied with seed coated with biofertilizers and organic matrix	77
4.3.4	Level of nitrate, nitrite, ammonium and phosphate content in leaves of wheat (<i>T. aestivum</i> L.) plant applied with seed coated with biofertilizers and organic matrix	78
4.3.5	Grain and straw yield production of wheat on application of biofertilizers in seed coated form with organic matrix	80
4.4.	Characterization of organic matrix and immobilized form of <i>A. chroococum</i> and <i>B. subtilis</i> with various organic matrix as super granular biofertilizer	81
4.4.1	Scanning electron microscope energy dispersive x-ray (SEM EDX) analysis	81

4.4.2	Fourier transform infrared spectrophotometer (FTIR) analysis	86
4.4.3	Microbial growth and biochemical properties in organic matrix immobilized super granules	90
4.4.4	Microbial growth of super granules at storage condition and different temperature	92
4.5.	Growth and productivity of wheat (<i>T. aestivum</i> L. cv. PBW-502) applied with charcoal based biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) immobilized with different organic matrix with enhanced dose (triple dose) on 30, 60, 90 and 120 days after sowing (DAS)	94
4.5.1	Growth parameters of wheat applied with optimised dose of biofertilizers immobilized with organic matrix analysed on 30, 60, 90 and 120 (DAS)	94
4.5.2	Level of nitrate, nitrite, ammonium and phosphate content in rhizospheric soil, of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers	100
4.5.3	Level of nitrate, nitrite, ammonium and phosphate content in roots of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers	102
4.5.4	Level of nitrate, nitrite, ammonium and phosphate content in leaves of wheat (<i>T. aestivum</i> L.) plant applied with enhance doses of free form of biofertilizers	104
4.5.5	Yield and productivity of wheat (<i>T. aestivum</i> L.) plant applied with free form of biofertilizers	106
4.6.	Growth and productivity of wheat (<i>T. aestivum</i> L. cv. PBW-502) applied with charcoal based immobilized biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) in enhanced doses (triple dose) with different organic carrier and binders on 30, 60, 90 and 120 days after sowing (DAS)	108
4.6.1	Growth parameters of wheat applied with charcoal based immobilized biofertilizers in enhanced doses (triple dose) with different organic carrier and binders on 30, 60, 90 and 120 days after sowing (DAS)	108
4.6.2	root transverse section examine under scanning electron microscope	111
4.6.3	Level of nitrate content assimilation in the soil and wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	112
4.6.4	Level of nitrite content assimilation in the soil and wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	113
4.6.5	Level of ammonium content assimilation in the soil and wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	114
4.6.6	Level of phosphate content assimilation in the soil and wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	116
4.6.7	Level of NRA content in root and leaves of wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	117
4.6.8	Level of dehydrogenase activity (DHA) in rhizospheric soil, of	118

	wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	
4.6.9	Level of alkaline phosphatase activity (mg/g/hr) in soil, of wheat (<i>T. aestivum</i> L.) plant parts on application of immobilized biofertilizers	118
4.6.10	Grain, straw yield and harvesting index of wheat crop by the application of biofertilizers with organic matrix	119
4.7.	Growth and productivity of wheat (<i>T. aestivum</i> L. cv. PBW-502) applied with charcoal based biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) in triple dose under the stress condition (salinity and high cadmium) on 30, 60, 90 and 120 days after sowing (DAS)	121
4.7.1	Growth parameters of wheat plant applied with immobilized and optimized biofertilizer on 30, 60, 90 and 120 days after sowing (DAS)	121
4.7.2	Level of nitrate, nitrite, ammonium and phosphate content in rhizospheric soil, of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers under stress of salinity and cadmium	125
4.7.3	Level of nitrate, nitrite, ammonium and phosphate content in roots of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers under stress of salinity and cadmium	127
4.7.4	Level of nitrate, nitrite, ammonium and phosphate content in leaves of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers under stress of salinity and cadmium	129
4.7.5	Yield and productivity of wheat (<i>T. aestivum</i> L.) plant applied with immobilized form of biofertilizers under stress of salinity and cadmium	131
4.8.	Growth and productivity of wheat (<i>T. aestivum</i> L. cv. PBW-502) applied with charcoal based biofertilizers (<i>a. chroococcum</i> and <i>b. subtilis</i>) in triple dose under drought condition on 30, 60, 90 and 120 days after sowing (DAS)	132
4.8.1	Growth parameters of wheat applied with immobilized biofertilizer under drought stress, recorded on 30, 60, 90 and 120 days after sowing (DAS)	132
4.8.2	Availability of nitrate, nitrite, ammonium and phosphate content in the soil on application of immobilized biofertilizers super granules under drought stress condition	137
4.8.3	Availability of nitrate, nitrite, ammonium and phosphate content in the roots on application of immobilized biofertilizer based super granules under drought stress condition	139
4.8.4	Availability of nitrate, nitrite, ammonium and phosphate content in the leaves by the application of immobilized biofertilizers based super granules under drought stress condition	141
4.8.5	Grain and straw yield of wheat (<i>T. aestivum</i> L.) plant applied with immobilized biofertilizers based super granules under drought stress condition	143

4.8.6	Changes in Physico-Chemical Properties of Soil by the Application of Biofertilizers in free form, Seed Coated form and Immobilized within Organic Matrix	144
4.9.	Cost Analysis	145
<hr/>		
V.	Discussion	146-158
<hr/>		
VI.	Summary and Conclusions	159-163
<hr/>		
	REFERENCES	164-219
<hr/>		
	PUBLICATIONS	-
<hr/>		

LIST OF TABLES

Table	Particulars	Page No.
2.1	Leaching, runoff, volatilization and emission losses of greenhouse gases of nutrients from the agricultural fields by application of commercial chemical fertilizers.	13
2.2	Micro-organisms are use in different crop as biofertilizers which enhance the growth and productivity	18
3.1	The detailed layout of biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) treatments in free form with enhance dose for dose optimization as follows	35
3.2	The detailed layout of free form, seed coated with biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>), in enhance dose for dose optimization as follows	36
3.3	The detailed layout of seeds coated with biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) and organic matrix (vermicompost), in enhance dose for dose optimization as follows	36
3.4	The detailed layout of various organic matrix based immobilized biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) triple dose for optimization of specific organic matrix as follows	36
3.5	The detailed layout of various organic matrix based immobilized biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) triple dose immobilized with different binders as follows	37
3.6	The details layout of organic matrix (vermicompost) based immobilized biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) triple dose immobilized with different binders applied under abiotic stress e.g. salinity and high levels of cadmium concentration as follows	38
3.7	The details layout of organic matrix (vermicompost) based immobilized biofertilizer (<i>A. chroococum</i> and <i>B. subtilis</i>) triple dose entrapped with different binders applied under abiotic stresses e.g. salinity and high cadmium levels condition as follows	38
3.8	The details layout of organic matrix (vermicompost) based entrapped biofertilizer (<i>A. chroococum</i> and <i>B. subtilis</i>) triple dose bind with different binders applied under drought stress condition as follows	38
4.1.1	Effect of free form of charcoal based <i>A. chroococum</i> and <i>b. subtilis</i> biofertilizers on plant growth parameters of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoots on 30, 60, 90 and 120 DAS	56
4.1.2	Effect of free form of charcoal based <i>A. chroococum</i> and <i>B. subtilis</i> biofertilizers on plant growth parameters of wheat (<i>T. aestivum</i> L. cv. PBW-502) roots on 30, 60, 90 and 120 DAS	57
4.2.1	Effect of organic matrix immobilized biofertilizers on growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoot on 30, 60, 90 and 120 DAS	63
4.2.2	Effect of organic matrix immobilized biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i>) on growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) roots on 30, 60, 90 and 120 DAS	65

4.3.1	Effect of coated seed with organic matrix and biofertilizers on plant growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoot on 30, 60, 90 and 120 DAS	73
4.3.2	Effect of application of <i>A. chroococcum</i> and <i>B. subtilis</i> and organic matrix as seed coated form, on plant growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) roots at 30, 60, 90 and 120 DAS	75
4.4.1	Percent weight and Percent atomic of clay soil	82
4.4.2	Percent weight and percent atomic of neem leaves powder	82
4.4.3	Percent weight and percent atomic of FCF-I	83
4.4.4	Percent weight and percent atomic of immobilized form of biofertilizers with cow dung and binder <i>acacia</i>	83
4.4.5	Percent weight and percent atomic of immobilized form of biofertilizers with cow dung and binder molasses	83
4.4.6	Percent weight and percent atomic of immobilized form of biofertilizers with cow dung and binder jaggery	84
4.4.7	Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure and binder <i>acacia</i>	84
4.4.8	Percent weight and percent atomic of immobilized form of biofertilizers with vermicompost and binder molasses	84
4.4.9	Percent weight and percent atomic of immobilized form of biofertilizers with vermicompost and binder jaggery	85
4.4.10	Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure and binder <i>acacia</i>	85
4.4.11	Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure use molasses as binder	85
4.4.12	Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure and jaggery	86
4.4.13	Fourier transform infrared spectrophotometer (FTIR) analysis of organic matrix (clay soil, neem leaves), conventional chemical fertilizer (urea and DAP) and organic matrix immobilized granular biofertilizers (cow dung, vermicompost and farm yard manure)	89
4.4.14	Bacterial colonies in organic matrix immobilized super granules, soil and biochemical properties	91
4.4.15	Microbial counting in various organic matrix based granular biofertilizer at storage condition and temperature	93
4.5.1	Effect of organic matrix immobilized biofertilizers (<i>A. chroococcum</i> and <i>B. subtilis</i>) on plant growth of Wheat (<i>T. aestivum</i> L. cv. PBW-502) shoot on 30, 60, 90 and 120 DAS	95
4.5.2	Effect of organic matrix immobilized biofertilizer on growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoots on 30, 60, 90 and 120 DAS	97
4.6.1	Effect of immobilized biofertilizers on growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoots on 30, 60, 90 and 120 (DAS)	109
4.6.2	Effect of immobilized biofertilizers on growth of wheat (<i>T. aestivum</i> L. cv. PBW-502) roots on 30, 60, 90 and 120 (DAS)	110
4.7.1	Effect of immobilized biofertilizers under stress of salinity and cadmium, on plant growth parameters of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoot on 30, 60, 90 and 120 DAS	122

4.7.2	Effect of immobilized biofertilizers under stress of salinity and cadmium, on plant growth parameters of Wheat (<i>T. aestivum</i> L. cv. PBW-502) roots on 30, 60, 90 and 120 DAS	124
4.8.1	Effect of free form, seed coated and immobilized forms of charcoal based biofertilizers on plant growth parameters of wheat (<i>T. aestivum</i> L. cv. PBW-502) shoot on 30, 60, 90 and 120 DAS under drought stress	133
4.8.2	Effect of charcoal based <i>A. chroococcum</i> and <i>B. subtilis</i> biofertilizers on plant growth parameters of wheat (<i>T. aestivum</i> L. cv. PBW-502) Roots on 30, 60, 90 and 120 DAS under drought stress	135
4.8.3	Changes in physico-chemical properties of soil on application of free form of biofertilizers, seed coated form and immobilized form with organic matrix	144
4.9.1	Net loss/gain in terms of economic return for wheat (<i>T. aestivum</i> L.) cultivated using biofertilizers in free form, seed coated form and immobilized organic matrix based super granular forms	145

LIST OF FIGURES

Figures	Particulars	Page No.
2.1	Environmental degradation due to the application of chemical fertilizers in agricultural field (Rawat et al., 2010, 2012; Yang et al., 2011; Cui et al., 2012; Gogoi and Baruah, 2012; Duan et al., 2014; Ram et al., 2014; Sapkota et al., 2014)	16
3.1	Standards curve of ammonium	42
3.2	Standards curve of nitrite	43
3.3	Standards curve of nitrate	44
3.4	Standards curve of phosphate	45
4.1.1	The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the soil on 30, 60, 90, and 120 days after sowing (DAS).	59
4.1.2	The enhance dose of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the roots on 30, 60, 90, and 120 days after sowing (DAS)	60
4.1.3	The nitrate, nitrite, ammonium and phosphate content in the plants leaves on 30, 60, 90, and 120 DAS the seeds	61
4.1.4	The application of enhance doses of free form biofertilizers on soil significantly increased yield and straw weight.	62
4.2.1	The enhance dose of biofertilizers on the nutrient availability of nitrate content in the soil, roots and leaves on 30, 60, 90, and 120 DAS the seeds	67
4.2.2	The enhance doses of biofertilizers on the nutrient availability of nitrite content in the soil, roots and leaves on 30, 60, 90, and 120 days after shown (DAS) the seeds	68
4.2.3	Levels of ammonium ($\mu\text{g g}^{-1}$) in soil of <i>T. aestivum</i> L. at 30, 60, 90 and 120 days after sowing.	69
4.2.4	The seed coated with biofertilizers significantly effect on the nutrient availability of phosphate content in the soil roots and leaves at on 30, 60, 90, and 120 days after shown (DAS) the seeds.	70
4.2.5	Effect of enhanced dose of biofertilizers on yield of wheat <i>T. aestivum</i> L. at after harvesting	71
4.3.1	Seed coated with biofertilizers and organic matrix significantly increased nutrient availability in the soil on 30, 60, 90, and 120 days after shown (DAS) the seeds.	77
4.3.2	The seed coated with biofertilizers and organic matrix increased nutrient availability in the roots on 30, 60, 90, and 120 days after shown (DAS)	78
4.3.3	Seed coated with biofertilizers organic matrix increased nutrient availability in the leaves on 30, 60, 90, and 120 days after shown (DAS) the seeds	79
4.3.4	Effect of seed coated organic matrix and biofertilizers on yield of wheat <i>T. aestivum</i> L. at after harvesting.	80

4.4.1.	SEM EDX analysis of clay soil	82
4.4.2.	SEM EDX analysis of neem leaves powder	82
4.4.3.	SEM EDX analysis of FCF; (RD; urea and DAP)	83
4.4.4.	SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFCD(<i>Acacia</i>)-III	83
4.4.5.	SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFCD (Molasses) - III	83
4.4.6.	SEM EDX analysis of organic matrix immobilized form of biofertilizers BFCD (Jaggery)-III	84
4.4.7.	SEM EDX analysis of organic matrix immobilized form of biofertilizers EV(<i>Acacia</i>)-III	84
4.4.8.	SEM EDX analysis of organic matrix immobilized form of biofertilizers EV(Molasses)-III	84
4.4.9.	SEM EDX analysis of organic matrix immobilized form of biofertilizers, EV(Jaggery)-III	85
4.4.10.	SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFFY(<i>Acacia</i>)-III	85
4.4.11	SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFFY(molasses)-III	85
4.4.12	SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFFY(Jaggery)-III	86
4.4.13	FTIR transmittance spectra of clay soil	87
4.4.14	FTIR transmittance spectra of neem leaves powder	87
4.4.15	FTIR transmittance spectra of free form of charcoal based biofertilizer	87
4.4.16	FTIR transmittance spectra of free form of urea and DAP	88
4.4.17	FTIR transmittance spectra of organic matrix immobilized form of Biofertilizers with cow-dung	88
4.4.18	FTIR transmittance spectra of organic matrix immobilized form of biofertilizers with vermicompost	88
4.4.19	FTIR transmittance spectra of organic matrix immobilized form of Biofertilizers with farm yard manure	89
4.5.1	The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the soil on 30, 60, 90, and 120 days after sowing (DAS).	102
4.5.2	The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the roots on 30, 60, 90, and 120 days after sowing (DAS).	104
4.5.3	The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the leaves on 30, 60, 90, and 120 days after sowing (DAS).	106
4.5.4	The application of enhance dose of free form biofertilizers on soil significantly recorded yield and straw weight	107
4.6.1	Transverse section of root examine under Scanning Electron Microscope	111
4.6.2	Levels of Nitrate ($\mu\text{g g}^{-1}$) in dry soil, roots and leaves of <i>T. aestivum</i> L. at 30, 60, 90 and 120.	113
4.6.3	Levels of nitrite ($\mu\text{g g}^{-1}$) in dry soil, roots and leaves of <i>T. aestivum</i> L. at 30, 60, 90 and 120 DAS in different treatments	114

4.6.4	Ammonium content on soil and plant parts at 30, 60, 90 and 120 DAS	115
4.6.5	Levels of phosphate ($\mu\text{g g}^{-1}$) in dry soil and plant tissue of <i>T. aestivum</i> L. at 30, 60, 90 and 120 DAS in different treatments	117
4.6.6	Levels of NRA ($\mu\text{g g}^{-1}$) in dry soil and plant tissue of <i>T. aestivum</i> L. at 30, 60, 90 and 120 DAS in different treatments	117
4.6.7	Dehydrogenase activity ($\mu\text{g TPF}/24 \text{ hr}$) in soil of experimental plot cultivated with <i>T. aestivum</i> L. at 60 and 90 DAS in different treatments.	118
4.6.8	Alkaline phosphate activity (mg/g/hr) in soil of experimental plot cultivated with <i>T. aestivum</i> L. at 60 and 90 DAS in different treatments	119
4.6.9	Effect of organic matrix based co- immobilized biofertilizers on seed yield, straw yield and harvesting index at 120 (DAS) old plants	120
4.7.1	The effect of optimized dose of biofertilizers on nutrient availability in the soil on 30, 60, 90, and 120 days after sowing	127
4.7.2	The effect of optimized dose of biofertilizers on nutrient availability in the soil on 30, 60, 90, and 120 days after sowing.	129
4.7.3	Nutrient concentrations in the leaves on 30, 60, 90, and 120 DAS the seeds.	131
4.7.4	Application of optimized dose of biofertilizers in immobilized form on soil significantly increased grain yield and straw yield	131
4.8.1	Effect of immobilized biofertilizers on the nutrient availability in the soil on 30, 60, 90, and 120 days after shown (DAS).	138
4.8.2	The immobilized biofertilizers increased nutrient availability in the roots on 30, 60, 90, and 120 days after shown (DAS).	141
4.8.3	Nutrient concentrations in the leaves increased on 30, 60, 90, and 120 DAS	143
4.8.4	Grain and straw yield significantly increased on application of immobilized biofertilizers on soil.	143
5.1.1	Yield of wheat crop affected by enhanced dose of biofertilizer with different mode of application at pot and field conditions	148

LIST OF ABBREVIATIONS

Annova	Analysis of variance
NH ₄ ⁺	Ammonium
cm.	Centimetre
CFU	Colony forming unit
°C	Degree centigrade
d	Day
DAS	Days after shown
DHA	Dehydrogenase activity
DW	Dry weight
FAO	Food and Agricultural organization
FYM	Farm yard manure
FW	Fresh weight
g	Gram
hr	Hour
INMP	Integrated nutrient management plant
Kg	Kilogram
Kg ha ⁻¹	Kilogram per hectare
mg g ⁻¹	Milligram per gram
mt	Metric tonnes
μ	Micro
μ mole	Micro mole
μg g ⁻¹	Micro gram per gram
nm	Nanometer
NRA	Nitrate reductase activity
N	Nitrogen
NO _x	Nitrogen oxide
N ha ⁻¹	Nitrogen per hectare
NO ₃	Nitrate
NUE	Nutrient use efficiency
OD	Optical density
OP	Osmotic Pressure
P	Phosphorous
PO ₄ ⁺³	Phosphate
K	Potassium
PGPR	Plant growth promoting rhizobacteria
RBD	Random block design
SD	Standard deviation
SE	Standard error
t ha ⁻¹	Tonnes per hectare



CHAPTER-1

INTRODUCTION

Chapter 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important and strategic cereal crop for the majority of world's growing populations. It is the most important staple food of about two billion people (36% of the world population). It is cultivated over a wide range of the climatic conditions. India counts a third largest producer of wheat in the world, with production of about 80 million metric ton wheat in 2014 (UN, FAO Statistic 2015).

Wheat (*T. aestivum* L.) belongs to the grass family Poaceae (*Gramineae*). Beside wheat Poaceae family also includes many other major crops such as, barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), rye (*Secale cereale* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.). *Triticeae* is one of the tribes containing more than 15 genera and 300 species including wheat and barley. Simmonds (1976) reported that one to several flowered spikelets are sessile and are alternate on opposite sides of the rachis forming a true spike Linnaeus (1753) was the first person who classified the wheat. Later on Sakamura (1918) reported the chromosome number sets (genomes) for each commonly recognized type. This was a turning point in *Triticum* classification after which the wheat was classified into three groups. Diploids having 14 (n=7), tetraploids having 28 (n=14) and the hexaploids having 42 (n=21) chromosomes. *T. aestivum*, *T. durum* and *T. compactum* are the other major species which are generally known as bread wheat (Briggle, 1967).

Though wheat is grown under a wide range of climatic conditions, it is best adapted to temperate regions with rainfall between 30-90 cm. Winter and spring wheats are the two major types of the crop, with the severity of the winter determining whether a winter or spring type is cultivated. Winter wheat is sown in the fall, spring wheat is generally sown in the spring but can also be sown in the fall, where winters are mild. Therefore, today wheat is grown all over the world, with different varieties sown according to different climates. In 2015, the world's main wheat producing regions were EU, China, India, Russian Federation, United States, Canada, Pakistan, Ukraine, Australia and Turkey (United State Department of Agricultural Database, 2015).

Most of the currently cultivated wheat varieties belong to hexaploid wheat (*T. aestivum* L.), which is known as common bread wheat and valued for bread making. The greatest portion of the wheat flour produced is used for bread making. Wheat grown in dry climates is generally hard type, having protein content of 11-15 percent and strong gluten (elastic protein). The sticky gluten of bread wheat entraps the carbon dioxide (CO₂) formed during yeast fermentation and enables leavened dough to rise. The hard type of wheat produces flour best suited for bread making. The softer type produces flour suitable for cakes, crackers, cookies, pastries and household flour. Durum wheat (*T. turgidum* L.), which is the main tetraploid type (Gelinas and McKinnon, 2006; Feldman et al., 2007; Shewry et al., 2009) is also important, as its large and very hard grains yield low gluten flour which is the main source of semolina suitable for pasta, couscous, burghul etc. The glutenin protein fraction is important in determining gluten quality and dough elasticity in wheat flour (Shewry et al., 2000; Don et al., 2006; Pirozi et al., 2008; Jiang et al., 2009; Kumar et al., 2015a, 2015b). Since wheat quality is of increasing importance for producers, it is necessary to understand the adverse effects of the interaction of these environmental constraints (Wollenweber et al., 2003, 2005, White et al., 2006).

The growing population of India is approx. 1.26 billion (www.worldmeter.info). However, the latest estimated demand for wheat production for the year 2020 is approximately 87.5 million tons (Joshi et al, 2007; Lobell et al., 2013). The growing demand for food is increasing (Laik et al., 2014; Singh et al., 2014) because the world population is rising and it predicted to rise from 6.5 billion approx. at present to 10 billion by 2050. The growth, productivity and yield of wheat are basically dependent on the chemical fertilizers (Singh et al., 2010; Ryan et al., 2012; Laik et al., 2014; Kumar et al., 2014a, b; Singh et al., 2014). The productivity of a crop is controlled by many factors i.e. environmental conditions (temperature and moisture etc.), nutrients such as nitrogen, phosphorous and potassium (NPK) (Fischer, 2000; Borlaug, 2003; Stewart et al., 2005; Ryan et al., 2012; Jia et al., 2014).

Usually high productivity of crop grains is accompanied with extensive utilization of agrochemicals for improvement of soil fertility; plant productivity and controlling plant diseases (Singh et al., 2014; Kumar et al., 2014a, b; Laik et al., 2014).

These agricultural practices has caused drastic effects on environment and public health such as increased environmental pollution, ecological damage, degraded soil fertility, developed resistant races of pathogens and increased human health risks and increased cost production (Gerber et al., 2005; Mitsch and Day, 2006; Tawfik et al., 2006; Ghormade, 2011). The most important factor is that the environmental stress and excessive and imbalanced use of chemical fertilizer has led to think about the use of organic fertilizers in intensively growing areas for sustainable crop production system (Wollenweber et al., 2003, 2005, White et al., 2006).

Environmental factors are considered to be the main source of yields reductions. Drought is one of the most common environmental stress that affects growth, development of plants through alterations in metabolism and gene expression, grain development (Leopold, 1990), reduction in yield (Blum and Sinmena, 1994). It continues to be a challenge to agricultural scientists in general and to plant breeders in particular, despite many decades of research. Improvement of productivity of wheat cultivars under drought condition becomes one of the important stress problems.

In many arid and semi-arid areas of the world where sustainability of agriculture is limited by salinity, Field salinization is an increasing problem worldwide. It is estimated that the world's cropland and irrigated land may affect by salinity (Qadir et al., 2000; Hernan and Alamansa, 2002). Salinity affects plant growth by reducing water uptake due to osmotic effect as influenced by the concentration of certain ions that have a characteristic toxic effect on plant metabolism (Patra and Panda, 1998; Weggler et al., 2000; Yang et al., 2009). Crop plants are more severely affected by salinity at early stage such as barley, wheat and corn are more sensitive to salinity during seed germination. Some keys to agricultural success in semi-arid areas include soil biology potential to maintain soil fertility, and to guard against erosion and water limiting (Hamidia et al., 2012; Dimkpa et al., 2009; Zarea et al., 2012).

Anthropogenically manufactured cadmium (Cd) is one of the heavy metals that contaminate soil and water and is of particular concern because it can accumulate in the food chain (Bashan and Holguin, 1994; 1995; Ouzounidou et al., 1997; Muhling and Lauchli, 2003). Cd pollution has increased drastically due to of industrial and agricultural practices such as power stations heating system, metal working industries or

urban traffic. It is widely used in electroplating, pigments, plastic stabilizers and Ni – Cd batteries (Santia di Toppi and Gabriell, 1999). Cd is one of the most toxic heavy metals and has been recognized as environmental contaminants with a significant role in various man and animal disease (Pinto et al., 2004). It is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto et al., 2004). Cd is a non-essential element the negatively affects plant growth and development. Cd accumulates and it translocate easily from root to shoot (Bashan and Levanony, 1987a,b). It inhibits the plant growth in terms of fresh weight and plant biomass, number of roots, root length (Safi et al., 2009). Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoot (Hernandez et al., 1996). Cd uptake is enhanced when plant grown in soil with a higher NaCl content (Weggler et al., 2000).

Fertilizers are essential components of modern agriculture because they provide essential plant nutrients (Adesemove et al., 2009). On earth, Nitrogen constitutes largest volume of air (78.08%). Nitrogen is the most important structural element of all the living organisms that plays the basic role in cell metabolism, growth, reproduction and transmission of heritable characters. But animals and the majority of plants are unable to fix atmospheric nitrogen. Excessive uses of readily available conventional chemical fertilizers on agricultural land are the main source of ground water contamination (Rawat et al., 2012). Nitrate leaching from arable land, which causes contamination of groundwater, has become a matter of worldwide concern. Nutrient losses due to leaching, run off, volatilization and fixation after fertilizer addition to the soil (Tilman et al., 2002; Jagadeeswaran et al., 2005).

Phosphorous (P) is required by plants for normal growth, however, in most part of the world soil is deficient in readily available P whereas P is present in insoluble metal chelate forms (Gyaneshwar et al., 2002; Vassilev et al., 2006a, b) therefore costly phosphatic fertilizers are applied in large quantity to fulfil the requirement of P (Shenoy and Kalagudi, 2005, Shigaki et al., 2006; Vassilev et al., 2006a, b). P fertilizers generally used are costly and contaminate environment not only after application on field but highly corrosive and explosive sulphuric acid (Reddy et al., 2002; Shigaki et al., 2006; Vassilev et al., 2006a). Phosphate solubilizing bacteria (PSB) plays fundamental roles in biogeochemical phosphorus cycling in natural and agricultural

ecosystem; PSB can transform the insoluble phosphorus to soluble forms HPO_2^{-4} and H_2PO^{-4} by acidification, chelation, exchange reactions, and polymeric substances formation (Delvasto et al., 2006). PSB plays an important role in phosphorus nutrition by enhancing its availability to plant through release from inorganic and organic soils P pools by solubilizing and mineralizing (Narula et al., 2000; Vessey, 2003; Yadav et al., 2011). Therefore, the use of PSB in agricultural practice would not only offset the high cost of manufacturing phosphatic fertilizers but would also mobilize insoluble P from soil to which they were applied.

Increasing soil nutrient availability reduces the dependence on costly chemical fertilizers in integrated nutrient management system (Adil et al., 2005; Jadia and Fulekar, 2008; Lazcano et al., 2011; Singh et al., 2013). Nevertheless, microbial inoculants have been widely adopted in various crop production systems but it is often hard to reproduce their beneficial effect consistently (Gajalakshmi and Abbasi, 2004; Shaharoon et al., 2008; Boonsiri et al., 2009; Singh et al., 2011; kumar et al., 2012; Minaxi et al., 2013; Ashok et al., 2014; Kumar et al., 2014a, b; 2015a, b). With growing concern over depleting nutrients in soil, the use of biofertilizers as a nutrient management strategy needs rethinking and alternate technology needs to be developed. In this context, biofertilizers may be a suitable option (Oliveira et al., 2009; Sharma et al., 2011; Kumar et al., 2015a, b).

At present, there is a need for developing an efficient nutrient management system with the use of organic manures, organic fertilizers and biofertilizers to maintain soil fertility and for better crop production (Shah et al., 2009; Abedi et al., 2010; Singh et al., 2011; Joe et al., 2012; Patra et al., 2014; Tamilselvi et al., 2015). The *Azospirillum* inoculation has reported to save about 20 units of N-fertilizer and that was became economically feasible by reducing the chemical fertilizers use required for improving the nitrogen content and counteracting the effects of salinity (Saad et al., 2009). For the sustainable agriculture system, it is imperative to utilize renewable inputs which can maximize the ecological benefits and minimize the environmental hazards such as in agro-ecological restoration, there is a need of reducing N loss via volatilization, erosion, leaching etc. (Hartemink, 2005; Ramos and Casasnovas, 2006).

To reduce pollution, restoration of land and wetlands and excessive use of our non-renewable resources such as petroleum, which are used in the chemical fertilizers production, an alternative method for agriculture must be developed. For this reason, environmental friendly product such as biofertilizers should be used when targeting agro-ecological restoration and sustainable ecosystem, which are the component of ecological engineering. Sustainable agriculture aims at long term maintenance of natural resources and agricultural productivity with minimal adverse impact on the environment. It emphasizes optimal crop production with minimal external inputs, reducing dependence on commercial inputs (fertilizers and pesticides) and substituting them with internal resources (Adesemoye et al., 2009; Ferreira et al., 2013; Zang et al., 2014; Kumar et al., 2014a,b; Kumar et al., 2015a,b). In order to reduce the negative effects of toxic chemicals, there is a globally continual emphasis on sustainable and less chemical dependent organic agriculture. For example, applications of various soil microbial inoculants are one of the most promising alternatives for chemical fertilizers (Arpana and Bagyaraj, 2007). The concept of adding phosphate-solubilizing microbes to fertilizers as providers of soluble phosphorus presents an economically and environmentally promising strategy (Rafique and Rashid, 2006). N₂-fixing and phosphate solubilizing bacteria (PSB) are important for crop plants as they increase N and P uptake and play a crucial role (Zahir et al., 2004; Zaidi et al., 2009; Adesemoye et al., 2010).

Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements NPK from unavailable to available from through biological process such as N fixation and solubilization of rock phosphate (Narula et al., 2000; Sahu and Jana, 2000; Cakmakic et al., 2001; Vessey, 2003). Biofertilizers decrease dependency on use of chemical N fertilizers by harvesting the atmospheric nitrogen through biological processes (Jagadeeswaran et al., 2005; Kumar et al., 2014a, b; Ashok et al., 2014). Inoculant necessitates the presence of a diverse set of traits that can help its colonization of the rhizosphere and survival under varying environmental conditions (Desbrosses et al., 2012).

Biofertilizers are important not only for the reduction of dinitrogen in air and soil but also for getting better yield in sustainable agriculture. Associate root colonizer

such as *Pseudomonas*, *Azospirillum* and *Bacillus* are able to grow rapidly with easily degradable substrates like monomeric carbohydrate or organic acids, whereas *R. leguminosarum* is able to colonize efficiently with the plant roots of leguminous and non-leguminous plants. These microbes have the unique ability to induce nitrogen fixation in agro-ecosystem (Schloter et al., 1997). In many crops, yield and N concentration in plant increased by inoculation with *Azotobacter* sp. (De Freitas, 2000; Kumar et al., 2001, Emtiazi et al., 2004). *A. chroococcum* as soil inoculants is not only effective in N fixation but also has other properties such as production of plant growth hormones (Remus et al., 2000). *Bacillus* inoculants significantly improved available P status of soil after harvest of wheat (Dadhich et al., 2011).

Plant growth responses depend on the inoculant strains and soil organic matters, the effective response observed by *Bacillus* spp., *Pseudomonas putida* and *Rhodobacter* spp., when applied with organic matter (Cakmakic 2006; Tapias et al., 2012; Kaur and Reddy 2014; Minj and Singh 2015). Application of organic materials (agricultural by-products, crop residue, tea residue, sawdust, vegetable-market waste, waste mushroom media, food processing waste, fly-ash, and municipal refuse) inoculated biofertilizers to soil can improve the soil quality, supply nutrients to plants and soil microbes, and reduce environmental pollution (Yang, 2003; Yang and Chen, 2003; Gaiind and Gaur, 2004; Zayed et al., 2005a, Biswas and Narayanasamy, 2006; Cheng et al., 2009). Microbes have various enzyme activities responsible for decomposing complex organic matrix (Yang, 2003). The animal wastes can be mixed with various biological bulking materials, such as and then with high soluble phosphorus content (Yang, 2003). To reduce N loss from agricultural land, it is essential to substitute slow-release fertilizers for readily available fertilizers and to establish proper application rates of manure (Maeda et al., 2003; Kumar et al., 2014a, b; Ashok et al., 2014). Application of biofertilizers on wheat crop significantly responds to nitrogen and increasing nutrient levels. Biofertilizer and N levels significant as spike number/m², grain weight/spike, spike length, grain and straw yields (El- Gizawy, 2009; Ahmed et al., 2011).

Organic matter can promote the dispersal and activity of applied plant growth promoting rhizobacteria, P mobilization and supply of crops in P deficient soils (Krey et al., 2011, 2012). Application of the PSB such as *Bacillus*, *Enterobacter*, *Pseudomonas*,

Aspergillus, *Trichoderma* and *Glomus* around the roots of plants, in soils, been shown to release soluble phosphorus, promote plant growth, and protect plants from pathogen infection (Zayed and Abdel-Motaal, 2005a,b; Biswas and Narayanasamy, 2006; Ouahmane et al., 2007). Biofertilizer and manure on the absorption and with the enhanced absorption efficiency, can naturally meet most of the plant's nutritional requirements, improve the efficiency of nutrient absorption and replace the chemical fertilizers in the sustainable agricultural production systems (Sharma et al., 2011; Gaggia et al., 2013; Ashok et al., 2014; Kumar et al., 2014a, b).

The socioeconomic and ecological benefits, improvement of soil quality are the significant aspects, as they contribute to a broad attributes including food quality and safety, human and animal health, environmental quality and agricultural sustainability (Parr et al., 2002). The use of non-chemical fertilizers and pesticides is one of the common practices that have been introduced with alternative agricultural systems and is largely based on the use of biofertilizers and bio pesticides. Plant roots secrete “food” for bacteria and fungi, which attract nematodes to the roots and nematodes, eat bacteria and fungi, and excrete Nitrogen, Sulphur and Phosphorus in a form that the plants can use (URS, 2001). Inoculation with biofertilizers generally increased NPK and Ca contents in the rhizospheric soil in compared with the uninoculated form. The inoculation with *Azospirillum* saved about 20 units of N-fertilizer and that saving was made economically feasible by decreasing the chemical fertilizers needed, improving the nitrogen content and counteracting the effects of salinity also (Saad et al., 2009).

The present plan of work was designed to prepare and develop commercially viable, new eco-friendly with low cost, organic matrix immobilized slow release biofertilizer super granules with the above perspective by optimizing the dose requirements of the commercially available charcoal based *Azotobacter chroococcum* and *Bacillus subtilis* consortium and its immobilization in the various combinations of the organic matrix materials during the storage and application of biofertilizers in soil. The Organic matrix based carrier of microbes is beneficial to the biofertilizers as it provides a good shelter for its multiplication and activities. Our aim was to enhance the growth, productivity and yield of winter (*T. aestivum* L.) by the application of optimal

dose of microbial consortium based super granules as sole fertilizer in place of the synthetic chemical fertilizers, urea and DAP.

There are seven main research objectives which are formulated as follows:

1. Dose optimization of *A. chroococcum* and *B. subtilis* strain applied as soil amendments in the terms of nitrogen and phosphorus availability in soil and plants, plant productivity and yield of wheat in the earthen pots.
2. Dose optimization of *A. chroococcum* and *B. subtilis* applied as seed coating for the cultivation of wheat in terms of availability of nitrogen and phosphate in soil and plants, as well as its effect on the productivity and yield of the crop in the earthen pots.
3. Optimization of the organic matrix carrier materials e.g. cow dung, vermicompost or Farm Yard Manure (FYM) for immobilization of optimum doses of *A. chroococcum* and *B. subtilis* for their enhanced efficacy in terms of microbial population, nutrient availability and plant yield in earthen pots and experimental plots.
4. Optimization of the different organic materials e.g. *acacia* gum, molasses and jaggery as binder for the immobilization of microbial biofertilizers and its enhanced efficacy in organic super granules.
5. Application of the best performing super granules for higher efficacy of the microbial biofertilizers and better wheat yield in the experimental plots.
6. Studies on the performance of best performing biofertilizer super granules under the abiotic stresses e.g. salinity, drought and high cadmium levels in pot conditions.
7. Cost-benefit analysis of the newly developed biofertilizer granules applied for wheat cultivation.



CHAPTER-2

REVIEW OF LITERATURE

Chapter 2

REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L., Family-Poaceae) cultivation started in India 9000 years ago (Feldman, 2001). Wheat is a predominant winter (Rabi) crop of north western plain zone and central zone of India. India is the second largest producer of wheat in the world (FAO, 2004; 2012; Sapkota et al., 2014). The total area under the crop is about 29 million hectares in the country (Majumdar et al., 2013). Wheat production suffers from variability in yield from year to year and from location to location. It is a permanent constraint to agricultural production in many developing countries and an occasional cause of losses of agricultural production in developed ones (Ceccarelli and Grando, 1991; Ceccarelli et al., 2007). World food production is limited primarily by – (i) Climatic factors (ii) Anthropogenic aspect.

2.2. Climatic factors

In connection with the anthropogenic influence and global change of climate there is an actual problem of plant adaptation to the odd environments (Smith, 1997; Ovrutskaya, 2012). Climatic factors significantly affect the production of crops (Gupta et al., 2002; IPCC, 2007). On global scale, 90% of all agriculturally used land is influenced by abiotic or biotic stress factors. Under stress conditions the plant faces a dilemma, similar to all other living organisms, existing under the influence of the surrounding environment, which in many cases provides conditions far or extremely far from optimal for their growth, development and yielding (Ceccarelli and Grando, 1996).

2.2.1. Drought

It has been predicted that global warming will cause more frequent and prolonged drought periods (IPCC, 2007). Drought is a widespread abiotic stress factor that lowers agricultural production due to reduction in grain yield by drought and heat stresses (Zhang et al., 2013). Drought is considered to be one of the most important agricultural problems, affecting plant growth, development and productivity (Ceccarelli and Grando, 1996; Vinocur and Altman 2005). Current research data on climate change shows that, increasing temperature combined with drought will affect many parts of the globe in near future (Esen Sekmen et al., 2012).

Drought stress leads to disruption of water potential gradients, loss of turgor, disruption of membrane integrity, and denaturation of proteins (Banderska et al., 2012). Proline as a key osmo-regulating solute in plants, play an overriding role in osmotic pressure adjustment of the cell sap concentration under water stress condition (Sharma and Dubey, 2005). Drought affects changes in water soluble sugar contents in plant tissues (Małek et al., 2012). Increase of glucose levels an indicator of the intensity of drought stress (Krzyzaniak and Lemanowicz, 2013). Limited water supply can also affect plant species and lead to contribution in it (Hoffmann and Hercus, 2000). Drought leads to stomata closure and restriction of CO₂ input, which causes imbalances between excitation energy driving electron transport and electron consumption in Calvin-cycle reactions (Flexas et al., 2004; Tausz et al., 2004). This imbalance causes the formation of harmful reactive oxygen species (ROS) and (photo-) oxidative stress (Tausz et al., 2004). In wheat, extremes of high temperature and water deficit during the critical grain filling period not only cause severe grain yield losses (Zhao et al., 2007), but also reduce the grain quality (Gooding et al., 2009). Under rainfed conditions, wheat crop frequently suffer from drought resulting in a significant loss of yield (Trethowan and Pfeiffer, 1999; Hossain et al., 2012). Drought, the result of low precipitation or high temperature, is thus one of the main problems underlying the success of modern agriculture around the globe; it affects the growth, development and production of plants (Hasanuzzaman et al., 2012; Hossain et al., 2012). Drought is uneven phenomenon that influences plants differently depending on the development stage at the time of its occurrence (Lopez et al., 2003; Martiniello and da Silva, 2011; Hossain et al., 2012).

Drought stress affects plant–water relationships on both the cellular and whole plant levels leading to the specific and nonspecific phenotype and physiological responses (Beck, 2007). Growth reduction under drought stress conditions has been well characterized in several plant species such as barley (Samarah, 2005), maize (Kamara et al., 2005), rice (Lafitte et al., 2007), and wheat (Rampino et al., 2006). Fresh weight and water content are common growth parameters that are severely affected by drought (Jaleel et al., 2009). Drought stress adversely affects plant growth, yield and productivity and also at cellular and molecular level (Pereyra et al., 2006). Vulnerability was considered as a function of sensitivity, wellbeing state relative to its damage

threshold and exposure. Vulnerability was calculated considering severe droughts in the selected years and the expected vulnerability considering the expected frequency of drought (Farhangfar et al., 2015).

2.2.2. Salinity

Abiotic factors such as shortage of water, extremely high or low temperatures or excessive salinity cause similar effects to those resulting from tissue damage or secondary stresses like osmotic or oxidative stress (Patra and Panda, 1998; Weggler et al., 2000; Yang et al., 2009). Abiotic stress factors inhibit the proper protein folding, hydrogen peroxide (H₂O₂) is produced by the effects of drought and salinity stresses, causes irreversible damage on the plant metabolism by oxidation of cellular components (Mittler, 2002; Wang et al., 2003; Hu and Schmidhalter, 2005; Hussain et al., 2008; Dimkpa et al., 2009; Khaled and Fawz, 2011; Mahdy, 2011; Uzilday et al., 2012). Increasing concentration of NaCl in perlite contributes to the increase of antioxidant activity in most studied plant species and can affect germination, seedling growth either by creating an osmotic pressure (OP) that prevents water uptake or by toxic effects of sodium and chloride ions on the germinating seed (Hasegawa et al., 2000; Ashraf, 2004; Soltani et al., 2006; Zarea et al., 2012).

2.3. Anthropogenic Aspects

2.3.1 Use of chemical fertilizers and losses

Input of agrochemicals for improvement of soil fertility, controlling of plant diseases and enhancing the crop productivity (Bakht et al., 2009; Duan et al., 2014; Kumar et al., 2014a,b; Ram et al., 2014; Singh et al., 2014; Sapkota et al., 2014). These agricultural practices, excessive use of chemical fertilizers causes drastic effects on environment and public health, increase environmental pollution, ecological damage, degrades soil fertility and increase production cost (Gerber et al., 2005; Mitsch and Day 2006). Conventional chemical fertilizers are more efficient for crop productivity in a given unit area, but a significant amount of applied fertilizers i.e. 30-50% approximately get lost by leaching, runoff, volatilization and emission losses (Menendez et al., 2008; Monem et al., 2010; Myrbeck et al., 2012; Gogoi and Baruah, 2012). These losses count to economic loss, environmental degradation and health hazards (Rawat et al., 2010, 2012). There is need to develop some sound alternatives to enhance crop productivity without harm the environment.

Wheat is the major cereal crop of India, covering about 27.0 m ha of cultivated agricultural land and consuming large amounts of nitrogenous chemical fertilizers (Dwivedi and Thakur, 2000; Babu et al., 2001; Quyen et al., 2002; Satyanarayana et al. 2002; Singh et al., 2006; Sharma et al., 2008; Liew et al., 2010). This has caused serious environmental concerns, such as emission of NO_x, ammonia volatilization, leaching of nitrate and other reactive nitrogen species in ground and surface waters, resulting in water pollution and eutrophication of streams and lakes (Golloway et al., 2008; Gupta et al., 2008; Velmurugan et al., 2008; Weligama et al., 2010; Rawat et al., 2012; Kumar et al., 2013b) (**Table-2.1.**). A survey showed that a large area in India including Uttar Pradesh, Punjab, and Haryana has been affected by nitrate pollution of ground water (Rawat and Singh, 2010). Thus, it appears that the large increase in nitrogen fertilizers is a cause for concern from the environmental, economic, and resource conservation points of view and warrants improvement in efficiency of fertilizer use.

Table 2.1. Leaching, runoff, volatilization and emission losses of greenhouse gases of nutrients from the agricultural fields by application of commercial chemical fertilizers

Fertilizers used	Leaching and Emission Losses of Nitrogen	References
chemical fertilizers on wheat crop, its losses by leaching and emission		
135 Kg N ka ⁻¹	NO ₃ ⁻ N leaching	Delin and Stenberg (2014)
0-200 Kg ha ⁻¹	NO ₃ ⁻ N leaching	Zhou and Bahl (2014)
200 Kg N ha ⁻¹	NO ₃ ⁻ N leaching N loss	Yang et al. (2014), Hartmann et al. (2014)
120 Kg N ha ⁻¹ , 75 Kg ha ⁻¹ , 45 Kg ha ⁻¹	NO ₃ ⁻ leaching	Zhang et al. (2013)
130 Kg N ha ⁻¹ , 90 Kg P ₂ O ₅ ha ⁻¹ , 36 Kg K ₂ O ha ⁻¹	NO ₃ ⁻ leaching and N ₂ O emission	Zhou et al. (2012)
270 Kg N ha ⁻¹ , 105 Kg P ₂ O ₅ ha ⁻¹ and 60 Kg K ₂ O ha ⁻¹	NO and N ₂ O emission	Cui et al. (2012)
170 Kg N ha ⁻¹ of ammonium nitrate and urea	N ₂ O emission	Gu et al. (2011)

Urea, diammonium phosphate and potassium sulphate 390-60-30 Kg N-P-K Kg ha ⁻¹	N ₂ O and NO emission	Liu et al. (2011)
Ca(NO ₃) ₂ , 4H ₂ O-23.5-188.0 mg N/column	NO ₃ ⁻ leaching in different soil layer	Weligama et al. (2010)
Urea 0 to 240 Kg hm ⁻² , P ₂ O ₅ 135 Kg hm ⁻² , K ₂ O-105 Kg hm ⁻²	NO ₃ ⁻ N leaching in successive soil depth (0-200 cm.)	Shi and Yu (2008)
Coated urea 75 Kg N ha ⁻¹ , 150 Kg N ha ⁻¹ , urea 125 Kg N ha ⁻¹ to 300 kg N ha ⁻¹	NH ₃ and NO ₃ ⁻ losses	Xiou- zhi et al. (2007)
Ammonium nitrate, urea, single super phosphate	NO ₃ ⁻ N leaching	Yang et al. (2006)
N- fertilizers	leaching of NO ₃ ⁻	Jalali (2005)
180 Kg N ha ⁻¹	CH ₄ , N ₂ O emission	Weiske et al. (2001)
Urea 250 Kg N ha ⁻¹ , superphosphate 20 Kg P ha ⁻¹	NO ₃ ⁻ and NO ₂ ⁻ leaching	Riley et al. (2001)
Urea 460 Kg N ha ⁻¹ , Floranid 32-320 g N Kg ⁻¹ , Mow compost 10.8g N Kg ⁻¹	NO ₃ ⁻ leaching	Diez et al. (1997)
chemical fertilizers on rice (<i>Oryza Sativa</i> L.) crop, its losses by leaching and emission		
Ammonium sulphate 300 Kg N ha ⁻¹	NH ₄ ⁺ , NO ₃ ⁻ leaching	Zhao et al. (2014)
–	CH ₄ , N ₂ O	Zang et al. (2014)
180 Kg N ha ⁻¹	CH ₄ , N ₂ O	Datta and Adhya (2014)
–	NO ₃ ⁻ leaching	Tan et al. (2013)
0-204 Kg N ha ⁻¹	CH ₄ , N ₂ O	Shang et al. (2011)
Ammonium sulphate 100-200 Kg N ha ⁻¹	NH ₄ ⁺ - N, NO ₃ ⁻ -N leaching	Luo et al. (2011)
Urea 0-375 Kg N ha ⁻¹	NH ₄ ⁺ - N, NO ₃ ⁻ -N leaching	Peng et al. (2011)
Kg N ha ⁻¹	CH ₄ , N ₂ O	Zhang et al. (2010)
0 - 100 Kg N ha ⁻¹	CH ₄ , N ₂ O	Qin et al. (2010)
0 - 270 Kg N ha ⁻¹	CH ₄ , N ₂ O	Ma et al. (2009)
0 - 40 Kg N ha ⁻¹	CH ₄ , N ₂ O	Datta et al. (2009)

0 - 300 Kg N ha ⁻¹	CH ₄ , N ₂ O	Li et al. (2009)
0 - 225 Kg N ha ⁻¹	CH ₄ , N ₂ O	Kreya et al. (2007)
0 - 270 Kg N ha ⁻¹	CH ₄ , N ₂ O	Ma et al. (2007)
0 - 120 Kg N ha ⁻¹	CH ₄ , N ₂ O	Bhatia et al. (2005)
chemical fertilizers on corn (<i>Zea mays</i> L.) crop, its losses by leaching and emission		
20-178 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Zhou and Bahl (2014)
–	NO ₃ ⁻ leaching	Wang et al. (2014)
240 Kg N ha ⁻¹	N ₂ O	Migliorati et al. (2014)
Urea 250 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Sanz-Cobena et al. (2012)
309-642 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Perego et al. (2012)
60- 268 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Sorensen and Ruback (2012)
0- 300 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Tafth and Sepaskhah (2012)
250 Kg N ha ⁻¹	N ₂ O, NO	Sanz- Cobena et al. (2012)
75-200 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Huang et al. (2011)
210 Kg N ha ⁻¹	N ₂ O	Liu et al. (2011)
0 - 247 Kg N ha ⁻¹	CH ₄ , N ₂ O	Halvorson et al. (2010)
90 - 225 Kg N ha ⁻¹	N ₂ O	Adviento-Borbe et al. (2010)
125 Kg N ha ⁻¹	N ₂ O	Parkin and Hatfield (2010)
146 Kg N ha ⁻¹	N ₂ O	Vanterea et al. (2010)
0 -180 Kg N ha ⁻¹	N ₂ O	Almaraz et al. (2009)
0 Kg N ha ⁻¹	N ₂ O	Guo et al. (2009)
70 Kg N ha ⁻¹	CH ₄ , N ₂ O	Phillips et al. (2009)
0-260 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Diez-lopez et al. (2008)
50 - 150 Kg N ha ⁻¹	N ₂ O	Wagner-Riddle et al. (2007)
140 - 210 Kg N ha ⁻¹	CH ₄ , N ₂ O	Adviento-Borbe et al. (2007)
0 - 215 Kg N ha ⁻¹	N ₂ O	Parkin and Kasper (2006)
0 - 291 Kg N ha ⁻¹	N ₂ O	Swiney and Robertson (2005)
0-150 Kg N ha ⁻¹	NO ₃ ⁻ leaching	Diez et al. (1997)

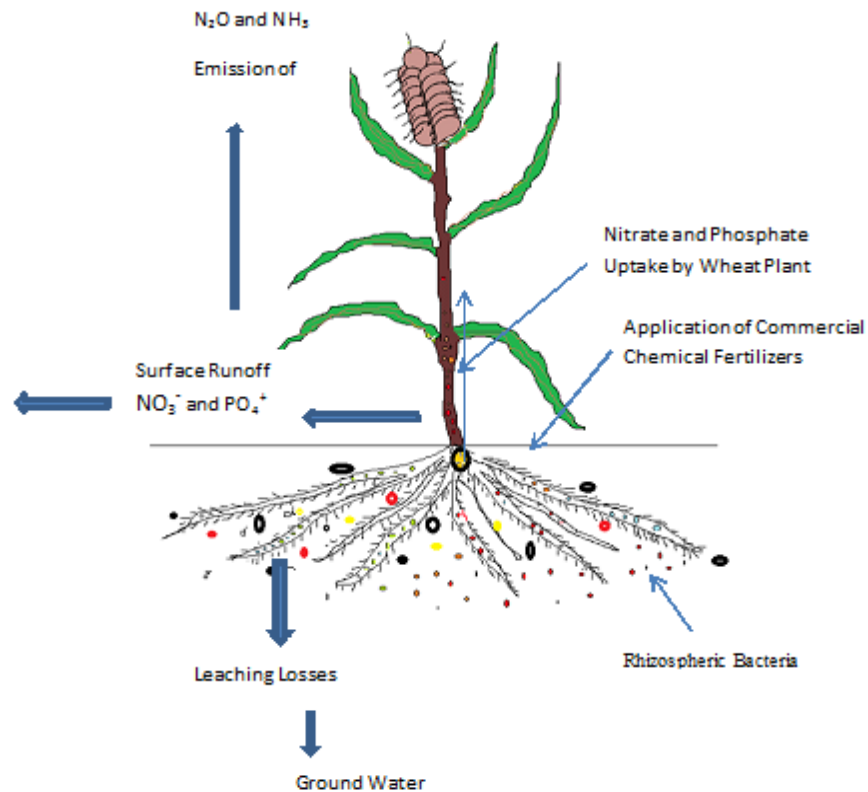


Figure 2.1. Environmental degradation due to the application of chemical fertilizers in agricultural field (Rawat et al., 2010, 2012; Yang et al., 2011; Cui et al., 2012; Gogoi and Baruah, 2012; Duan et al., 2014; Ram et al., 2014; Sapkota et al., 2014).

2.3.2 Cadmium toxicity

Cadmium (Cd) contaminates the soil and water and this is of particular concern because it can accumulate in the food chain (Kollarova et al., 2012). Cd is a toxic heavy metal taken up by wheat root showed the effects on growth of root length and shoot height. Cd toxicity also effects the seed germination and seedling biomass (Amirjani, 2012). Cd uptake by roots and transported to shoots, negatively affecting physiological processes. Casparian strips and suberine lamellae as apoplastic barriers are important components of endodermal and hypodermal cell walls in roots critical for the control of solute transport. Lignin is the biopolymer forming these barriers, mainly Casparian strips Cd uptake by the root and its transport to the shoot are apparently related to the development of root apoplasmic barriers (Schreiber et al., 1999; Vatehova et al., 2012).

2.4. Application of Biofertilizers as an Ecofriendly Alternative

Microbes have been used as an alternative to chemical fertilizers to increase soil fertility, replace chemical fertilizers, pesticides and related agrochemicals and also enhance the crop production in sustainable farming system, more useful, economically and environmentally (Wu et al., 2005). The use of selected plant growth-promoting rhizobacteria (PGPR) strains as a bio-fortification strategy for wheat, not only leads to 50% savings of N fertilizer but also represent a major, low-cost and sustainable option for enhancing micronutrient concentration in wheat grains (Rana et al., 2012). Biofertilizers play an important role in providing nutrition by enhancing its availability to plant through solubilizing and mineralizing (Yadav et al., 2011; Ardakani et al., 2011). Bacteria inoculation significantly increased number of spike/m², plant height, spike length, 1000 kernel weight, and grain yield (Gizaway, 2009; Ahmed et al., 2011), dry matter (Sundara et al., 2002; Gupta et al., 2013). P and K-dissolving bacteria PDB (*Bacillus megaterium* var. *phosphaticum*) and KDB (*Bacillus mucilaginosus* and *B. subtilis*) increased fresh and dry weight, P and K availability, uptake nutrient and the plant growth (Abou et al., 2012; Zafar et al., 2012), in soil stress condition, bacteria possess stress tolerance along with the plant growth- promoting traits (Tiwari et al., 2011) also *B. subtilis* and *B. cereus* gave the most frequent positive growth responses (wheat seedling, root weight, shoot weight and shoot length) (Ryder et al., 1999). The various type and strain has been applied on different plant species for better growth promotion (**Table 2.2.**).

Biofertilizers have illustrating the interrelationships of nitrogen fixation with increased phosphate (P) uptake by plants, it greatly promote the green technology for agriculture (Karivaradharajan et al., 2013). Organic matrix entrapped slow release bio-fertilizer (OMEB) slowly supplied nitrogen to plant and decreased the losses of nutrients resulting more nitrogen (N) available to plant for better yield production and reduced the increasing rate of NPK added to the soil (Shaharoona et al., 2008; boonsiri et al., 2009; Singh et al., 2011).

Among biofertilizers are PGPR, which are free-living rhizosphere bacteria that promote plant growth by a variety of mechanisms (Vessey, 2003; 2007; Adesemoye et al., 2010). The specific mechanism involved in PGPR-elicited enhanced nutrient uptake (Adesemoye et al., 2009). It was proposed that PGPR promoted the growth of the plant

and increased the root surface area or the general root architecture, better roots then released higher amounts of C in root exudates, the increase prompted more microbial activity and the cycle of events made more N available for plants uptake (Schloter et al., 1997; Gopalakrishnan et al., 2009; Naher et al., 2009).

Role of PGPR in plant growth promotion and biological control of soil borne pathogens have been intensively investigated (Kloepper et al., 1989; Kumar et al., 2005). Integration of PGPR with traditional organic fertilizers in the field may prove to be effective means to increase the solubility of insoluble phosphorous ions and other minerals to plants with simultaneous reduction in diseases incidence. They take systemic and simultaneous account of environmental aspects, quality of the produce and profitability of agriculture (Maene, 2000).

There are several PGPRs currently commercialized, whose growth-promoting activity in crop plants have been demonstrated in several ways including production of iron-sequestering siderophores and antimicrobial compounds that hinder colonization of hosts by phytopathogen (Weller, 1998). (Yao et al., 2010) found that *Pseudomonas putida* could protect against salt stress and promote cotton seedling growth. Inoculation of biofertilizers, solubilized precipitated mineral, provide nutrients for production of plant growth hormones, thereby enhancing the plants ability to take up nutrients from soil and increasing yield (Glick, 1995).

Table 2.2. Micro-organisms are used in different crop as biofertilizers which enhance the growth and productivity

Bacteria	Plants	Effect on plant	Mode of Application	References
<i>P. putida</i>	<i>Agaricus bisporus</i>	hyphal growth	Soil	Chen et al. (2013)
<i>Paenibacillus Lentimorbus</i> , and <i>B. subtilis</i>	<i>Phaseolus vulgaris</i>	increase in the phenolics' content and lignin accumulation	Seed inoculation	Martins et al. (2013)
<i>Lolium perenne</i>	<i>Lolium perenne</i> L.	maintenance and increased performance of turfgrass surfaces	Plant treatment	Gaggia et al. (2013)
<i>Janibacter</i>	<i>Medicago sativa</i> L.	plant growth	Seed inoculation	Guinazu et al. (2013)

<i>Endophytic bacteria</i>	<i>Oryza sativa</i> L.	shoot height, and root length	Seed inoculation	Duangpaeng et al. (2012)
<i>Azospirillum brasilense</i>	<i>Chlorella vulgaris</i>	lipid and pigment content	microalgae immobilized	Francisco et al. (2012)
<i>A. brasilense</i> , and <i>B. pumilus</i>	<i>Mesquite amargo</i> , <i>Prosopis articulate</i> , <i>palo verde</i> <i>Parkinsonia microphylla</i> , and <i>Parkinsonia florida</i>	Plant height, number of branches, and diameter of the main stem	Seed inoculation	Bashan et al. (2012)
<i>B.megaterium</i> , <i>B.mucilaginosus</i> and <i>B. subtilis</i>	<i>Zea mays</i> L.	plant growth (shoot and root growth)	Plant treatment	Seoud and Megeed (2012)
<i>Rhanelia sp.</i>	<i>Z. mays</i> L.	increases in shoot dry weight	Seed inoculation	Montanez et al. (2012)
<i>Burkholderia sp.</i> <i>B.megaterium</i> and <i>Sphingomonas sp.</i>	<i>Z. mays</i> L.	plant growth	Soil inoculated	Sheng et al. (2012)
<i>A. brasilense</i>	<i>Z. mays</i> L.	Increase in plant height (19%), plant dry weight (16%), grain yield (31%), stover yield (17%) and nitrogen uptake (18%)	Cell treatment	Joe et al. (2012)
<i>P. fluorescens</i> and <i>Burkholderia cepacia</i>	<i>T. aestivum</i> L.	Plant growth	Seed inoculation	Saxena et al. (2011)
<i>B. subtilis</i>	<i>Lycopersicon esculentum</i> Mill	Plant biomass production and yield	Seed inoculation	Hariprasad et al. (2011)
<i>P. fluorescens</i> , <i>B. subtilis</i> and <i>A. brasilense</i>	<i>Mentha piperita</i>	crop productivity and EO yield	Plant	Santoro et al. (2011)
<i>P. fluorescens</i>	<i>L. esculentum</i>	Yield	Seed and root tip treatments	Manikandan et al. (2010)
<i>A. ficuum phytase</i>	<i>Cajanus cajan</i>	Plant biomass production	Seed inoculation	Patel et al. (2010)
<i>P. putida</i>	<i>Gossypium hirsutum</i> L.	Growth-promotion	Seed inoculation	Yao et al. (2010)
<i>Sphingobacterium</i> , <i>B. sp.</i> ,	<i>Z. mays</i> L.	Plant elongation Plant biomass	Soil application	Marques et al. (2010)

<i>Achromobacter</i> , and <i>Ralstonia</i> ,		production P and N accumulation		
<i>P. trivialis</i>	<i>Galega orientalis Lam.</i>	increase nodule numbers and nitrogen content	Seed inoculation	Egamberdieva et al. (2010)
<i>Serratia proteamaculans</i>	<i>Cicer arietinum L.</i>	improving growth and yield	Seed inoculation	Shahzad et al. (2010)
<i>P. mendocina</i>	<i>Lactuca sativa L. cv.</i>	Antioxidant enzymes, proline	Soil application	Kohler et al. (2009)
<i>A. brasilense</i> and <i>P. fluorescens</i>	<i>T. aestivum L.</i>	grain yield and rhizosphere colonization of wheat	Seed	Naiman, et al. (2009)
<i>P. aeruginosa</i>	<i>Sesamum indicum L.</i>	oil yield	Seed inoculation	Kumar et al. (2009)
<i>Streptomyces acidiscabies</i>	<i>Vigna unguiculata</i>	Chlorophyll, lipid	Soil application	Dimkpa et al. (2009)
<i>P. aeruginosa</i>	<i>Sesamum indicum L.</i>	Yield	Seed inoculation	Kumar et al. (2009)
<i>Methylobacterium sp.</i>	<i>Oryza sativa L.</i>	rice seedlings growth and lateral root	Seed inoculation	Kumara et al. (2009)
<i>B. pumilus</i>	<i>Chlorella vulgaris</i>	growth yielded	Plant inoculation	Hernandez et al. (2009)
<i>A. brasilense</i> and <i>Brayrhizobium japonicum</i>	<i>Z. mays L. and Glycine max L.</i>	seed germination, nodule formation	Seed inoculation	Cassan et al. (2009)
<i>P. syringae</i>	<i>C. arietinum</i>	minerals uptake like P, Mn, and Fe and plant growth	Seed	Sahni et al. (2008)
<i>P. putida</i> and <i>P. fluorescens</i>	<i>Pisum sativum L.</i>	Plant growth	Soil and Seed inoculation	Arshad et al. (2008)
<i>M. endolithica</i>	<i>Phaseolus vulgaris L.</i>	plant growth	Soil inoculation	Tarabily et al. (2008)
<i>Rhizobium sp.</i> and <i>Burkholderia sp.</i>	<i>Schizolobium amazonicum</i>	plant growth	Soil inoculation	Siviero et al. (2008)
<i>Pseudomonas</i> and <i>Bacillus</i>	<i>Camellia sinensis</i>	tea yield	Soil application	Saravanakum ar et al. (2007)
<i>P. fluorescens</i>	<i>Catharanthus roseus</i>	growth parameters	seedling treatments	Jaleel et al. (2007)
<i>Megaterium, B. subtilis</i> and <i>P. corrugata</i>	<i>Z. mays L.</i>	yeild	Root colonization	Kumar et al. (2007)
<i>Pseudomonas sp.</i>	<i>T. aestivum</i>	increase root and	Soil	Sheng and

	L.	shoot growth	inoculation	Gong (2006)
<i>Paenibacillus polymyxa</i>	<i>S. indicum</i> L.	plant growth and the grain yield	Seed inoculation	Ryu et al. (2006)
<i>A. lipoferum</i>	<i>Z. mays</i> L.	Plant biomass production	Seed	Zemrany et al. (2006)
<i>Bacillus species, Paenibacillus polymyxa, P. putida and Rhodobacter capsulatus</i>	<i>Beta vulgaris</i>	root weight and sugar yield	Seed inoculation	Cakmakci et al. (2006)
<i>Achromobacter piechaudii</i>	<i>L. esculentum</i> and <i>Capsicum annuum</i>	plant growth	Plant inoculation	Mayak et al. (2004)
<i>P. agglomerans Mycobacterium sp. Mycoplana bullata and P. fluorescens</i>	<i>T. aestivum</i> L.	higher N, P, and K contents	Root and Shoot inoculation	Egamberdiyeva and Ho flich (2003)
<i>P. fluorescens</i>	<i>L. esculentum</i> Mill	Root establishment	Seed inoculation	Siddiqui et al. (2003)
<i>P. fluorescens</i>	<i>C. arietinum</i> L. and <i>Vigna radiata</i> L.	plant growth	Seed inoculation	Sindhu et al. (2002)
<i>Enterobacter cloacae</i> and <i>P. Putida</i>	<i>L. esculentum</i>	Root and shoot Growth	Seed inoculation	Grichko and Glick (2001)
<i>P. corrugata</i>	<i>Amaranthus paniculatus</i>	Root-shoot length and root-shoot biomass	Seed inoculation	Pandey et al. (1999)
<i>A. chroococcum</i>	<i>Eleusine coracana</i>	higher root-shoot length, root-shoot biomass, biological yield and grain yield	Seed inoculation	Pandey et al. (1999)
<i>Burkholderiu cepaciu,</i> and <i>P. fluorescens</i>	<i>Sorghum bicolor</i>	plant growth	Seed inoculation	Chiarini et al. (1998)

Immobilized microbial biofertilizers with lower doses of the conventional chemical fertilizers i.e. urea and DAP, and are very effective in enhanced growth productivity, yield, and quality of wheat (Kumar et al., 2014a, b 2015a, b). The inoculation with genus *Bacillus* (*B. licheniformis* and *B. pumilus*) is enhancing the growth of *Pinus pinea* plants, increases rhizosphere microbial communities (Probanza

et al., 2001), it produce gibberellin for promoting the growth of plant (Probanza et al., 2001; Probanza et al., 2002).

Phosphate solubilizing (PS) activities of *Bacillus*, *Rhodococcus*, *Arthrobacter*, *Serratia chryseobacterium*, *Delftia*, *gordonia* and *Phyllobacterium*, especially *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. have been reported after confirming their capacity to solubilize considerable amount of tricalcium phosphate in the medium. Phosphate Solubilizing bacteria (PSB) are used for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for the field application (Chen et al., 2006). Immobilization of *B. pumilus* from arid land soils significantly enhanced the growth of the fresh water green microalga (*Chlorella vulgaris*) (Hernandez et al., 2009). The use of PSB as inoculants simultaneously increases P uptake by the plant. *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most effective phosphate solubilizers (Rodriguez and Fraga, 1999). The synergistic effects of soil fertilization with rock P and K materials and co-inoculation with P and K-dissolving bacteria [PDB (*B. megaterium* var. *phosphaticum*), KDB (*B. mucilaginosus* and *B. subtilis*)] on the improvement of P and K uptake, P and K availability and growth of plant grown under limited P and K soil conditions have been reported (Abou-el-Seoud and Abdel-Megeed, 2012). *B. subtilis* is used as the microbial pest control agent (MPCA) (Felici et al., 2008), chlorophyll content, shoot length, root length and leaf area were significantly increased by *B. subtilis*, spray or on the soil application and it also significantly reduced the negative effect of ozone and indicating that it has capacities in plant protection against environmental stress (Holzinger et al., 2011). *B. fusiformis* exhibit highest nitrogenase activity and *A. chroococcum*, *P. fluorescent*, *B. fusiformis* and *Mesorhizobium ciceri* produced indole acetic acid (IAA), siderophore and hydrogen cyanide (HCN) in which IAA production has a promising potential for their use as a plant growth promoting rhizobacteria (Park et al., 2010; Ahmad et al., 2008; Duangpaeng and Phetcharat, 2012). Solubilization of phosphate, Siderophore production, antifungal activity and HCN production was more common trait of *Bacillus* (Rodriguez and Fraga, 1999; Ahmad et al., 2008).

2.4.1. Drought tolerant microbes

The increased frequency of droughts as a result of climate change has already been shown to reduce crop yields (IPCC, 2007; Gornall et al., 2010). The complexity of abiotic stress tolerance is the task of introducing new tolerant varieties (Wahid et al., 2007). An alternative source is to induce stress tolerance by using various biological agents (Conrath et al., 2006; Omar et al., 2009) such as PGPR which colonize in rhizospheric soil and enhance the growth of plants by direct and indirect mechanisms (Lucy et al., 2004; Dimkpa et al., 2009).

Use of biofertilizers (*B. amyloliquefaciens* and *A. brasilense*) based sustainable techniques to improve drought stress tolerance of wheat which tolerant to osmotic stress, fixates nitrogen were the best inoculation for yield of wheat grains (Omar et al., 2000, 2009). Wheat growth under drought stress conditions through priming with beneficial bacteria considered PGPR has ability to attenuate several stress consequences in plants which strongly supports the potential of such an approach to control drought stress in wheat (Kasim et al., 2013) droughts condition in soil deplete, soil C and N stocks (Borken and Matzner, 2009).

PGPR can enhance plant growth by alleviating soil stresses. The bacteria of the genus *Azospirillum* are the most researched PGPR detected in the rhizosphere of many crop plants and also in wheat crop field under drought stress (Kloepper, 2004; Cohen et al., 2008). They are able to produce plant hormones such as auxin, and proteins like polyamines, fix N, increase root growth and enhance growth of plant under the stress condition (Ramos et al., 2002; Bhaskara Rao and Charyulu, 2005; de-Bashan et al., 2005; Russo et al., 2008; Cassan et al., 2009). Production of plant hormones by *Azospirillum brasilense* can increase root growth through enhancing nutrient uptake (Russo et al., 2008; Pereyra et al., 2009). The beneficial effects of *Azospirillum* on enhanced wheat yield and reduced chemical fertilization, including N fertilizer, are of important agricultural and environmental significance and can resist the unfavourable effects of stress. (Bashan et al., 1990; Bashan and Holguin, 1994; 1995, Castellanos et al., 2000; El-Komy et al., 2003; Pereyra et al., 2006, 2009; Spaepen et al., 2008; Arzanesh et al., 2009; Pereyra et al., 2009; Fischer et al., 2010).

In intensified stress the soil microorganisms have ability to alleviate the stress (Arzanesh et al., 2011). Such conditions are common in the arid and semi-arid areas of the world. Inoculation with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas (Marulanda et al., 2007). Root-colonizing non-pathogenic endophytic bacteria can increase plant resistance to biotic and abiotic stress factors (Dimkpa et al., 2009). Plant-associated microorganisms are involved in symbiotic and associative microbial activities that help in plants to establish in their environment (Morrissey et al., 2004). IAA is quantitatively the most abundant phytohormone secreted by most plant associated bacteria such as *Pseudomonas sp.*, *Bacillus sp.*, *Azotobacter*, and *Enterobacter* species. *Bacillus*, play positive role in plant growth promotion investigate by many researchers (Spaepen et al., 2007, 2009; Duangpaeng and Phetcharat, 2012; Kumar et al., 2013; 2014a, b; 2015b). The *A. chroococcum* inoculations influenced plant growth and the nitrogen content of various plant parts, the plant growth promotion due to stimulation of native microbial communities of the rhizosphere/rhizoplane region were also found (Pandey et al., 1999).

2.4.2. Salinity tolerant microbes

Growth and productivity of rice and soil inhabiting microbial population is negatively affected by soil salinity (Nautiyal et al., 2013). To improve sustainable techniques using biofertilizers (*B. amyloliquefaciens* and *A. brasilense*) which tolerant to osmotic stress, fixates nitrogen were the best inoculation for plant growth, yield of wheat grains and has control of salt stress (Omar et al., 2000, 2009; Tiwari et al., 2011). *Pseudomonas* spp. may be used as plant-growth-promoting rhizobacterium, produced plant-growth-promoting siderophores, IAA, phosphate solubilizing enzymes, and fungal cell wall degrading enzymes such as protease and chitinase (Nain et al., 2010; Naik et al., 2008). Several PGPB strains are also known to induce abiotic stress tolerance in some plants such as salt and drought stress in wheat (Ashraf et al., 2007; Creus et al., 2004). *Azotobacter* strains to protect maize plants against salt damage; they were use as inoculant carriers of a bacterial consortium constituted with respect to improving plant performance and soil physical and microbiological properties (Rivera-Cruz et al., 2008). *Azotobacter* strains were evaluated, and the most tolerant to salinity, the use of nitrogen-fixing plant growth-promoting bacteria may represent an important biotechnological approach to decrease the impact of salinity in crops (Tapias et al.,

2012; Jha and Subramanian, 2014). Nitrogen-fixing *A. chroococcum*, P solubilizer (*B. megaterium*) and K solubilizer (*B. mucilaginous*) bacteria significantly increased the growth, biomass, seedling height and increased the nutritional assimilation of plant (total N, P and K) and also improved soil properties, such as organic matter content and total N in soil (Wu et al., 2005). The *Azospirillum sp.*, *A. chroococcum* and *P. fluorescens*, was found most effective in increasing the total dry weight, root and shoot length.

Plants have been protected from salt stress in soils by treating crop seeds and seedlings with PGPR (Yue et al., 2007; Saravanakumar and Samiyappan, 2007), which is an emerging technology designed to improve the productivity of agricultural systems. This technology aligned with the principles of sustainable agriculture is in use nowadays and limits the increased use of pesticides and fertilizers (Reed and Glick, 2004). In addition to decreasing *Azospirillum* population, increased salinity and drought significantly decreases the activity of nitrate reductase in plants and bacteria resulting in nitrate accumulation and hence decreased plant growth. As previously mentioned, *Azospirillum* is also able to produce plants hormones such as auxin and gibberellins in addition to the production of nitrate reductase even under stress, resulting in enhanced plant N and other nutrient uptake of (Hamdia and El-Komy, 1997; El-Samad et al., 2004; El-Komy et al., 2003; Cohen et al., 2008; Hamdia et al., 2000; Spaepen et al., 2007, 2008).

Growth and productivity of rice and soil inhabiting microbial population is negatively affected by soil salinity (Nautiyal et al., 2013). However, some salt resistant, rhizosphere competent bacteria like *Bacillus amyloliquefaciens* improve plant health in saline stress and increased plant growth (Nautiyal et al., 2013). Salt stress increased proline concentration, particularly in plants inoculated with PGPR. Increased salinity stress raised significantly the antioxidant enzyme activities, including those of ascorbate peroxidase and catalase of *Jatropha* leaves. The PGPR strain induced a higher increase in these antioxidant enzymes in response to salinity. Inoculation with selected PGPR could serve as a useful tool for alleviating salinity stress (Nadeem et al., 2013; Patel and Saraf, 2013).

2.5. Organic carrier

Biofertilizers have been identified as alternative to chemical fertilizers (Kundu et al., 2009; Wu et al., 2005; Perez- Montano et al., 2014; Ramesh et al., 2014). There are many methods for storage and application of biofertilizers. The first patent (“Nitragin”) was registered for plant inoculation with *Rhizobium* sp. (Nobbe and Hiltner, 1896). The practice of mixing “naturally inoculated” soil with seeds became a recommended method of legume inoculation in the USA (Smith, 1995). Two major breakthroughs in plant inoculation technology occurred in the late 1970s: (i) *Azospirillum* was found to enhance non-legume plant growth (Diibereiner and Day, 1976) by directly affecting plant metabolism (Bashan and Holguin, 1997a, b), and (ii) biocontrol agents, mainly of the *Pseudomonas fluorescens* and *P. putida* groups, began to be intensively investigated (Defago et al., 1992; Kloepper and Schroth, 1981; Glick, 1995; Glick and Bashan, 1997). The inoculated bacteria sometimes cannot find an empty niche in the soil for survival except in sterilized soil, a condition which does not exist in large-scale agriculture.

There is requirement for carrying the biofertilizers, for storage and long condition, provide healthy nutrition. There are several types and good amount of organic wastes which can carry easily biofertilizers. Organic farming and use of biofertilizers are often considered as alternative sources. It needs perfection, support/carrier for bacterial support to prevent direct stress from environment (Packowski and Berryhill, 1979; Hegde and Brahmprakash, 1992; Halsall, 1993; Rivera-Cruz et al., 2008; Jannoura et al., 2014). The bacterial formulations in the form of super granules i.e. biofertilizers Entrapped with organic matrix (SGBs) have a potential to manage high crop productivity without the problem and risk associated with it (Adil et al., 2005; Densilin et al., 2011; Sharma et al., 2012; Kumar et al., 2012; 2013a; 2014b; 2015a). In this organic farming the use of organic matrix such as vermicompost has the high nutrient quality. The vermicompost is naturally enhancing the efficiency of biofertilizers. Vermicompost generated from organic solid wastes, as it has high nutrients value which enhance soil fertility (Adil et al., 2005; Jadia and Fulekar, 2008; Lazcano and Dominguez, 2011; Singh et al., 2013), it is especially rich in NPK (nitrogen, phosphorus and potassium), micronutrients and beneficial soil microbes too (Channabasanagowda et al., 2007; Kizilkaya et al., 2012). The super

granular form of biofertilizers (*A. chroococcum* and *B. subtilis*) provides the essential nutrients to the plant during applications (Dahiya et al., 2004; Kumar et al., 2012; 2013a, b; 2014a, b; 2015a). It enriches the soil fertility and enhanced the crop productivity. The amendment of organic carrier is recent interest in eco-friendly and sustainable agricultural practices to improve the crops yield (Harish et al., 2006; 2009; Kumar et al., 2012; 2013a, b; 2014a, b; 2015a; Minj and Singh, 2015).

Application of biofertilizers, a common practice for crop cultivation throughout the world as it increases plant productivity and grain yield over no fertilizers (Rajpar et al., 2006; Zaman et al., 2010; Xin-Kai et al., 2012; Sapkota et al., 2014), farm yard manures (Gopinath et al., 2008; Nayak et al., 2012; Otinga et al., 2013), green manures (Patra et al., 2006; 2009; Yadav et al., 2013) and microbial inoculants (Ogut et al., 2005; Sary et al., 2009; Kaur and Reddy, 2014; Kumar et al., 2014 a,b).

The alternate source to increase the efficacy of applied nutrient is use of nitrification inhibitor e.g. neem leaves, which subsequently decrease the release of free ammonia from the granules (Mohanty et al., 2008; Abbasi et al., 2011). Granular form of organic matrix based slow release fertilizers slowly provide nitrogen to soil and plants and decreases the losses of nutrients caused by rapid availability of soluble chemical fertilizers and the slow release fertilizers for replacement of chemical fertilizers in agricultural field (Pathak et al., 2010; Yang et al., 2011; Singh et al., 2013; Yang et al., 2015).

Biofertilizers immobilization in an organic matrix containing cow dung, clay soil, neem leaf powder, rice bran, and acacia gum (nontoxic and biodegradable organic materials) as a carrier prepared in the form of super granules, enhances growth, productivity, and yield in rice (Dahiya et al., 2004; Kumar et al., 2012; 2015a Ashok et al., 2014), Indian mustard (Sharma and Singh, 2011) and wheat (Kumar et al., 2013a, b; 2014a, b; 2015b). Several biofertilizers have been applied as slow release forms enhance the growth and productivity of wheat crop (Kumar et al., 2014, 2015).

Application of composted organic fertilizers (poultry manure) resulted in less galling and nematode multiplication and improving plant growth (Siddiqui, 2004). The several investigations demonstrate that the newly prepared organic matrix entrapped biofertilizers (OMEB) significantly enhanced the efficacy of *Azotobactor* and *Bacillus*

spp. (Kumar et al., 2012; Ashok et al., 2014; Kumar et al., 2014, 2015a, b). The effect of OMEB on growth parameters; biomass, nutrient uptake and yield productivity of wheat crop. They were significantly increased and found good amount of microbial activities (Sharma et al., 2011, 2012; Ashok et al., 2014; Kumar et al., 2014, 2015a, b). The OMEB-applied plants showed better growth and nutritional quality than free biofertilizers. The cost of OMBE was slightly higher than that for the conventional biofertilizers, but it was less expensive than the conventional chemical fertilizer. The OMEB can replace synthetic chemical fertilizers in extensive cropping areas where there is major concern for economic, environmental and health issues. Even though the grain yield was slightly lower than with the recommended dose of conventional urea, the straw yield was almost the same with the application of OMEB.

2.6. Integrated Nutrient management

Production of crops has undergone enormous change in recent years due to development of innovative technologies including integrated nutrient management practices using biofertilizers (Aseri et al., 2008). Integrated plant nutrient systems (IPNS), farmyard manure (FYM), and use of biofertilizers are often regarded as alternatives to synthetic fertilizers (Mahajan et al., 2003; Singh et al., 2008, 2011; Affendy et al., 2011; Sharma et al., 2011b, 2012a). Integrated nutrient management system (INMS) has been recommended for crop cultivation by use of organic matrix (Singh et al., 2011; Sharma et al., 2011, 2012; Ashok et al., 2014; Kumar et al., 2014, 2015a, b). Slow release, controlled release and specially fertilizers (Singh et al., 2008 a, b; Singh et al., 2011; Chander, 2012) are new generation plant nutrients, which can be efficient and ecofriendly though most of the formulations are expensive and farmers in controlled agricultural economy or poor economy are unable to afford its use for most of the crops. N₂-fixing and phosphate solubilizing bacterial strains, dissolved P, fixed N₂ (Cakmakci et al., 2006) and significantly increased by the bacterial inoculation with low- and high-OM soil. Plant growth responses were variable and dependent on the inoculants strain, soil organic matter content, growing stage, harvest date and growth parameter evaluated. The effect of PGPR was greater at early growth stages than at the later. Effective *Bacillus* species, *Paenibacillus polymyxa*, *P. putida* and *R. capsulatus* may be used in organic and sustainable agriculture (Cakmakci et al., 2006). An integrated approach wherein microbial inoculants applied along with reduced level of

fertilizers so as to obtain better growth and yield is essentially required (Mohiuddin et al., 2000; Stephan and Nybe, 2003).

The organic fertilizers and various kinds of customized fertilizers e.g., slow release fertilizers, controlled released fertilizers, urease and nitrification inhibitors as well as microbial biofertilizers have a better retention and continuity of release of the nutrients in plants rhizosphere, therefore, their application can reduced the environmental losses of the expensive plants nutrients loaded during the crop cultivation (Dahiya et al., 2004; Sieling et al., 2006; Emilsson, 2007; Zaman and Blennerhassett, 2010; Grant et al., 2012; Steng et al., 2014). A direct correlation between the application of NPK fertilizers and crop productivity have been reported for wheat cultivation (Yadav, 2003; Kumar and Nanwal, 2006; Osborne, 2007; Brar et al., 2013; Duan et al., 2014), which leads to the leaching, volatization and emission losses (Akiyama, 2000; Weber et al., 2001; Wei-xin et al., 2007; Jiang et al., 2010; Liu et al., 2014; Plaza-Bonilla et al., 2014) biofertilizers, e.g., *A. chroococcum*, *B. subtilis*, *Azospirillum*, *Acetobacter* (Kumar et al., 2001, Ogut et al., 2005; Lavakush et al., 2014; Perez-Montano et al., 2014; Ramesh et al., 2014) and organic fertilizers (Sharma and Prasad, 1999; Yadav et al., 2013; Laik et al., 2014; Singh et al., 2014) have been applied to the wheat fields as alternative and eco-friendly nutrients. The integrated nutrient management practices and use of customized fertilizers have also been attempted for (Kumar and Nanwal, 2006; Sharma et al., 2011; Nayak et al., 2012; Das et al., 2014). However, these eco-friendly and economically as well as environmentally sustainable form of fertilizers are not able to replace the main stream chemical fertilizers due to their limitations in meeting the nutrient requirements of the crop plants, adequate promotion policies and lack of determination of the responsible systems, the problems of easy availability to the farmers as well as the cultural constraints in farmers to readily accept the new alternatives. Hence more studies on dose responses, uptake potential, leaching and emission losses and technical interventions to develop more efficient alternative fertilizers are the urgent need for maintaining sustainable productivity of wheat in extensively utilized agro- ecosystems like rice- wheat cropping system of Indo-Gangetic plains (Sharma et al., 2011; Laik et al., 2014; Singh et al., 2014; Sapkota et al., 2014).

Presently the microbial bio-fertilizers are also not able to replace synthetic fertilizers due to lower efficiency of its recommended doses and conventional forms (Ogut et al., 2005; Kundu et al., 2009). Use of such nutrients improves the soil health and quality of products. Strains of *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Bacillus*, and *Acetobacter* have been developed as biofertilizers for cereals, pulses, vegetables, oilseeds, cotton, and sugarcane (Mahajan et al., 2003; Ogut et al., 2005; Broschat and Moore, 2007). Although biofertilizers offers an ecologically sound alternative to chemical fertilizers, their efficacy is significantly lower in increasing crop yield as compared with synthetic conventional fertilizers. Thus, this is a major hindrance in realizing the ultimate goal of increasing productivity by use of biofertilizers. The microbial biofertilizers are known to be more effective, when applied with vermicompost generated from organic wastes, as it has high nutrient value to enhance microbial population and activity and subsequently soil fertility (Adil et al., 2005; Jadia and Fulekar, 2008; Lazcano et al., 2011; Singh et al., 2013). Application with vermicompost and PGPR in agricultural field showed an increase in acid and alkaline phosphatase. Some studies are available, which indicates that the use of such fertilizers is beneficial for wheat cultivations and soil environment (Patra et al., 2009; Yang et al., 2011; Kumar et al., 2013).

The organic matrix based granules significantly enhanced nitrogenous species assimilation of *T. aestivum* L. plants over the free form of Urea and DAP treated plants (Kumar et al., 2014 a, b). It has been reported that application of chemical fertilizers especially urea and DAP is a common practice for wheat cultivation throughout the world as it increases plant productivity and grain yield over no fertilizers (Rajpar et al., 2006; Zaman et al., 2010; Xin-Kai et al., 2012). It has been also reported that application of farm yard manures (Gopinath et al., 2008; Srensen and Ruback 2012; Otinga et al., 2013), green manures (Patra et al., 2006) and microbial inoculants (Ogut et al., 2005; Arshad et al., 2008; Sary et al., 2009; Montanez et al., 2012; Kaur and Reddy 2014) increase the plant growth and highest yield production over the conventional chemical fertilizers during wheat cultivation.

Harmful environmental effects resulting from the use of chemical fertilizers have spurred research into integrated nutrient management strategies which can include the use of specific micro-organisms to enhance nutrient use efficiency (NUE) (Dahiya

et al., 2004; Oak 2005; El-Sirafy et 2006; Akhtar et al., 2009; Shah et al., 2009; Abedi et al., 2010; Singh et al., 2011; Ashok et al., 2014; Kumar et 2012; Kumar et 2013b; 2014b; 2015a, b). The PGPR have been reported to enhance nutrient uptake by plants, though scientists have reported that PGPR have ability to enhance N uptake by the plant in addition with this together it can be reduced the amounts of chemical fertilizers (Adil et al., 2005; Chang and Yang 2006; Ahmad et al., 2008; Adesemoye et al., 2009; 2010; Desbrosses et al., 2012; Guinazu et al., 2013; Dong et al., 2014). Adesemoye et al. (2010) reported that the application of PGPR could help in plant growth promotion, reduce leaching of N during the growing season and reduce residual N in soils after harvest. Increase the efficacy of applied nutrient by use of nitrification inhibitor and slow release bio-fertilizer for replacement of chemical fertilizers and management of nutrient supply in agriculture (Pathak et al., 2010; Singh et al., 2013).

This brings need/demand of good farming techniques in agriculture and its management (Joshi et al., 2007; Sarwar et al., 2008; Singh et al., 2010; Nayak et al., 2012; Ryan et al., 2012; Yadav et al., 2013; Majumdar et al., 2013; Laik et al., 2014; Sapkota et al., 2014; Audrey, and Philippe, 2015; Dinis et al., 2015). The concept of organic farming has existed since the 1980s, and now it becomes the focus of significant attention by policy-makers, consumers, environmentalists and farmers (Stolze and Lampkin, 2009; Mzoughi, 2014; Palsova et al., 2014; Vincent and Fleury, 2015; Dinis et al., 2015). In India, the use of plant and animal wastes as a source of plant nutrient was the accepted practice (Tein et al., 2014; Jannoura et al., 2014; Charis et al., 2015). The importance and aim of organic manures and green manure crops have revived in modern agriculture (Chandra, 2005; Lazcano and Dominguez, 2011; Yadav et al., 2013; Tein et al., 2014; Todorova and Ikova, 2014). Hence, now a day's scholars and farmers are reworking back towards primitive farming methodology i.e. system of the organic farming in sustainable manner is increased in the agricultural field (Mzoughi, 2014; Talmhaiochta and Mara., 2014; Palsova et al., 2014; Atlason et al., 2015; Dinis et al., 2015; Lazzaro et al., 2015; Kontopoulou et al., 2015; Allaire et al., 2015). In organic framing the use of organic manure, green manure, organic waste, is used as organic fertilizers. Compost and composted manure can be added to improve fertility of soil (Kumar et al., 2013; 2014a; 2015a, b; Ashok et al., 2014). The demand of grains, cereals and vegetables grown by organic farming is day by day increasing.

It appears that the efficacy of conventional chemical fertilizer can be improved by immobilizing it with organic matrix. It can be seen as a successful attempt towards developing alternatives of synthetic N fertilizers, which cause significant economic, environmental and health related concerns. The previous study demonstrated that the application of farm yard manure, green manure and biofertilizer (*Azospirillum*) along with reduced doses of urea increase soil microbial properties in optimal irrigation and no tillage conditions (Sharma et al., 2011; 2012). Commercial preparation of coated urea (Shoji and Gandenza, 1992, Xioa- Zhi et al., 2007) have been shown that lower doses of chemical fertilizers can be more yield producing with certain technical interventions. Yang et al. (2011) have also showed that more wheat productivity can be obtained with less amount of urea if applied as a control release formulations. The half of the recommended dose of urea entrapped in an organic matrix can produced more wheat with better nutritional qualities (Kumar et al., 2013).

Today biofertilizer application is a common practice for wheat cultivation throughout the world as it increases plant productivity and grain yield over no fertilizers. The recommended dose of a nutrient is generally decided by field trials in the given agro-climatic conditions, however many factors e.g. soil type, availability of organic matter and nutrients in soil and other agro-climatic and genetic factors may affect the nutrient use efficiency (NUE) for specific crops in specific agricultural fields.

2.7. Organic binders

The use of biofertilizers affect the mineralization and solubilization of nutrients in the agricultural field (Kundu et al., 2009; Ardakani et al., 2011; Piromyou et al., 2011; Yadav et al., 2011; Panhwar et al., 2012; Singh et al., 2013; Kumar et al., 2014a, b; Yadav et al., 2014). By the application of biofertilizers and organic matrix increased the wheat crop productivity in crop field (Naher et al., 2009; Abedi et al., 2010; Youssef et al., 2014; Kumar et al., 2013a, b, c; 2014; 2015a, b). But the direct application of biofertilizer as in soil inoculation or seed inoculation may get disposed or harm it by other microbes, protozoan and many other stresses (low nutrient profile, harsh soil condition, environmental stresses etc.) they can't tolerate (Heijnen et al. 1992; Rodriguez-Navarro et al., 1991; Bashan and Levanony, 1988; Van-Elsas et al., 1986; Mamta, 2010; Piromyou et al., 2011; Sharma et al., 2012; Lavakush et al., 2014; Kumar et al., 2015 a, b; Zaidi, et al., 2015).

Organic matrix applied in agricultural field in granular biofertilizers form, reduces the environmental losses of the expensive plants nutrients loaded during the crop cultivation (Dahiya et al., 2004; Sieling et al., 2006; Emilsson, 2007; Zaman et al., 2010; Grant et al., 2012; Steng et al., 2014; Shaharoon et al., 2008; boonsiri et al., 2009; Singh et al., 2011; Kumar et al., 2012; 2013a, b; 2014a, b). SGBs based slow release formulations enhances the efficiency of biofertilizers. In this formulations the different kind of substrate including, acacia gum, molasses and jaggery have been use as co-binders to produce slow release fertilizers (SRFs).

Binder in preparation of super granules plays an important role (Sharma et al., 2011; 2012; Kumar et al., 2012; 2013a, b; 2014 a, b; 2015 a, b). Molasses is used as binder of lime stone (Jim and Robert 2001) and is a by-product of fruits (Ahmedna et al., 2000). Molasses and jaggery has their own property which provides the various nutrients such as carbon source, sucrose as a medium growth to the microbes (Kirk and Othmer 1967; Bhosale and Gadre, 2001; Kalogiannis et al., 2003; Aksu and Tugba-Eren 2005; Li et al., 2007; Oliveira et al., 2007; Ren et al., 2010; Kucukasik et al., 2011; Cappelletti et al., 2012; Abou-Taleb et al., 2014; Trivedi and Shah 2014).

In this experiment we have evaluate the beneficial and low cost super granules. This kind of formulation increases the efficacy of applied PGPR as slow release biofertilizers and it has excellent ability to manage nutrient supply in agricultural field (Adesemoye et al., 2009; Singh et al., 2013; Ashok et al., 2014). It greatly promotes the green technology for agriculture (Swarnalakshmi et al., 2013) in sustainable manners. Basically release of nutrients depends on the degree of affinity between binders and organic matrix materials, which define the release of nutrients from granules in the soil. In this paper effects of different binders used to immobilise *A. chroococcum* and *B. subtilis* with organic matrix at optimized dose for wheat cultivation in the field experiment have been investigated. The objectives of this paper were: (1) To study the knowledge of organic matrix immobilized with biofertilizers for organic farming practices (2) To find out the relationship between binders and organic matrix materials. (3) The socio-economics effect of super granules (SGs).



CHAPTER-3

MATERIALS

AND

METHODS

Chapter 3

MATERIALS AND METHODS

3.1. Materials and Methods

3.1.1. Experimental Site

The experiments were conducted in the environmental field station (Net House) at Babasaheb Bhimrao Ambedkar University, Lucknow, India. It is situated of 123 m above the sea level and lies between 26.30⁰ and 27.10⁰ north latitude and 80.30⁰ and 81.13⁰ east longitudes and Latitude and Longitude of Mohanlalganj, Lucknow is 26.69⁰N and 80.98⁰E respectively during winter (Rabi) season.

3.1.2. Seed Source

The certified seeds of wheat (*Triticum aestivum* L. cv. PBW-502) were obtained from a local dealer of Lucknow city.

3.1.3. Immobilized of Organic Matrix Based Granular Slow Release Microbial Biofertilizers

The Biofertilizer consortium constituted of phosphate solubilizing bacteria (i.e. *Bacillus subtilis*; 2.47 Kg ha⁻¹) and nitrogen fixing bacteria (i.e. *Azotobacter chroococcum*; 2.47 Kg ha⁻¹). Organic matrix was prepared in comprising of clay soil (diameter of particles <0.002 mm; 50 kg ha⁻¹), vermicompost (2 ton ha⁻¹), cow dung, farm yard manure and neem (*Azadirachta indica*) leaves were collected locally. All the collected materials were dried separately in oven at 60 -70⁰C for 3 days and powdered in a grinder and were mixed in 1:1:1 ratio to prepare the granule. These organic materials were binded with 15% saresh (plant gum of *Acacia spp.*) and approximately 5 mm diameter small granules were prepared manually and dried at room temperature.

3.1.4. Preparation of Experimental Plots and Application of Organic Matrix Based Granular Slow Release Fertilizers Granules in the Plots and Experimental design

Various nutrient combinations were used under integrated nutrient management practices with organic matrix (clay soil, neem leaves, vermicompost, farm yard manure, cow dung in 1:1:1 ratio) and plant gum saresh (*Acacia spp.*) was used as binder. The

experiments were conducted in 2011-12, 2012-13 and 2013-14, during winter season (December to April). The field blocks of 3 m² (1.5 x 1.5 m) area were maintained. The experimental design consisted of a number of different treatments and each treatment was replicated three times in a randomized block design (RBD).



Plate: 3.1. Organic matrix such as clay soil, vermicompost, neem leaves immobilized with commercial charcoal based biofertilizers. Vermicompost is one of the best organic carriers among the FYM and cow dung

Table 3.1. The detailed layout of biofertilizer (*A. chroococum* and *B. subtilis*) treatments in free form with enhance dose for dose optimization as follows:

NF-	No fertilizer
CF-RD-	Chemical fertilizer recommended dose (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
BF- I-	Biofertilizer free form single dose (RD; 0.6 kg ha ⁻¹)
BF- II-	Biofertilizer free form double fold
BF- III-	Biofertilizer free form triple fold
BF- IV-	Biofertilizer free form fourth fold
BF- V-	Biofertilizer free form fifth fold
BF- VII-	Biofertilizer free form seventh fold

Table 3.2. The detailed layout of free form, seed coated with biofertilizer (*A. chroococum* and *B. subtilis*), in enhance dose for dose optimization as follows:

NF	No fertilizer
CF-RD	Chemical fertilizer recommended dose (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
BFS- I	Seed coated with biofertilizers single dose (RD; 0.6 kg ha ⁻¹).
BFS- II	Seed coated with biofertilizers upto twofold used <i>acacia</i> gum
BFS- III	Seed coated with biofertilizers upto threefold used <i>acacia</i> gum
BFS- V	Seed coated with biofertilizers upto fivefold used <i>acacia</i> gum
BFS- VI	Seed coated with biofertilizers upto sixfold used <i>acacia</i> gum
BFS-VII	Seed coated with biofertilizers upto sevenfold used <i>acacia</i> gum

Table 3.3. The detailed layout of seeds coated with biofertilizer (*A. chroococum* and *B. subtilis*) and organic matrix (vermicompost), in enhance dose for dose optimization as follows:

NF	No fertilizer
CF-RD	Chemical fertilizer recommended dose (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
BF-1(SV)	Seed coated with biofertilizers single dose (RD; 0.6 kg ha ⁻¹) and vermicompost
BF-2(SV)	Seed coated with biofertilizers upto twofold with vermicompost and used <i>acacia</i> gum
BF-3(SV)	Seed coated with biofertilizers upto threefold with vermicompost and used <i>acacia</i> gum
BF-5(SV)	Seed coated with biofertilizers upto fivefold with vermicompost and used <i>acacia</i> gum
BF-6(SV)	Seed coated with biofertilizers upto sixfold with vermicompost and used <i>acacia</i> gum
BF-7(SV)	Seed coated with biofertilizers upto sevenfold with vermicompost and used <i>acacia</i> gum

Table 3.4. The detailed layout of various organic matrix based immobilized biofertilizers (*A. chroococum* and *B. subtilis*) triple dose for optimization of specific organic matrix as follows:

NF	No fertilizer
CF-RD	Chemical fertilizer recommended dose (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
BF-3	Free form of consortium of biofertilizers in triple dose. Recommended dose of biofertilizers (<i>A. chroococum</i> and <i>B. subtilis</i> RD; 0.6 kg ha ⁻¹ respectively).
BFCDCN	Immobilized form of consortium of biofertilizer with cow dung clay soil, neem leaves and binder saresh

BFCDN	Immobilized form of consortium of biofertilizer with cow dung, clay soil neem leaves and binder saresh
BFDC	Immobilized form of consortium of biofertilizer with cow, dung clay soil and binder saresh
BFCD	Immobilized form of consortium of biofertilizer with cow dung and binder saresh
BFVCN	Immobilized form of consortium of biofertilizer with vermicompost clay soil, neem leaves and binder saresh
BFVN	Immobilized form of consortium of biofertilizer with vermicompost , neem leaves and binder saresh
BFVC	Immobilized form of consortium of biofertilizer with vermicompost, clay soil and binder saresh
BFV	Immobilized form of consortium of biofertilizer with vermicompost and binder saresh
BFFYCN	Immobilized form of consortium of biofertilizers with farm yard manure, clay soil neem leaves and binder saresh
BFFYN	Immobilized form of consortium of biofertilizer with farm yard manure, neem leaves and binder saresh
BFFYC	Immobilized form of consortium of biofertilizer with farm yard manure, clay soil and binder saresh
BFFY	Immobilized form of consortium of biofertilizers with farm yard manure and binder saresh
BFCN	Immobilized form of consortium of biofertilizer with clay soil neem leaves and binder saresh
BFC	Immobilized form of consortium of biofertilizer with clay soil and binder saresh

Table 3.5. The details layout of organic matrix (vermicompost) based immobilized biofertilizer (*A. chroococum* and *B. subtilis*) triple dose immobilized with different binders as follows:

NF	No fertilizer
CF-RD	Chemical fertilizer recommended dose (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
BF- III	Biofertilizer free form triple dose (RD; 0.6 kg ha ⁻¹)
IVJ- III	Immobilized form of biofertilizer with vermicompost and binder jaggery
IVM- III	Immobilized form of biofertilizer with vermicompost and binder molasses
IVS- III	Immobilized form of biofertilizer with vermicompost and binder saresh

Table 3.6. The details layout of organic matrix (vermicompost) based immobilized biofertilizer (*A. chroococum* and *B. subtilis*) triple dose immobilized with different binders applied under abiotic stresses e.g. salinity and high levels of cadmium concentration as follows:

NF	No fertilizer
FCF	Conventional chemical fertilizers (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
FBOM	Free form of consortium of biofertilizer (<i>A. chroococum</i> , and <i>B. subtilis</i>) in threefold of recommended dose (RD; 0.6 kg ha ⁻¹) with free form of organic matrix apply at after 30 DAS (Salinity; 100 mM Kg ⁻¹ soil)
IVS	Immobilized form of vermicompost apply after 30 DAS used <i>acacia</i> as binder (100 mM Salinity+5 µM Cd; Kg ⁻¹ soil)
IVJ	Immobilized form of vermicompost apply after 30 DAS used jaggery as binder (Salinity; 100 mM Kg ⁻¹ soil)
IVJ	Immobilized form of vermicompost apply after 30 DAS used jaggery as binder (100 mM Salinity+5 µM Cd; Kg ⁻¹ soil)
SCVJ	Seed inoculate with biofertilizer and coat with vermicompost used jaggery as binder + free form of organic matrix (100 mM Salinity+5 µM Cd; Kg ⁻¹ soil)
IVJ	Immobilized form of vermicompost apply after 30 DAS used jaggery as binder (Cd; 5 µM Kg ⁻¹ soil)
IVM	Immobilized form of vermicompost apply after 30 DAS used molasses as binder (Salinity; 100 mM Kg ⁻¹ soil)
IVM	Seed inoculate with biofertilizer and coat with vermicompost used molasses as binder (100 mM Salinity+5 µM Cd; Kg ⁻¹ soil)

Table 3.7. The details layout of organic matrix (vermicompost) based Immobilized biofertilizer (*A. chroococum* and *B. subtilis*) triple dose bind with different binders applied under drought stress condition as follows:

NF	No fertilizers
FCF	Conventional chemical fertilizers (Urea; 150 kg ha ⁻¹ and DAP; 75 kg ha ⁻¹)
FBF	Free form of consortium of biofertilizer (<i>A. chroococum</i> , and <i>B. subtilis</i>) in threefold of recommended dose
BFOM(NI)	Free form of biofertilizers with free form of organic matrix. (normal irrigation)

SCVJ (NI)	Seed colonization with biofertilizer and coated with vermicompost use binder jaggery+ free form of organic matrix (normal irrigation)
FOM (NI)	Free form of organic matrix apply after 30 DAS (normal irrigation)
IVJ (NI)	Immobilized form of biofertilizer in with vermicompost, clay soil neem leaves and binder jaggery apply after 30 DAS (normal irrigation)
BFOM(D)	Free form of biofertilizers and free form of organic matrix apply after 30 DAS (Drought condition)
IVJ (D)	Immobilized of with vermicompost, clay soil neem leaves and binder jaggery apply after 30 DAS (Drought condition)
SCVJ (D)	Seed colonization with biofertilizer in vermicompost use jaggery as binder (Drought condition)
FOM (D)	Free form of organic matrix apply after 30 DAS (Drought condition)

3.2. Method of Biofertilizers Application

3.2.1. Seed Inoculation

Seeds were sterilized by soaking them in 0.1% HgCl₂ solution for 5 minutes to avoid any fungal/bacterial contamination and then washed thoroughly with running tap water followed by deionized water.

3.2.2. Soil Application

In soil application of biofertilizer apply as free form and also in form of organic matrix based granular slow release fertilizers.

3.3. Maintenance of Crop Growth Conditions

The seeds of wheat (*T. aestivum* L. cv. PBW 502) were sown at a depth of 4-5 cm. Thinning of plants was carried out after 25 days of sowing. The plant to plant distance of 10 cm was maintained and the distance between field blocks of 1.5 feet was maintained. In addition to irrigations were given as per after 21, 60 and 90 days of sowing. Hoeing-cum-weeding was done after one month of sowing. The crop was harvested when the colour of siliquae changed from green to light yellow. The harvested crop was tied in bundles and kept for sun drying after proper labelling.

3.4. Measurement of the Growth Parameters

3.4.1. Root Length and Shoot Length

Root length and shoot lengths were measured by erecting the plant parts on a graph paper and measured by using graph scales.

3.4.2. Leaf Number and Number of Roots

Leaf number and number of roots were counted manually at regular intervals.

3.4.3. Fresh Weight

The fresh weight of plant parts was recorded at regular intervals using single pan electric balance. The plant parts were washed with distilled water and excessive water was removed by placing within two layers of filter paper before weighing.

3.4.4. Dry Weight

The dry weight of the same tissue was recorded after drying it in hot air oven at 60⁰C for 48 hr. when the dry weight became constant.

3.5. Measurement of the yield parameters

3.5.1. Number of Tillers per Plant

Tillers of selected plants from each plot were counted (n=6) and then mean number of tillers per plant were calculated.

3.5.2. Grain and Straw Yield (t ha⁻¹)

The grain and straw yield of each plot was recorded in tonnes after cleaning the threshold produce and expressed in t ha⁻¹.

3.6. Enumeration of Bacteria by Plate Count or Serial Dilution Agar Plate

The plate count technique is one of the most routinely used procedures because of the enumeration of viable cells by this method. This method is based on the principle that when material containing bacteria are cultured, every viable bacterium develops into a visible colony on a nutrient agar medium. The number of colonies, therefore, is the same as the number of organism contained in the sample. In this procedure a small measured volume (or weight) is mixed with a large volume of sterile water or saline called the diluent or dilution blank. Dilutions are usually made in multiples of ten. Different dilution (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶ and 10⁻⁷) of 1 g soil sample were

prepared. Appropriate dilution (1 ml) (10^{-2} to 10^{-7}) was added to sterile petriplates for isolation of microbial colonies. Three petriplates were used for each dilution. Nutrient medium (15 ml) was added to each plate containing the diluted sample. The contents of each plate were mixed to distribute the microbial cells throughout the medium. The plates were allowed to solidify and incubated in an inverted position for 24 h at 37°C . The number of colonies in the plates was counted by the method- To facilitate calculations, the dilution is written in exponential notation a single dilution is calculated as follows:

$$\text{Dilution} = \frac{\text{volume of the sample}}{\text{total volume of the sample and the dilution}}$$

Serial dilutions are prepared by transferring a known volume of the dilution to second dilution blank and so on. Once diluted, the specified volume of the dilution sample (1 ml or 0.1 ml) from various dilutions is added to sterile petri plates (in triplicate for each dilution) to which molten and cooled ($45\text{-}50^{\circ}\text{C}$) suitable agar medium is added. The colonies are counted on a Quebec colony counter. The number of organisms developed on the plates after an incubation period of 24-48 hours per ml is obtained by multiplying the number of colonies obtained per plate by the dilution factor, which is the reciprocal of the dilution. Count of soil samples were determined by viable plate count method.

$$\text{number of cells/ml} = \frac{\text{number of colonies}}{\text{amount plated} \times \text{dilution}}$$

Requirements-

- Sample
- 9 ml dilution blanks (7)
- Sterile petri plates (12)
- Sterile 1ml pipette (7)
- Nutrient agar medium (200 ml)
- Colony counter

Nutrient agar (pH 7.0)

Peptone : 5.0 g

Beef extract : 3.0 g NaCl : 5.0 g

Nutrient agar : 15.0 g

Distilled water : 1000.0 ml.

3.7. Estimation of Ammonium Content

Ammonium was estimated using weatherburn, 1967 by using Nessler's reagent method.

Reagent required: -

1. Nessler's Reagent : 1 g iodine

Procedure

Crush 1 g of sample in 6 ml distilled water. Centrifuge it and obtain the supernatant. Supernatant is used as the sample. Take 2.5 ml of sample. Add 1.5 ml Nessler's reagent. Red to brown colour appears. Take O.D. at 420 nm.

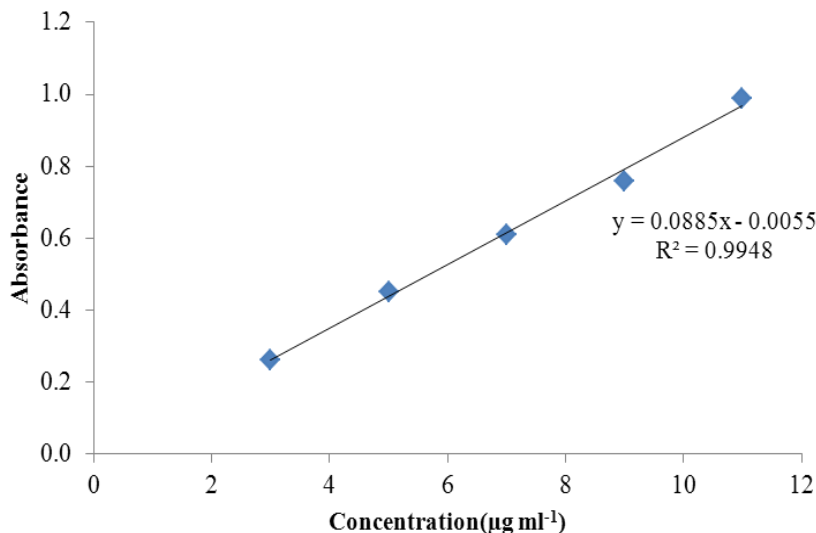


Figure 3.1. Standards Curve of Ammonium

Calculation:

Ammonium ($\mu\text{g ml}^{-1}$) = K-factor x Absorbance (O.D.).

K- Factor = Absorbance (O.D.) / Concentration.

3.8. Estimation of Nitrite content

Under normal growing conditions with sufficient light as a source of energy, enzyme systems in green plants rapidly reduce nitrate-N (NO_3^-) to intermediate compounds that are subsequently converted into amino-nitrogen. (J.R. Brown)

For nitrite estimation **Stevens and Oaks, 1973** method is used.

Reagent required: -

1. **NaNO₂ stock solution:** - dissolved 0.001 g of NaNO₂ in 100 ml distilled Water.
2. **1 % sulphailamide (LR) solution (4-amono benzene sulphonamide (C₆H₈N₂O₂S))**
:- Dissolved 1 g sulphanilamide in 100 ml 1N HCl.
3. **0.01% NED (N -1-naphthyl ethyldiamine dihydrochloride (GR) C₁₂H₁₆C₁₂N₂:-**
Dissolved 0.01 g of NED in 100 ml distilled water.

Procedure: - Standard with NaNO₂

Reference: - 0.1 ml distilled water + 1 ml sulphanilamide + 1 ml 0.02 % NED.

1 g of sample crushed with 6 ml of distilled water. Centrifuge at 5000 rpm for 10 minutes. Take out 0.1 ml of supernatant and Add 1 ml of 1 % sulphanilamide and Add ml of 1 ml of 0.02 % NED and after 10 minutes pink colour developed. Take OD at 540 nm.

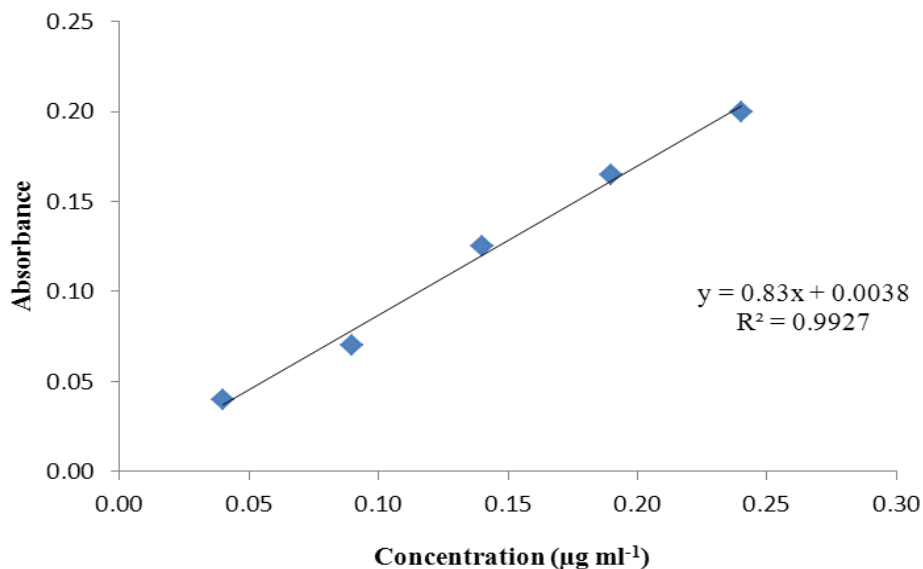


Figure 3.2 Standards Curve of Nitrite

Calculation

Nitrite (µg ml⁻¹) = K-factor x Absorbance (O.D.)

K- Factor = Absorbance (O.D.) / Concentration

3.9. Estimation of Nitrate content

For nitrate estimation **Catalado method (Catalado et al., 1975)** is used.

Reagents preparation: -

1. **Stock solution of KNO₃**: - 0.1g KNO₃ + 100 ml distilled water.
2. **5 % salicylic acid**: - 5 g salicylic acid + 100 ml conc. H₂SO₄.
3. **2N NaOH**:- 20 g. NaOH + 250 ml distilled water.

Procedure: - standard with KNO₃.

Reference: - 0.1 ml of distilled water + 0.4 ml of 5 % salicylic acid + 9.5 ml 2N NaOH.
1 g. sample crushed with 6 ml of distilled water. Centrifuge at 5000 rpm for 10 minutes.
Take out 0.1 ml of supernatant and Add 0.4 ml 5 % salicylic acid and then add 9.5ml 2N NaOH. Orange-yellowish colour appears after 20 minutes. Take OD at 410 nm.

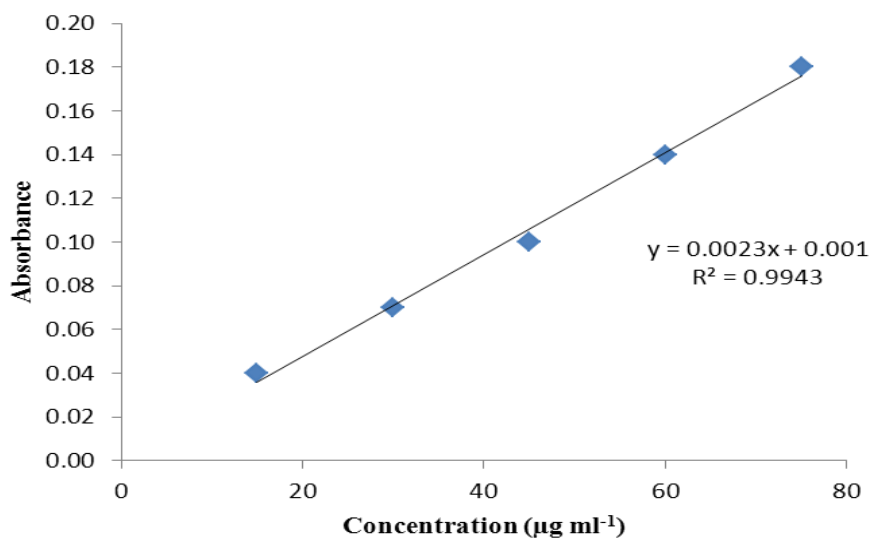


Figure 3.3. Standards Curve of Nitrate

Calculation:

Nitrate (µg ml⁻¹) = K- factor x Absorbance (O.D.)

K- Factor = Absorbance (O.D.) / Concentration

3.10. Estimation of Phosphate Content

Phosphate content was estimated by ammonium molybdate and stannous chloride method.

Reagent preparation

1. Ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$): 1.25 g in 50 ml Conc. H_2SO_4 .
2. Stannous chloride (SnCl_2) : Dissolve 0.61 g in 25 ml of glycerol, $\text{C}_3\text{H}_8\text{O}_3$

Crush 2 g of sample in 12 ml distilled water. Centrifuge it and obtain the supernatant. Supernatant is used as the sample. Take 10 ml of sample. Add 0.4 ml of ammonium molybdate and 1 drop of stannous chloride. Take reading at O.D. of 680 nm.

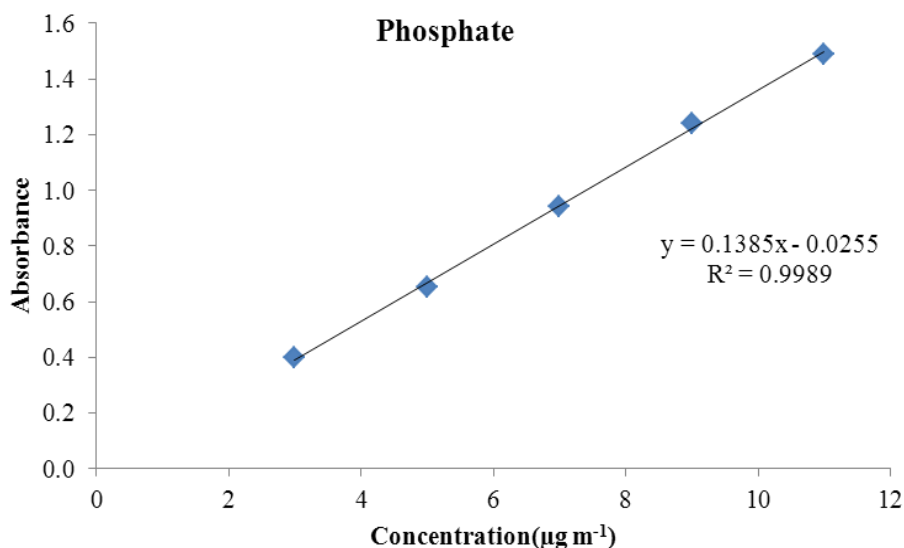


Figure 3.4. Standards Curve of Phosphate

Calculation

Phosphate ($\mu\text{g ml}^{-1}$) = K-factor x Absorbance (O.D.)

K- Factor = Absorbance (O.D.) / Concentration

3.11. Estimation of Nitrate Reductase Activity**Reagents-**

a) Phosphate buffer 0.1M

b) Grinding medium- Tris- 0.1M

Cysteine- 0.01M

EDTA- 0.0003M

-pH of grinding medium was 7.3 – 7.8

c) 0.1M KNO_3 – dissolved 1.01 g KNO_3 in 100 ml DW

d) 2 mM NADH- dissolved 14 g NADH 0.014 g in 10 ml DW

e) 1% Sulphanilamide in 1.5N HCL

- f) 0.02% N-(1-Naphthyl), Ethylenediamine (HCL) NNED – dissolved in 100 ml DW
- g) Standard potassium nitrite solution (0.01M) – dissolved 0.85 g pure KNO_2 in 100 ml DW in a 100 ml volumetric flask.

Enzyme Extraction-

Removed the shoot portion or leaves from the plants immerse quickly in cold water (2°C).

- ❖ Blotted dry, weight and cut in to small pieces.
- ❖ Grinded in an ammimixes at max speed for 10mins in grinding medium (4 ml/g tissue).
- ❖ Press & homogenate through cheese cloth and centrifuge at 20000 rpm for 10 mins.
- ❖ Decant the supernatant, stored at $3-5^{\circ}\text{C}$.

Enzyme Assay-

- 1) Pipette out 1 ml of phosphate buffer + 0.2 ml KNO_3 + 0.5 ml NADH + .01 ml DW
- 2) Initiate the assay by adding 0.2 ml of enzyme extract. Set the blank similarly with water instead of enzyme extract.
- 3) Incubate at 27°C for 15mins.
- 4) Stop the reaction by adding 1ml of sulphanilamide
- 5) Add 1 ml of NNED reagent, mix by inverting the tubes & incubate for 5mins.
- 6) Centrifuge at 1500 rpm for 10 min to remove turbidity if any.
- 7) Measure the absorbance against the blank at 540 nm.
- 8) Prepare the standard curve with diluted KNO_2 solution with series of test tubes, make the volumes in each to 2 ml with water and proceed from steps 4 to 7.

3.12. Protein Estimation-

Protein estimation was determined by Lowry et.al. (1951).

- 1) Reagent A- reaction mixture- 2% Na_2CO_3 in 50 ml mixed with 50 ml of 0.1N NaOH solution.
- 2) Reagent B- 10 ml of 1.56% CuSO_4 solution mixed with 10 ml of 2.3% sodium potassium tartrate solution.
- 3) Reagent C- prepare analytical reagent by mixing 2 ml of reagent B with 100 ml of reagent A.
- 4) 10% TCA
- 5) NaOH (1N)

Procedure -

- ❖ 100 mg (0.1 g) sample crushed in 3 ml of 10% chilled TCA.
- ❖ Cooling centrifuged at 10000 rpm for 10 min at 4⁰C
- ❖ Discard supernatant take pellets.
- ❖ Dissolve pellets in 3 ml of 1N NaOH, heated for 30 mins.
- ❖ Cool it again centrifuged at 10000 rpm for 10 min.
- ❖ 0.5 ml supernatant + 6 ml of reagent C, keep it for 10-15 min. at room temp.
- ❖ In this, reagent D (0.5 ml) Folin phenol reagent was added mixed thoroughly constant shaking.
- ❖ After 30 min OD was measured at 660 nm

3.13. Scanning Electron Microscope Energy dispersive X-ray (SEM EDX) Based Analysis

Organic matrix and organic matrix Immobilized granule samples were oven dried (60⁰C) and powdered with pestle and mortar separately. The samples were mounted on a stub with double sided sticky carbon tape. A very thin film of gold and palladium was deposited on the surface of the samples to make them electrically conductive using vaccum coating unit. This extremely fine coating was done through the evaporation of Au-Pd plate under inert atmosphere. After that, the samples were mounted on electron microscope stubs and examined and photographs were taken at different magnification by stereo scanning electron microscope at 30 tilt and 10 KV. The SEM EDX analysis was carried out with the help of a computer. Model: JSM- 6490 LV, Make: JEOL, Japan.

3.14. Fourier Transform Spectroscopy (FTIR) Based Analysis

Organic matrix and organic matrix Immobilized granules were oven dried (60⁰C) and powdered with pestle and mortar separately. The powdered samples were mixed with potassium bromide (KBr) in 1:90 of sample and KBr. The pellets/ tablets for FTIR spectroscopy were prepared using high pressure (150 pascal). The transmittance spectra were measured between 400 to 4000 cm⁻¹ by using fourier transform infrared spectroscopy (FTIR), **model: Nicolet TM 6700, Make, Thermo Scientific, USA.**

3.15. Soil Analysis

Soils were sampled from top 0-15 cm soil, between the plants roots was collected and then analysed.

3.15.1. pH (pouvoir hydrogen or hydrogen power)

Equipment: pH meter

Procedure

4 g of soil sample was taken and mixed with 20 ml of distilled water.

The suspension is stirred at regular intervals of 30 minutes and pH was recorded.

3.15.2. Electrical Conductivity:

Instrument: Conductivity meter

20 g of soil is shaken intermittently with 40 ml of distilled water in a 150 ml conical flask for 1h and allowed to stand. The conductivity of the supernatant liquid is measured with the help of conductivity meter.

3.15.3. Water Holding Capacity

Known amount of dried and sieved soil is taken in a pre-weighed filter paper and saturated with water. After the saturation, the soil is weighed again and placed in oven along with filter paper at 105⁰C till constant weight. Water Holding Capacity was calculated by using the formula:

$$\text{WHC (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,

W1 = Weight of dried soil in g.

W2 = Weight of saturated soil in g.

3.15.4. Organic Carbon

Chemicals and Reagents:

Potassium dichromate, 1N: Dissolve exactly 49.04 g of AR (Analytical grade) K₂Cr₂O₇ (dried at 105⁰C) in 1 litre of distilled water.

Ferrous ammonium sulphate 0.5N (approx.): Dissolve 196 g of the hydrated crystalline FeSo₄ (NH₄)₂.6H₂O per litre containing 20 ml of conc. H₂SO₄.

Diphenylamine indicator: Dissolve 0.5 g (C₆H₅)₂ NH in a mixture of 20 ml of water and 100 ml of Conc. H₂SO₄ conc. sulphuric acid, H₂SO₄ (Sp. Gravity 1.840 Ortho - phosphoric acid (85%) and or sodium fluoride.

Procedure

(I) Standardisation of ferrous ammonium sulphate solution

Take 10 ml of 1N Potassium dichromate solution in a 250 ml of conical flask. Then very carefully add 150 ml of Conc. H₂SO₄. This will generate heat. Swirl the mixture and allow it to cool. Add 200 ml of distilled water. Then add 10 ml of ortho-phosphoric acid and 1 ml of indicator solution and mix thoroughly.

Add ammonium ferrous sulphate from the burette, swirl the flask until the colour changes from blue to green. These data are also used as the blank reading.

(II) Test for Organic Carbon

The oven dried soil was ground and completely passed through a 0.2 mm sieve and 0.50 g of the sample was placed at the bottom of a dry 500 ml conical flask. 10 ml of Potassium dichromate was added in the conical flask and the flask was swirled gently to disperse the soil in the dichromate solution. 20 ml of Conc. H₂SO₄ was carefully added from a measuring cylinder. Swirled two to three times. The flask was allowed to stand for 30 minutes. 200 ml of distilled water and 10 ml of ortho-phosphoric acid was added to get a sharper end point of the titration. After the addition of 1mL of Diphenyl indicator the contents were titrated with ferrous ammonium sulphate solution till the colour flashed from blue-violet to green. Simultaneously a blank is run without soil.

Calculation

$$(a) \text{ Organic Carbon (\%)} = \frac{10 (B-T)}{B} \times 0.003 \times \frac{100}{S}$$

Where,

B= Volume of ferrous ammonium sulphate required for blank titration in ml.

T=Volume of ferrous ammonium sulphate needed for soil sample in ml.

S= Weight of soil in g.

3.15.5. Organic matter

$$(b)\text{Organic matter} = \text{organic carbon} \times 1.724$$

3.15.6. Total Nitrogen

Total Nitrogen was estimated by Kjeldahl method. The nitrogen in the sample was converted into ammonium sulphate nitrogen when treated with sulphuric acid using potassium selenate and copper sulphate as catalyst. An excess of alkali is then added and the ammonia distilled into an excess of boric acid solution was determined by titration with standard sulphuric acid. One to Five gram of dried sample was taken into a 500 ml Kjeldahl flask, 20 ml of concentrated H₂SO₄ and one Kjeltab CuSO₄ and K₂CuSO₄ were added (containing CuSO₄ and K₂CuSO₄ to the flask. After thorough mixing, slow digestion was done until frothing ceased and after 30 min the liquid became clear, the sample was estimated for N by Auto Kjeltec 1030 analyzer and direct reading in percent was obtained.

3.15.7. Available Nitrogen

Reagents used

- i. Potassium permanganate solution (0.32%)
- ii. Sodium hydroxide solution (2.5%)
- iii. Sulphuric acid (0.02 N)
- iv. Boric acid solution (2%)
- v. Methyl red indicator

Soil sample (2.0 g) was taken in distillation flask and added with 20 ml distilled water. Then 100 ml of potassium permanganate solution and 100 ml sodium hydroxide solution were added to it and immediately fitted up in the distillation apparatus. 20.0 ml of sulphuric acid was pipetted out in a conical flask and the end of the delivery tube was dipped in it. The ammonia gas was distilled from the distillation flask and collected in about 30 ml of filtrate. Then five drops of methyl red indicator were added and titrated against 0.02 N sulphuric acid solutions. The available nitrogen was calculated by the titrant used.

3.15.8. Available Phosphorous (P_2O_5)

Reagents:

- i. Sodium bicarbonate (1/2 N) pH 8.5
- ii. Activated carbon; Darco G-60
- iii. Ammonium molybedate

Working solution

Stannous chloride ($SnCl_2 \cdot 2H_2O$) (10.0 g) was dissolved in 25.0 ml of concentrated hydrochloric acid (HCl) and made upto 100 ml with distilled water.

Procedure for detection of available phosphorus in soil

Black Darco G-60 (2.0-3.0 g) and 50.0 ml of sodium bicarbonate (1/2 N) solution were added to 2.0 g dried powdered soil. It was shaken thoroughly for 30 minutes and then filtered through Wattman No. 40 filter paper. To 5.0 ml of filtered soil extract 5.0 ml of ammonium molybedate was added and this was diluted to about 20.0 ml with distilled water. Stannous chloride solution (working solution) (1.0 ml) was then added to it and the final volume was made upto 25.0 ml with distilled water and was shaken thoroughly. The colour intensity which was red was measured using the colorimeter at 660 nm after 10 minutes.

3.15.9. Available Potassium (K)

Reagents:

A. Ammonium acetate 1N: To 700 or 800 ml of water, 57.0 ml of concentrated acetic acid and then 68.0 ml of concentrated ammonium hydroxide was added. It was diluted to a volume of 1.0 litre and was adjusted to pH 7.0 by the addition of more ammonium hydroxide or acetic acid.

B. Potassium chloride 0.02 N: Dry potassium chloride (1.49 g) in water was dissolved in distilled water and was diluted to a volume of 1.0 litre.

C. Potassium chloride: 0.02 N KCl in 1 N ammonium acetate: Dry potassium chloride (KCl) (1.49 g) was dissolved in reagent A and diluted to a volume of exactly 1.0 litre with additional A solution.

D. Lithium chloride, 0.05 N: Dry lithium chloride (2.12 g) was dissolved in distilled water and diluted to 1.0 litre.

Procedure

Reagents B and D were used to prepare a series of standard potassium chloride solutions, each containing the same concentration of lithium chloride. A similar series of standard potassium solutions using reagents C and D and which was diluted by reagent A were prepared. The concentrations of potassium chloride were 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5 and 2.0 moles equivalent/L. The optimum concentration of lithium chloride varies with individual flame photometers but was usually 5.0 to 10.0 meq. /L. Standard solutions were made in water employed for the analysis of water and water extracts of soils; whereas, those made up in ammonium acetate solution were used for the analysis of ammonium acetate extracts of soils. The flame photometer was calibrated for operation over the concentration range 0.0 to 0.5 meq./l. of potassium, using the first 6 standard solutions of the appropriate series. The first and the last 4 solutions of the appropriate series were used to calibrate the instrument for operation over the concentration range 0.0 to 2.0 meq/l of potassium. An aliquot of the solution was pipetted out to analysis containing less than 0.1 meq. of potassium into a 50 ml volumetric flask. An amount of reagent D was added, which when diluted to a volume of 50.0 ml, gave a concentration of lithium chloride exactly equal to that in the standard potassium chloride solutions. It was diluted to volume with water or with A, and determined the potassium concentration by use of the flame photometer.

3.15.10. Alkaline Phosphate Activity**Method:**

1.0g of fresh soil was mixed with 0.2 ml of toluene, 4 ml of modified universal buffer (MUB) of pH 11.0 and 1.0 ml of 0.025 mol/l p-nitrophenol phosphate and incubated for 1 hr at 37⁰C. After incubation, the mixture was mixed well with 1.0 ml of 0.5 mol/l CaCl₂ & 4.0ml of 0.5 mol /l NaOH & then filtered through a filter paper. The concentration of product (p- nitrophenol) in the filtrate was determined colorimetrically at 400nm. Activity quantified by reference to a calibration curve construction using Phenol standards under the same condition describe above. It is expressed in mg/(g.h). Tabatabai, M.A.; Bremner J.M. (1969). Use of p-nitrophenylphosphate for assay of phosphatase activity. *Soil Biol Biochem*, 1:301-307.

3.15.11. Urease Activity

Urease activity was determined by Hofman, 1965.

Reagents-

- 1- 10% Urea- dissolved 10 g of urea and made up to 100 ml.
- 2- Citrate buffer-(pH 6.7) - dissolved 368 g of citric acid in 800 ml of water. Dissolved 295 g of KOH in 300 ml of water. Combined the two solutions, cooled, adjusted the pH 6.7 with 1 N KOH and diluted to 2000 ml with water.
- 3- 12.5% phenate-
 - a)- dissolved 62.5 g of phenol in the smallest volume of ethanol, added 2 ml of methanol and 18.5 ml of acetone. Diluted to 100 ml with ethanol and stored refrigerated.
 - b) - dissolved 27 g of NaOH in water and made up to 100 ml. Just before use mixed 20 ml of solution A and solution B and up to 100 ml.
- 4- Sodium hypochlorite (NaOCl) - diluted the commercial solution with water so that it contains 0.9% active chlorine (determined by addition of KI, acidification with HCl and back titration with $\text{Na}_2\text{S}_2\text{O}_3$ solution). The solution was stable.
- 5- Standard ammonium sulphate solution- (1.2 mg NH_3/ml)- dissolved 4.717 g of $(\text{NH}_4)_2\text{SO}_4$ in water and made up to 1000 ml. diluted 10ml of this solution to 1000 ml with water. 1ml of this solution contained 10 μg N.

Method

Enzyme Assay-

- 1- Pipette out ml of filtrate, 9 ml of water, 4 ml phenate solution and 3 ml of sodium hypochlorite solution into 50 ml volumetric flask.
- 2- Mixed well & allowed to stand for 20 min until the max colour is obtained.
- 3- Diluted to 50 ml with water & mixed well.
- 4- Read the OD at 630 or 580 nm within 60min against the reagent blank prepared in the same manner.
- 5- Construct the standard curve by pipetting 0, 1, 2, 4, 6, 8 and 10 ml of diluted standard ammonium sulphate solution (10 $\mu\text{gN}/\text{ml}$) into a series of 50 ml volumetric flasks, make up the volume in each flask to 10 ml with water, add 4 ml of phenate through step 2 to 4. Measure the absorbance against blank i.e., zero flask and plot optical density vs. concentration.

3.15.12. Dehydrogenase Activity

Method:

2 g of soil was placed in 18 mm×20 mm glass tube and incubated with 2 ml of 3% 2, 3, 5, tri-phenyl chloride (TTC) for 24 h at 30⁰C. After incubation, 10 ml acetone was added, and the suspension was homogenized with intermittent agitation for 2 h (once every half an hour) and then filtered in darkness. Reactive products were measured at 485 nm using a spectrophotometer. A sample without soil containing 2 ml buffer, which is instead of TTC, was used as control. Dehydrogenase activity is expressed in mg/(kg.d).

3.16. Cost Benefits Analysis

The cost of cultivation for each treatment under study was worked out for detailed assessment of the cost involved in the cultivation of mustard. The details of cost have been described in the result section.

3.17. Statistical Analysis

The data (n=6 for plant growth parameters and nutrient content) were analyzed statistically by one way analysis of variance (SPSS, Statistical Package and MS Excel) using Duncan's Multiple Range Test (DMRT) and *t* test to determine the significance of difference among treatments at probability (*p*) 0.05 and 0.01.



CHAPTER-4

RESULTS

Chapter 4

RESULTS

4.1 Growth and productivity of wheat (*Triticum aestivum* L. cv. PBW-502) as well as soil enrichment applied with organic matrix immobilized biofertilizers (*Azotobacter chroococum* and *Bacillus subtilis*)

4.1.1 Growth and productivity of wheat (*T. aestivum* L.) applied with enhance dose and free form of charcoal based biofertilizers (*A. chroococum* and *B. subtilis*), parameters recorded on 30, 60, 90 and 120 days after sowing (DAS)

There was a significant increase in growth parameters on application of free form of charcoal based biofertilizers (*A. chroococum* and *B. subtilis*; R/D = 0.6 kg h⁻¹) with enhanced doses (BF-I, BF-II, BF-III, BF-V, BF-VI and BF-VII.) and recommended dose of commercial chemical fertilizers; urea and DAP. Application of BF-III and BF-II increased shoot height, number of leaves, fresh and dry weight of shoot significantly (p<0.05) over their respective higher doses of free form of charcoal based biofertilizers (*A. chroococum* and *B. subtilis*) on 30, 60, 90 and 120 days after sowing (Table-4.1.1)

The application of biofertilizer significantly increased shoot height by BF-III was 25.73, 6.39 and 1.59% on 30 DAS, 26.68, 7.38 and 1.24% on 60 DAS, 22.47, 12.32 and 5.92% on 90 DAS and 23.12, 13.37 and 5.17% on 120 DAS over no added fertilizer, BF-I and BF-II. The number of leaves was increased by 48.95% in BF-III over NF, 38.80% over BF-I, 38.80% over BF-II and 34.76% over the BF-V on 90 DAS.

The percentage fresh weight of shoot was significantly recorded by 17.71, 5.42, 4.85, 2.28, 3.42 and 5.71%, on 120 days old plant by the application of BF-III over NF, BF-I, BF-III, BF-V, BF-VI and BF-VII. The dry weight of shoot was increased by 17.71, 5.71 and 5.14% on 120 days after sowing respectively by the application of BF-III over the no fertilizers, BF-I and BF-II respectively (Table 4.1.1).

Table 4.1.1 Effect of Free Form of Charcoal Based *A. chroococcum* and *B. subtilis* Biofertilizers on Plant Growth Parameters of Wheat (*T. aestivum* L. cv. PBW-502) Shoots on 30, 60, 90 and 120 DAS

Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Shoot Height (cm)				
NF	19.97±1.43 ^a	34.26±0.98 ^a	40.57±2.56 ^a	42.82±2.02 ^a
CF-RD	37.37±1.96 ^d	50.88±1.80 ^e	55.37±2.96 ^e	62.37±3.30 ^f
BF- I	25.17±0.74 ^{ab}	43.28±2.90 ^{bc}	45.88±1.24 ^b	48.25±1.01 ^b
BF- II	26.73±1.29 ^b	46.15±1.45 ^d	49.23±1.86 ^b	52.82±2.40 ^d
BF- III	26.89±2.10 ^b	46.73±1.69 ^d	52.33±1.55 ^{cd}	55.70±2.28 ^e
BF- V	27.23±2.08 ^{bc}	46.80±1.34 ^d	51.17±1.00 ^c	51.38±1.83 ^{cd}
BF- VI	27.25±1.44 ^{bc}	47.53±1.84 ^d	52.02±3.99 ^{cd}	51.12±1.89 ^{cd}
BF- VII	27.08±1.16 ^{bc}	44.60±1.60 ^b	50.37±1.54 ^a	49.90±2.26 ^{bc}
Number of leaves				
NF	2.17±0.41 ^a	3.00±0.00 ^a	4.17±0.41 ^a	4.17±0.41 ^a
CF-RD	5.67±0.52 ^c	9.67±1.37 ^f	10.50±2.07 ^d	12.17±2.56 ^d
BF- I	3.00±0.00 ^a	4.83±0.98 ^b	5.00±0.00 ^b	4.50±0.55 ^{ab}
BF- II	3.00±0.00 ^a	4.50±0.84 ^b	5.00±0.00 ^b	4.50±0.55 ^{ab}
BF- III	5.17±0.75 ^c	5.67±0.52 ^{bc}	8.17±1.47 ^c	5.00±0.00 ^c
BF- V	4.83±1.17 ^b	7.33±1.37 ^e	5.33±0.52 ^b	4.50±0.55 ^{ab}
BF- VI	5.17±0.41 ^c	6.33±1.03 ^d	5.00±0.00 ^b	4.50±0.55 ^{ab}
BF- VII	4.17±1.33 ^b	6.17±0.98 ^d	5.00±0.00 ^b	4.50±0.55 ^{ab}
Fresh weight of shoot (g)				
NF	0.76±0.05 ^a	1.79±0.06 ^a	2.40±0.19 ^a	2.88±0.06 ^a
CF-RD	1.71±0.06 ^b	3.85±0.06 ^c	5.55±0.08 ^c	5.79±0.06 ^d
BF- I	1.17±0.02 ^a	2.57±0.19 ^b	3.12±0.02 ^b	3.31±0.04 ^b
BF- II	1.18±0.03 ^a	2.71±0.11 ^b	3.19±0.03 ^b	3.33±0.07 ^b
BF- III	1.21±0.03 ^a	2.90±0.05 ^b	3.29±0.04 ^b	3.50±0.06 ^{bc}
BF- V	1.19±0.01 ^a	2.84±0.07 ^a	3.24±0.01 ^{ba}	3.42±0.03 ^a
BF- VI	1.19±0.02 ^a	2.83±0.09 ^b	3.21±0.01 ^b	3.38±0.02 ^{bc}
BF- VII	1.18±0.01 ^a	2.66±0.08 ^b	3.18±0.04 ^b	3.30±0.07 ^b
Dry weight of shoot (g)				
NF	0.15±0.01 ^a	0.36±0.01 ^a	0.60±0.05 ^a	1.44±0.03 ^a
CF-RD	0.34±0.01 ^a	0.77±0.01 ^d	1.39±0.02 ^d	2.90±0.03 ^d
BF- I	0.23±0.01 ^a	0.51±0.04 ^{bc}	0.78±0.00 ^b	1.65±0.02 ^b
BF- II	0.23±0.00 ^a	0.54±0.02 ^{bc}	0.80±0.01 ^{bc}	1.66±0.04 ^b
BF- III	0.24±0.01 ^a	0.58±0.01 ^{bc}	0.84±0.01 ^{bc}	1.75±0.03 ^c
BF- V	0.24±0.00 ^a	0.58±0.01 ^{bc}	0.82±0.01 ^{bc}	1.72±0.03 ^c
BF- VI	0.24±0.01 ^a	0.57±0.02 ^{bc}	0.82±0.01 ^{bc}	1.70±0.03 ^c
BF- VII	0.23±0.00 ^a	0.53±0.02 ^{bc}	0.80±0.01 ^{bc}	1.65±0.03 ^b

All the values are means of three replicates with two determinations (n=6) ±S.D.), One way ANOVA is performed to compare the means of different treatments at p < 0.05. Values followed by different letters are significantly differences between the treatments. [Where, NF= No fertilizer, CF-RD Recommended dose of urea and DAP, BF-I free form of biofertilizers single dose, BF-II free form of biofertilizers twofold, BF-III free form of biofertilizers threefold, BF-V free form of biofertilizers fivefold, BF-VI free form of biofertilizers sixfold and BF-VII free form of biofertilizers sevenfold]

Root length increased due to application on BF-III was 22.66, 20.92, 4.74, 7.49 and 934% on 120 DAS by the application of BF-III over NF, BF-I, BF-II, BF-V and BF-VI. The root length increased by 23.07, 2.02, 10.76, and 8.09% on 30 DAS by the application of BF-III over NF, BF-I, BF-V and BF-VI, by 34.00, 19.91, 13.42, 1.11, 8.94 and 10.51% on 60 d, 29.13, 19.37, 8.94, 9.21 and 13.27% on 120 DAS by the application of commercial free form of charcoal based biofertilizers (*A. chroococcum* and *B. subtilis*); BF-III over the NF, BF-I, BF-II, BF-V and BF-VI respectively. The percentage fresh weight of shoot was increased by 17.71, 5.42, 4.85, 2.28, 3.42 and 5.71%, fresh weight of roots were and 59.43, 8.49, 3.77, 7.54, 13.20 and 16.98% recorded in 120 days old plants by the application of BF-III over NF, BF-I, BF-III, BF-V, BF-VI and BF-VII respectively. Increased in dry weight of shoot was observed in 120 days after sowing on application of BF-III. The dry weight of shoot was increased by 17.71, 5.71 and 5.14% and dry weight of root was increased 17.85, 7.14 and 3.57% on 120 days after sowing respectively by the application of BF-III over the no fertilizers (Table 4.1.2).

Table 4.1.2 Effect of Free Form of Charcoal Based *A. chroococcum* and *B. subtilis* Biofertilizers on Plant Growth Parameters of Wheat (*T. aestivum* L. cv. PBW-502) Roots on 30, 60, 90 and 120 DAS

	Root length (cm)			
NF	1.90±0.22 ^a	2.95±0.14 ^a	4.20±0.18 ^a	5.23±0.23 ^a
CF-RD	2.95±0.29 ^a	5.58±0.26 ^d	6.97±0.25 ^{dc}	8.18±0.22 ^e
BF- I	2.42±0.22 ^a	3.58±0.24 ^b	5.22±0.23 ^b	5.95±0.16 ^b
BF- II	2.47±0.26 ^a	3.87±0.18 ^b	5.62±0.29 ^{bc}	6.72±0.39 ^c
BF- III	2.47±0.23 ^a	4.47±0.24 ^c	6.18±0.40 ^d	7.38±0.29 ^d
BF- V	2.23±0.22 ^a	4.42±0.26 ^c	6.33±0.43 ^d	6.70±0.53 ^c
BF- VI	2.27±0.27 ^a	4.07±0.19 ^c	5.93±0.53 ^{bc}	6.40±0.40 ^c
BF- VII	2.37±0.15 ^a	4.00±0.13 ^c	5.37±0.31 ^b	6.55±0.37 ^c
	Number of root			
NF	2.67±0.82 ^a	3.83±0.75 ^a	5.17±0.75 ^a	5.33±0.82 ^a
CF-RD	3.33±0.52 ^a	7.50±0.55 ^d	8.17±0.75 ^e	8.17±0.75 ^e
BF- I	2.33±0.52 ^a	5.67±0.52 ^b	5.67±0.52 ^a	5.67±0.52 ^{ab}
BF- II	3.00±0.00 ^a	7.00±0.89 ^d	7.00±0.89 ^d	6.83±0.75 ^{cd}
BF- III	3.67±0.52 ^a	7.83±0.41 ^d	7.17±0.98 ^d	7.17±0.75 ^a
BF- V	3.00±0.00 ^a	6.00±0.89 ^c	6.83±1.17 ^{bc}	6.67±1.21 ^{cd}
BF- VI	3.00±0.00 ^a	6.17±0.41 ^c	6.33±0.52 ^b	6.50±1.05 ^c
BF- VII	3.00±0.00 ^a	6.00±0.00 ^c	6.17±0.41 ^b	7.17±0.75 ^d
	Fresh weight of root (g)			
NF	0.07±0.01 ^a	0.20±0.02 ^a	0.36±0.03 ^a	0.43±0.03 ^a
CF-RD	0.17±0.01 ^a	0.52±0.02 ^d	0.93±0.04 ^f	1.14±0.09 ^e
BF- I	0.10±0.01 ^a	0.30±0.02 ^b	0.54±0.03 ^b	0.97±0.03 ^{bc}
BF- II	0.11±0.01 ^a	0.32±0.02 ^b	0.58±0.03 ^{bc}	1.02±0.04 ^d

BF- III	0.11±0.01 ^a	0.40±0.06 ^b	0.71±0.11 ^d	1.06±0.06 ^d
BF- V	0.12±0.01 ^a	0.35±0.02 ^b	0.62±0.04 ^d	0.98±0.08 ^{bc}
BF- VI	0.13±0.02 ^a	0.34±0.03 ^c	0.61±0.06 ^e	0.92±0.01 ^{bc}
BF- VII	0.11±0.01 ^a	0.34±0.04 ^b	0.60±0.07 ^d	0.88±0.04 ^b
Dry weight of root (g)				
NF	0.06±0.01 ^a	0.14±0.00 ^a	0.19±0.02 ^a	0.23±0.00 ^a
CF-RD	0.14±0.01 ^a	0.31±0.01 ^c	0.44±0.01 ^d	0.46±0.00 ^e
BF- I	0.09±0.00 ^a	0.21±0.02 ^b	0.25±0.00 ^b	0.26±0.00 ^b
BF- II	0.09±0.00 ^a	0.22±0.01 ^b	0.26±0.00 ^c	0.27±0.00 ^c
BF- III	0.09±0.00 ^a	0.23±0.01 ^b	0.26±0.00 ^c	0.28±0.01 ^d
BF- V	0.09±0.00 ^a	0.23±0.01 ^b	0.26±0.00 ^c	0.28±0.01 ^d
BF- VI	0.09±0.00 ^a	0.23±0.00 ^b	0.26±0.00 ^c	0.28±0.01 ^d
BF- VII	0.09±0.00 ^a	0.21±0.01 ^b	0.25±0.00 ^b	0.26±0.01 ^b

All the values are means of three replicates with two determinations (n=6) ±S.D.) (Other details are described in Table. 4.1.1)

4.1.2 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Rhizospheric Soil, of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Free form of Biofertilizers

The soil nitrate content increased by the application of BF-III was 15.30, 7.22, 6.10, and 6.36% at 60 and 36.38, 5.92, 3.8 and 14.15% at 120 DAS over the NF, BF-I, BF-II and BF-VII respectively. Similarly by the BF-II, 9.19, 1.11 and 0.25% over NF, BF-I and BF-VII BF was recorded respectively at 60 DAS (Figure 4.1.1)

The nitrite content in the soil, the application with BF-III has recorded significantly and it increase at different level by 53.99, 18.38, 10.29 and 21.28% and 37.84, 20.15, 12.94 and 28.76% at 60 and 120 DAS respectively over NF, BF-I, BF-II and BF-VII (Figure 4.1.1)

Ammonium content was recorded different level by BF-III by 41.04, 13.03, 8.2 and 14.15% over NF, BF-I, BF-II and BF-VII and 36.38, 4.53 and 0.07% over NF, BF-I and BF-VII at 60 respectively. Percentage of ammonium on BF-V by 39.03, 7.18 and 2.6% over NF BF-I and BF-II at 60 DAS was recorded significantly (Figure 4.1.1).

The phosphate content in the root, the application with BF-III has recorded significantly and it increase at different level by 82.56, 16.63 and 23.52% and 82.83, 7.29 and 10% over the NF, BF-VI and BF-VII at 60 and 120 DAS respectively (Figure 4.1.1).

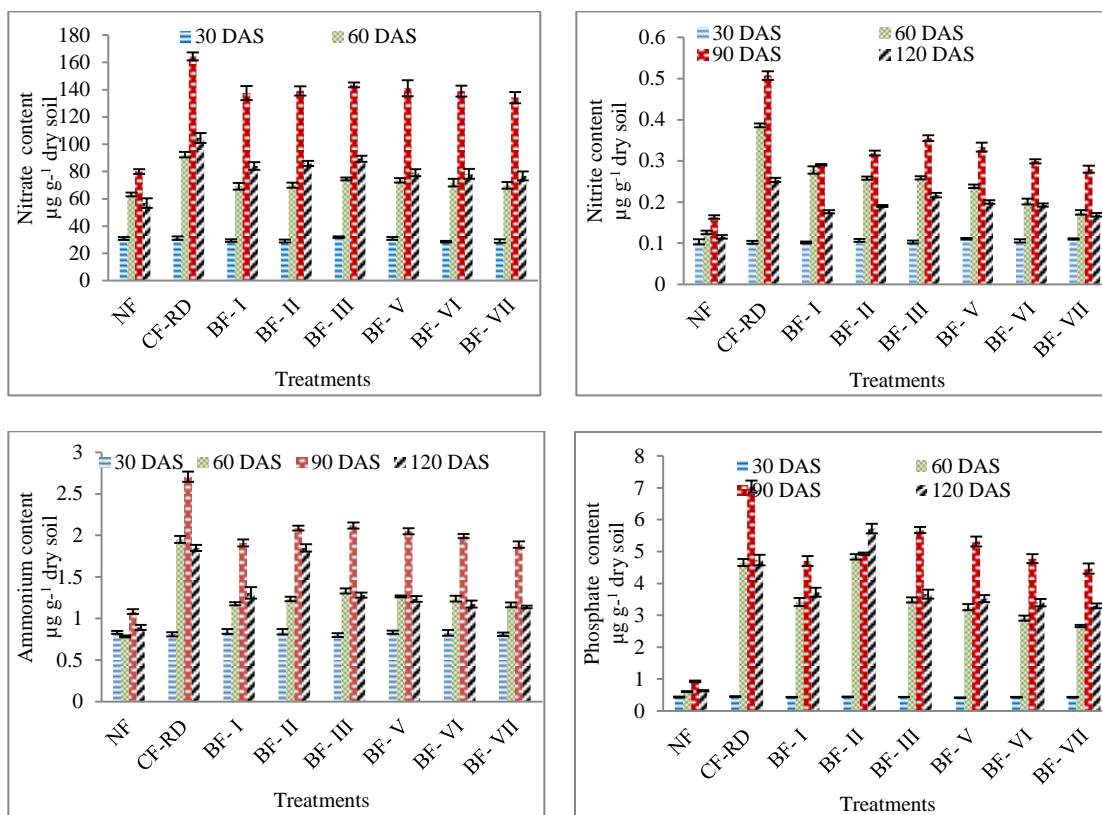


Figure 4.1.1 The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the soil on 30, 60, 90, and 120 days after sowing (DAS). All the values are means of three replicates with two determinations ($n=6 \pm S.E.$). (Other details are described in Table. 4.1.1)

4.1.3 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Roots of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Free form of Biofertilizers

Nitrate content in wheat roots applied with enhanced dose (1.8 kg ha^{-1}) of biofertilizers; BF-III increased by 45.77, 8.61, 4.48, 14.68 and 25.57% at 60 DAS and 46.31, 16.58, 7.57, 17.95 and 20.93% at 120 days after sowing respectively over NF, BF-I, BF-II, BF-VI and BF-VII (Figure 4.1.2).

The root nitrite increased by 61.46, 21.40, .04, 17.58, 25.72% at 60 DAS, 63.39, 8.32, .01, 20.84 and 23.72% at 90 DAS and 59.36, 11.06, 7.24, 13.58 and 23.11% at 120 DAS, respectively, with application of the triple dose; BF-III over that in NF, BF-I, BF-II, BF-VI and BF-VII (Figure 4.1.2).

The ammonium content in root of wheat plant was increased by 61.27, 1.46 and 21.47% at 90 DAS and 75.29, 36.13 and 12.78% 120DAS with the application on FB-III over NF, BF-I and BF-VII (Figure 4.1.2).

The root phosphate increased by 61.75, 16.97, 25.47, 17.34 and 23.33% at 60 DAS, 62.58, 19.97, 12.70, 4.26 and 9.41% at 90 DAS and 54.98, 21.22, 11.79, 14.32 and 18% at 120 DAS over applications on NF, BF-I, BF-II, BF-VI and BF-VII respectively with the application of FB-III (Figure 4.1.2).

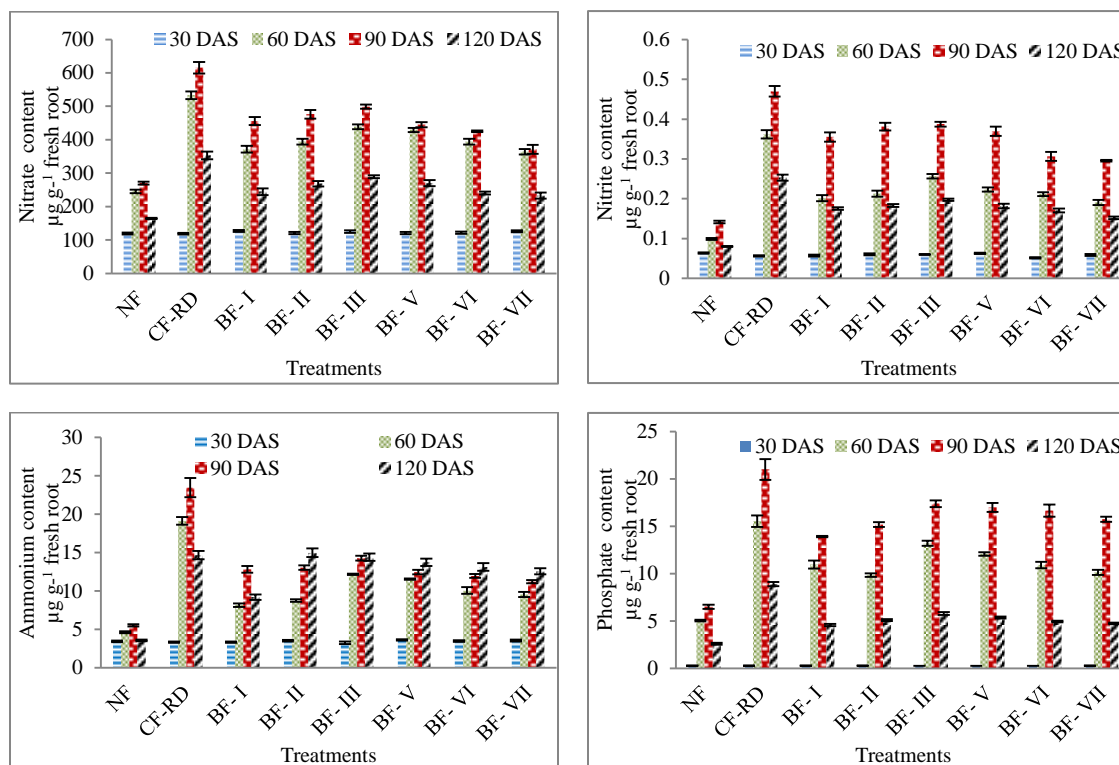


Figure 4.1.2 The enhance dose of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the roots on 30, 60, 90, and 120 days after sowing (DAS). All the values are means of three replicates with two determinations ($n=6$) \pm S.E). (Other details are described in Table. 4.1.1)

4.1.4 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Free form of Biofertilizers

Nitrate content in leaves applied with enhanced dose of free form of biofertilizers; BF-III increased by 41.82, 2.99 and 0.94% at 60 DAS, 51.38, 15.63 and .05% on 90 DAS, 42.86, 26.65 and 10.63% at 120 DAS respectively over NF, BF-I and BF-II (Figure 4.1.3).

Nitrite content in wheat leaves increased by 60.65, 21.26, 14.82, 9.6 and 17.04% at 60 DAS, 59.42, 5.26, 3.06, 5.79 and 10.29% at 90 DAS respectively applied with enhanced dose (1.8 kg ha⁻¹) of biofertilizers. Enhanced dose with BF-III has increased the soil nitrite content over application of NF, BF-I, BF-II BF-VI and BF-VII (Figure 4.1.3).

Ammonium content in leaves of wheat plant increased when applied with BF-III was 55.63, 13.29, 11.38, 12.51 and 12.63% at 60 DAS, 58.15, 21.66, 18.01, 16.92 and 27.95% at 90 DAS, 56.73, 10.81, 8.52, 3.68 and 4.2% at 120 DAS respectively over NF, BF-I, BF-II BF-VI and BF-VII (Figure 4.1.3).

Phosphate content in wheat leaves applied with BF-III was increased by 62.90, 12.23 and 11.56% at 60 DAS, 64.92, 12.59 and 7.51 % at 90 DAS, 53.37, 32.23 and 25.41% at 120 days after sowing respectively over NF, BF-I, BF-II. Similarly BF-II also increased phosphate content in leaves over BF-I (Figure 4.1.3).

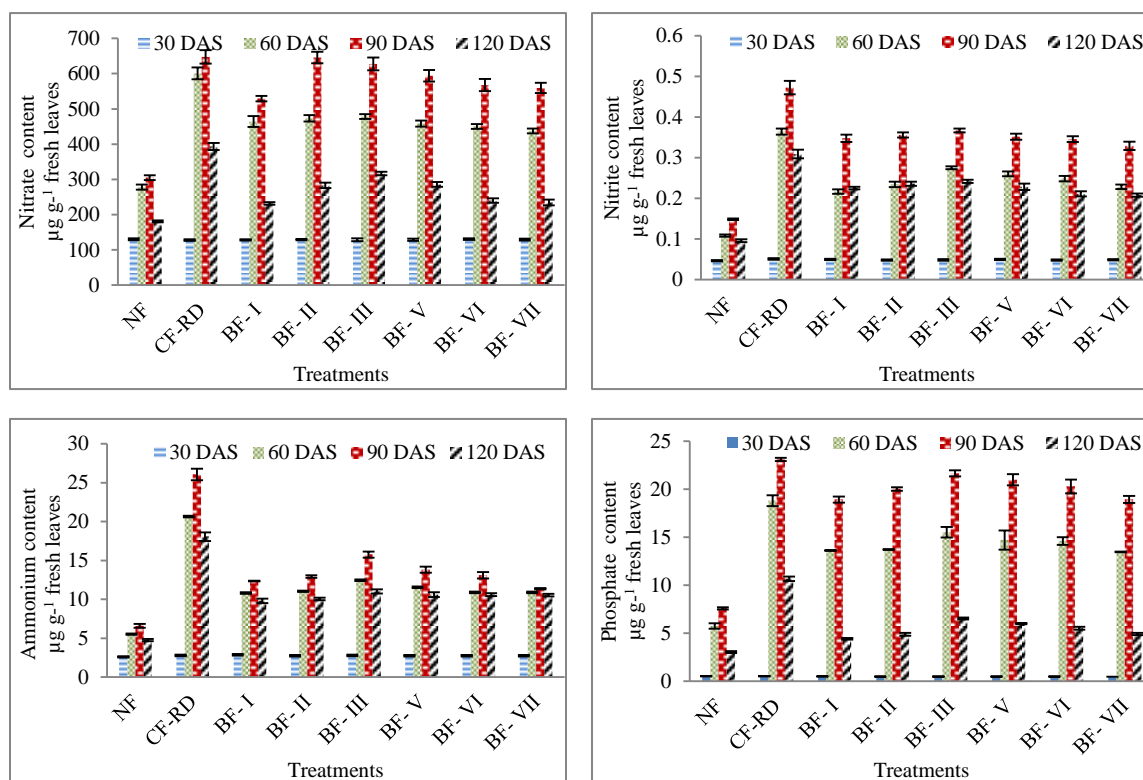


Figure 4.1.3 The nitrate, nitrite, ammonium and phosphate content in the plants leaves on 30, 60, 90, and 120 DAS the seeds. The level in leaves was significantly recorded with the application of BF-III followed by BF-II and BF-V. All the values are means of three replicates with two determinations (n=6) ±S.E). (Other details are described in Table. 4.1.1)

4.1.5 Yield and Productivity of Wheat (*T. aestivum* L.) Plant Applied with Free Form of Biofertilizers

Application of commercial free form of charcoal based biofertilizers (*A. chroococcum* and *B. subtilis*) increased the yield (g plant⁻¹). Enhance doses of biofertilizers increased percentage of grain yield by 17.46, 5.41, 4.84, 3.34 and 5.48% on application of BF-III over NF, BF-I, BF-II, BF-VI and BF-VII after harvesting at 120 DAS. By application of enhanced dose of biofertilizers BF-II was increased grain yield by 13.62 and 56% over application on NF and BF-I (Fig. 4.1.4).

The straw yield increased by 76.21, 3.43, 2.80, 2.94 and 3.09% on after harvesting with the application on BF-III over NF, BF-I, BF-II, BF-VI and BF-VII respectively. The percentage increased of yield in wheat of the experimental field applied with BF-II was 73.41% and .63% on straw yield over NF and BF-II respectively on after harvesting (Figure 4.1.4).

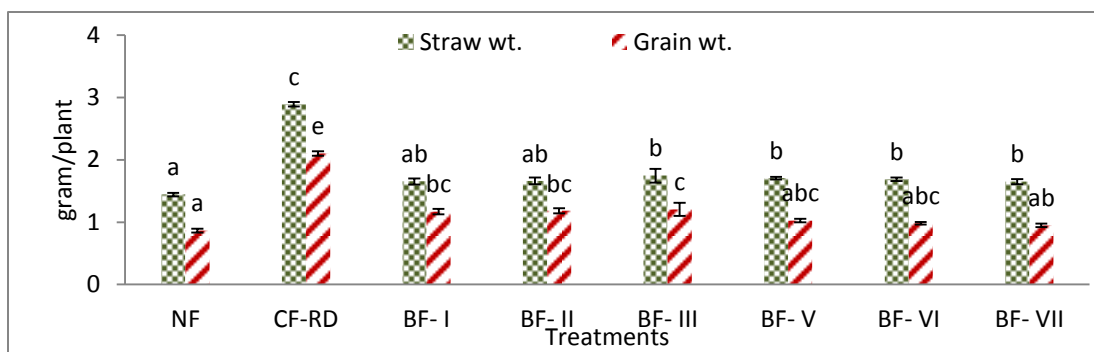


Figure 4.1.4 The application of enhance doses of free form biofertilizers on soil significantly increased yield and straw weight on application of BF-III followed by BF-II and BF-V. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significantly differences between the treatments at 5% level. (Other details are described in Table. 4.1.1)

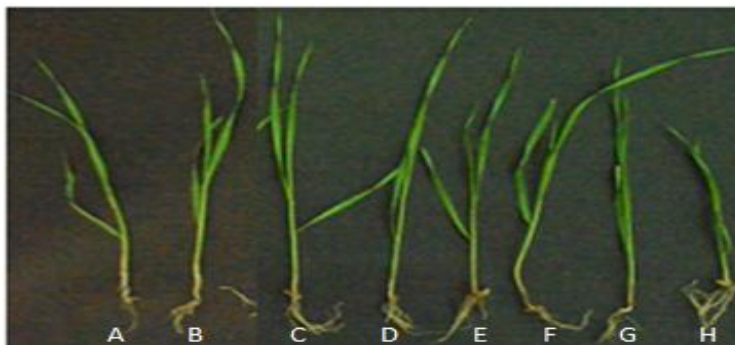


Plate 4.1.1 Effect of enhanced dose of biofertilizers on wheat plants at 30 DAS. A; No fertilizer, B; Recommended dose of urea and DAP, C; free form of biofertilizers single dose, D; free form of biofertilizers two fold, E; free form of biofertilizers threefold, F; free form of biofertilizers fivefold, G; free form of biofertilizers sixfold and H; free form of biofertilizers sevenfold

4.2 Growth and Productivity of Wheat (*T. aestivum* L.) Applied with Charcoal Based Biofertilizers (*A. chroococcum* and *B. subtilis*) in Enhanced Doses and Seed Inoculated form on 30, 60, 90 and 120 Days after Sowing

4.2.1 Growth Parameters of Wheat Applied with Biofertilizers in Enhanced Doses and Seed Coated form, Recorded on 30, 60, 90 and 120 Days after Sowing

The seed coated with biofertilizers enhanced the fresh and dry weight of shoot over the free form of commercial biofertilizer of different magnitude. All the treatments enhanced the shoot, number of leaves and shoot biomass (Table 4.2.1). The shoot height was increased by 29.67, 6.43, 3.53, 2.53 and 3.57% and 20.43, 4.92, 3.37, 3.08 and 3.71% at 60 and 120 days after sowing (DAS) when BFS-III applied over NF, BFS-I, BFS-II, BFS-VI and BFS-VII respectively. This increase was, however, only by 17.64 and 1.61% over the NF and BFS-I with application on BFS-II at 120 DAS.

The number of leaves was recorded significantly on application of BFS-III by 57.78, 38.46, 44.93 and 46.75% over NF, 33.33, 19.23, 27.54 and 28.57% over BFS-I, 11.11, 25, 26.09 and 37.66% over BFS-III at 30, 60, 90 and 120 DAS respectively. Application on BFS-III also significantly increased over BFS-V by 23.38%, BFS-VI by 28.57% and BFS-VII by 44.16% at 120 DAS (Table 4.2.1).

Fresh weight of shoot increased in presence of BFS-III by 74.1, 3.3, 2.73, 1.34, 2.86 and 3.01% over NF, BFS-I, BF-II, BFS-VI and BFS-VII and similarly BFS-II by 73.46, and 00.81% over the NF and BFS -I at the 120 DAS, whereas BFS-V is increased it by 1.4% over the BFS-II only. The dry weight of plant increased on application of BFS-III was 16.44, 4.23, 3.66 and 4.31% over NF, BFS-I, BFS-II, BFS-VI and BFS-VII at 120 DAS respectively (Table 4.2.1).

Table 4.2.1 Effect of Organic Matrix Immobilized Biofertilizers on Growth of Wheat (*T. aestivum* L. cv. PBW-502) Shoot on 30, 60, 90 and 120 DAS

Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Shoot Height (cm)				
NF	21.45±1.86 ^a	34.88±1.09 ^a	43.21±1.59 ^a	46.02±1.25 ^a
CF-RD	36.42±1.29 ^g	53.13±2.02 ^e	58.38±1.27 ^e	66.70±2.00 ^f
BFS- I	26.25±1.85 ^c	46.41±1.62 ^b	51.55±1.19 ^b	54.98±4.53 ^b
BFS- II	26.77±1.99 ^c	47.85±2.32 ^c	52.68±1.49 ^b	55.88±4.61 ^c
BFS- III	27.87±1.78 ^d	49.60±1.97 ^d	53.92±3.41 ^{bc}	57.83±3.11 ^e

BFS- V	30.40±1.58 ^f	49.63±1.45 ^d	54.58±2.42 ^d	57.80±2.22 ^c
BFS- VI	28.30±1.99 ^e	48.34±2.64 ^{cd}	54.22±1.38 ^{cd}	56.05±4.51 ^{cd}
BFS- VII	25.62±2.26 ^b	47.83±3.38 ^c	52.30±1.1	55.68±4.57 ^c
Number of leaves				
NF	3.17±0.41 ^a	5.33±0.82 ^a	6.33±1.03 ^a	6.83±1.17 ^a
CF-RD	6.33±1.03 ^{bc}	8.00±1.41 ^d	10.83±1.33 ^d	12.83±2.04 ^f
BFS- I	5.00±1.26 ^b	7.00±1.26 ^c	8.33±1.63 ^{bc}	9.17±1.47 ^d
BFS- II	6.67±1.51 ^c	6.50±1.22 ^b	8.50±1.22 ^{bc}	8.00±1.26 ^c
BFS- III	7.50±1.05 ^d	8.67±1.03 ^d	11.50±1.52 ^e	12.83±2.04 ^f
BFS- V	6.50±1.05 ^c	7.67±1.03 ^c	8.67±1.37 ^{bc}	9.83±1.72 ^{de}
BFS- VI	7.00±1.26 ^a	7.17±1.33 ^c	8.33±1.37 ^{bc}	9.17±2.14 ^d
BFS- VII	5.33±0.52 ^b	6.50±1.05 ^b	7.67±1.21 ^b	7.17±0.75 ^b
Fresh weight of shoot (g)				
NF	0.89±0.04 ^a	1.73±0.04 ^a	2.36±0.03 ^a	1.30±0.03 ^a
CF-RD	1.69±0.05 ^c	3.71±0.07 ^e	5.24±0.08 ^e	5.83±0.07 ^f
BFS- I	1.25±0.04 ^a	2.95±0.28 ^a	3.96±0.01 ^{bc}	4.86±0.01 ^{bc}
BFS- II	1.28±0.02 ^a	3.05±0.18 ^b	4.01±0.04 ^d	4.90±0.02 ^{bc}
BFS- III	1.33±0.02 ^b	3.45±0.09 ^{cd}	4.11±0.13 ^d	5.03±0.07 ^e
BFS- V	1.25±0.02 ^a	3.29±0.41 ^c	4.09±0.02 ^d	4.97±0.03 ^{cd}
BFS- VI	1.22±0.02 ^a	3.28±0.39 ^c	4.01±0.02 ^d	4.89±0.03 ^{bc}
BFS- VII	1.19±0.01 ^a	3.12±0.07 ^b	3.97±0.02 ^{bc}	4.88±0.02 ^{bc}
Dry weight of shoot (g)				
NF	0.19±0.01 ^a	0.45±0.01 ^a	0.80±0.06 ^a	1.92±0.04 ^a
CF-RD	0.43±0.02 ^d	0.96±0.02 ^d	1.85±0.03 ^c	3.86±0.04 ^d
BFS- I	0.29±0.01 ^{bc}	0.64±0.05 ^b	1.04±0.01 ^b	2.20±0.03 ^{bc}
BFS- II	0.30±0.01 ^c	0.68±0.03 ^b	1.06±0.01 ^b	2.22±0.05 ^{bc}
BFS- III	0.30±0.01 ^c	0.71±0.02 ^c	1.08±0.01 ^a	2.30±0.06 ^c
BFS- V	0.30±0.00 ^c	0.72±0.01 ^c	1.09±0.01 ^b	2.32±0.06 ^c
BFS- VI	0.30±0.01 ^c	0.72±0.01 ^c	1.10±0.01 ^b	2.33±0.04 ^c
BFS- VII	0.29±0.00 ^{bc}	0.66±0.02 ^b	1.06±0.01 ^b	2.20±0.05 ^{bc}

The enhanced doses of biofertilizers at 30, 60, 90, and 120 days after sowing (DAS) the seeds. Application of BFS-III shows the better growth followed by BFS-V and BFS-II. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significant differences between the treatments at 5% level. [Where NF; No fertilizer, CF-RD; Recommended dose of urea and DAP, BFS-I; single dose of biofertilizers in seed coated form, BFS-II; twofold of biofertilizers in seed coated form, BFS-III; threefold of biofertilizers in seed coated form, BFS-V; fivefold of biofertilizers in seed coated form, BFS-VI; sixfold of biofertilizers in seed coated form and BFS-VII; sevenfold of biofertilizers in seed coated form]

The plant growth parameters were significantly affected by the enhanced dose and biofertilizers in seed coated form. The application of enhanced dose of biofertilizers significantly increased the root length, number of roots and root biomass (Table 4.2.2). The root length was increased on 120 DAS by 31.43, 16.53, 12.24, 4.49 and 14.29% when BFS-III application was applied over NF, BFS-I, BFS-II, BFS-VI and BFS-VII respectively. The number of roots of wheat plant increased by 62.3, 24.59, 14.75,

22.95, 24.5 and 29.51% and 51.47, 27.94, 25, 30.88, 27.94 and 32.35% at 60 and 90 DAS over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII over respectively.

The fresh and dry weights of roots were also recorded significantly with application of enhanced dose of biofertilizers in seed coated form. Fresh weight of root increased in the presence of BFS-III was 48.24, 7.98, 3.57, 6.60, 8.42 and 8.58% at 60 DAS, 45.6, 11.67, 4.6, 5.04, 6.69 and 15.2% at 90 DAS and 56.91, 10.41, 5.13, 14.36, 16.92 and 20.51% at 120 DAS over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII respectively. The dry weight of plant root increased an application of BFS-III was 34.6, 15.65, 7.38, 13.92, 18.43 and 25.25% and 60.23, 15.61, 5.44, 21.62, 25.75 and 31.91% over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII at 60 and 120 DAS respectively (Table 4.2.2).

Table 4.2.2 Effect of Organic Matrix Immobilized Biofertilizers (*A. chroococcum* and *B. subtilis*) on Growth of Wheat (*T. aestivum* L. cv. PBW-502) Roots on 30, 60, 90 and 120 DAS

Root length (cm)				
NF	2.40±0.19 ^a	2.88±0.25 ^a	3.63±0.18 ^a	5.60±0.32 ^a
CF-RD	3.93±0.37 ^b	5.47±0.31 ^d	7.30±0.37 ^e	8.40±0.25 ^e
BFS- I	3.23±0.21 ^a	3.70±0.18 ^b	6.12±0.40 ^d	6.82±0.23 ^b
BFS- II	3.33±0.26 ^a	4.13±0.14 ^c	6.37±0.67 ^d	7.17±0.58 ^c
BFS- III	3.43±0.36 ^a	4.47±0.31 ^c	6.97±0.16 ^d	8.17±0.36 ^e
BFS- V	3.35±0.19 ^a	4.43±0.36 ^c	6.40±0.58 ^d	8.28±0.48 ^e
BFS- VI	3.20±0.28 ^a	4.33±0.26 ^c	5.67±0.42 ^{bc}	7.80±0.32 ^{cd}
BFS- VII	3.02±0.15 ^a	4.23±0.21 ^c	5.47±0.34 ^{bc}	7.00±0.42 ^c
Number of root				
NF	3.33±0.52 ^a	3.83±0.75 ^a	5.50±0.55 ^a	5.83±0.75 ^a
CF-RD	7.00±1.55 ^d	9.33±1.51 ^d	9.50±1.52 ^d	12.67±1.51 ^d
BFS- I	6.17±1.60 ^c	7.67±1.37 ^b	8.17±1.33 ^c	9.00±0.89 ^c
BFS- II	6.50±2.17 ^c	8.67±1.21 ^{bc}	8.50±1.05 ^c	9.50±1.52 ^c
BFS- III	8.00±1.79 ^e	10.17±2.40 ^d	11.33±1.75 ^e	13.33±2.42 ^e
BFS- V	6.17±1.17 ^c	7.83±1.72 ^b	7.83±1.17 ^{bc}	9.17±0.75 ^c
BFS- VI	6.33±1.51 ^c	7.67±1.63 ^b	8.17±0.98 ^c	8.67±1.21 ^{bc}
BFS- VII	5.67±1.21 ^b	7.17±0.75 ^b	7.67±1.37 ^{bc}	8.00±1.10 ^b
Fresh weight of root (g)				
NF	0.07±0.01 ^a	0.20±0.01 ^a	0.37±0.02 ^a	0.49±0.02 ^a
CF-RD	0.17±0.01 ^e	0.53±0.02 ^d	0.94±0.03 ^e	1.21±0.07 ^{ef}
BFS- I	0.10±0.01 ^b	0.36±0.00 ^b	0.60±0.03 ^c	1.02±0.03 ^a
BFS- II	0.11±0.01 ^{bc}	0.38±0.01 ^a	0.65±0.03 ^{cd}	1.08±0.04 ^e
BFS- III	0.11±0.01 ^{bc}	0.40±0.01 ^c	0.68±0.02 ^d	1.13±0.03 ^e
BFS- V	0.12±0.01 ^c	0.37±0.00 ^b	0.64±0.04 ^{cd}	0.97±0.03 ^{bcd}
BFS- VI	0.13±0.02 ^d	0.36±0.00 ^b	0.63±0.06 ^d	0.94±0.03 ^{bcd}
BFS- VII	0.11±0.01 ^{bc}	0.36±0.00 ^b	0.58±0.05 ^{bc}	0.90±0.03 ^{bcd}

Dry weight of root (g)				
NF	0.04±0.01 ^a	0.12±0.02 ^a	0.19±0.02 ^a	0.23±0.00 ^a
CF-RD	0.11±0.01 ^e	0.23±0.02 ^g	0.44±0.01 ^e	0.69±0.03 ^f
BFS- I	0.06±0.00 ^{bc}	0.15±0.01 ^c	0.34±0.03 ^{bcd}	0.49±0.03 ^{cd}
BFS- II	0.07±0.00 ^d	0.17±0.02 ^e	0.35±0.03 ^{cd}	0.55±0.03 ^e
BFS- III	0.08±0.04 ^{dc}	0.18±0.01 ^f	0.39±0.02 ^{cd}	0.58±0.03 ^e
BFS- V	0.07±0.00 ^d	0.16±0.02 ^d	0.34±0.02 ^{bcd}	0.46±0.03 ^{cd}
BFS- VI	0.07±0.00 ^d	0.15±0.01 ^c	0.34±0.03 ^{bcd}	0.43±0.03 ^{bc}
BFS- VII	0.06±0.00 ^{bc}	0.13±0.01 ^b	0.31±0.03 ^{bcd}	0.40±0.03 ^{bc}

The seed coated form and free form of biofertilizers significantly increased the yield of wheat. (Other details are described in Table. 4.2.1)

4.2.2 Level of Nitrate Content in Rhizospheric Soil, Roots and Leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Biofertilizers in Seed Coated Form

The enhanced dose of biofertilizers increased soil nitrate content in the plants' rhizosphere at the depth of 0-15 cm significantly. The soil nitrate increased by 15.31, 7.22, 6.11, 1.51, 3.72 and 6.36 % at 60 DAS and 44.28, 4.22, 3.08, 1.70, 3.27 and 6.53 % at 90 DAS over NF, BFS-I, BFS-V, BFS-VI and BFS-VII respectively with application on BFS-III. The application on BF-II also increased was 9.19, 1.11 and 0.25%, 41.20, 1.14 and 3.4% and 92.68, 50.34 and 70.45% at 60, 90 and 120 DAS respectively over NF, BFS-I and BFS-VII

The percentage increase of nitrate content in roots when applied with application on BFS-III was 44.04, 15.30, 10.16, 2.15, 0.03, 17.03% at 60 DAS, 45.77, 8.62, 4.49, 10.75, 14.65 and 25.57% at 90 DAS and 43.20, 15.47, 7.23, 6.72, 16.74 and 19.52% over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII respectively (Figure 4.2.1).

The nitrate content was significantly recorded in leaves of wheat plant by 41.82, 2.99, 0.95, 4.17, 5.83 and 8.48% at 60 days after sowing with application of the BFS-III over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII respectively. (Figure 4.2.1)

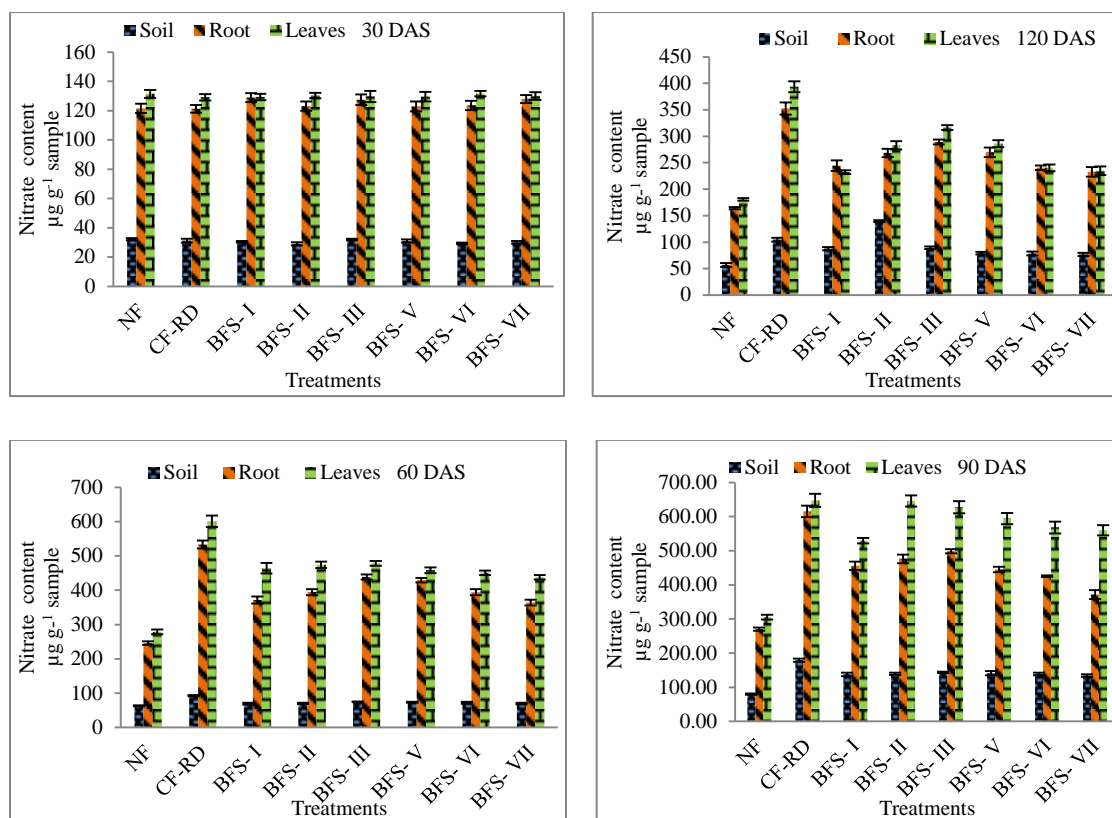


Figure 4.2.1 The enhance dose of biofertilizers on the nutrient availability of nitrate content in the soil, roots and leaves on 30, 60, 90, and 120 DAS the seeds. All the values are means of three replicates with two determinations ($n=6$) \pm S.E). (Other details are described in Table. 4.2.1)

4.2.3 Level of Nitrite Content in Rhizospheric Soil, Roots and Leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Biofertilizers in Seed Coated Form

An application of enhanced dose of biofertilizers increased soil nitrite on BFS-III was 53.99, 18.39, 10.30, 6.22, 15.89 and 21.29 % at 90 DAS and 46.67, 18.61, 11.95, 7.68, 11.06 and 21.94% at 120 days after sowing over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII respectively Seed coated with biofertilizers also increased nitrite content in soil by 43.69, 8.09, and 10.98% and 34.71, 6.6 and 9.98% on 90 and 120 DAS respectively over NF, BFS-I, and BFS-VII (Figure 4.2.2)

The root nitrite increased by 61.46, 21.40, 17.04, 13.09, 17.59, 25.72% at 60 DAS and 63.40, 8.32, 1.57, 4.65, 20.85, 23.73% at 90 DAS and 59.36, 11.06, 7.24, 7.86, 13.58, 23.11% at 120 DAS with the application of BFS-III over NF, BFS-I, BFS-

II, BFS-V, BFS-VI and BFS-VII respectively. Similarly BFS-II also increased nitrite content in root of wheat plant over NF and BFS-I (Figure 4.2.2).

Nitrite content in wheat leaves applied with BFS-III also increased by 60.65, 21.27, 14.82, 5.42, 9.66 and 17.05% at 60 DAS 59.42, 5.27, 3.07, 4.14, 5.80 at 90 DAS and 10.30% and 41.87, 16.69, 10.72, 6.89, 9.92 and 19.68% at 120 DAS over NF, BFS-I, BFS-II, BFS-VI and BFS-VII respectively on applied with BFS-III (Figure 4.2.2).

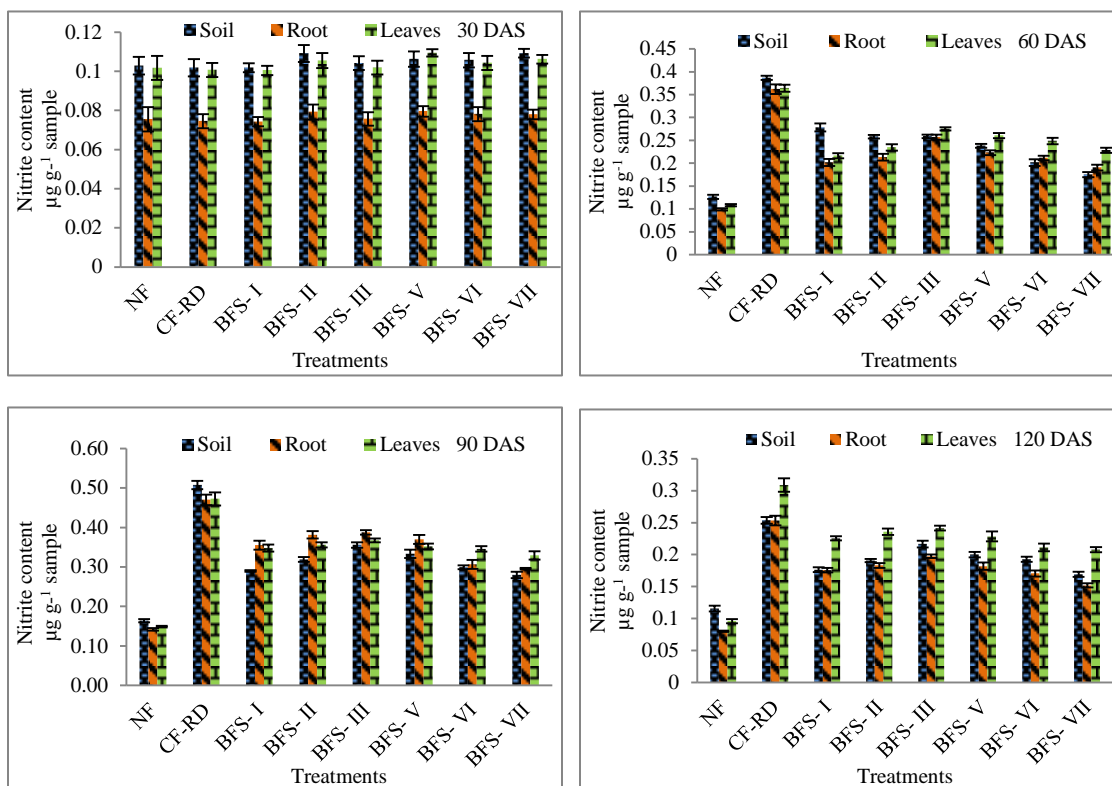


Figure 4.2.2 The enhance doses of biofertilizers on the nutrient availability of nitrite content in the soil, roots and leaves on 30, 60, 90, and 120 days after shown (DAS) the seeds. (Other details are described in Table. 4.2.1)

4.2.4 Level of Ammonium Content in Rhizospheric Soil, Roots and Leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Biofertilizers in Seed Coated Form

The soil ammonium increased by 41.04, 11.53, 7.33, 4.87, 6.95 and 12.52% and 48.92, 10.00, 1.50, 3.24, 6.05 and 10.92% with the application on BFS-III over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII at 60 and 90 DAS respectively. Application on BFS-II increased significantly was 33.7 and 4.20%, 47.41 and 8.5%, and 74.64 and 42.16% at 60, 90 and 120 DAS over NF and BFS-I (Figure 4.2.3).

The ammonium content in root of wheat plant was increased by 62.00, 32.95, 28.17, 5.21, 17.31 and 21.56% at 60 DAS and 61.27, 10.23, 8.56, 12.83, 16.16 and 21.47% at 90 DAS respectively on application of BFS-III over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII. Similarly BFS-II also increased ammonium content in root over NF, BFS-I and BFS-VII (Figure 4.2.3).

Ammonium content in leaves of plant was increase in applied with BFS-III by 55.64, 13.30, 11.38, 7.25, 12.51 and 12.63% and 58.10, 21.66, 18.02, 12.44, 16.93 and 27.95% on application of BFS-III at 60 and 90 DAS respectively over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII. Similarly the ammonium content significantly increased on 90 and 120 DAS over NF and BFS-I (Figure 4.2.3).

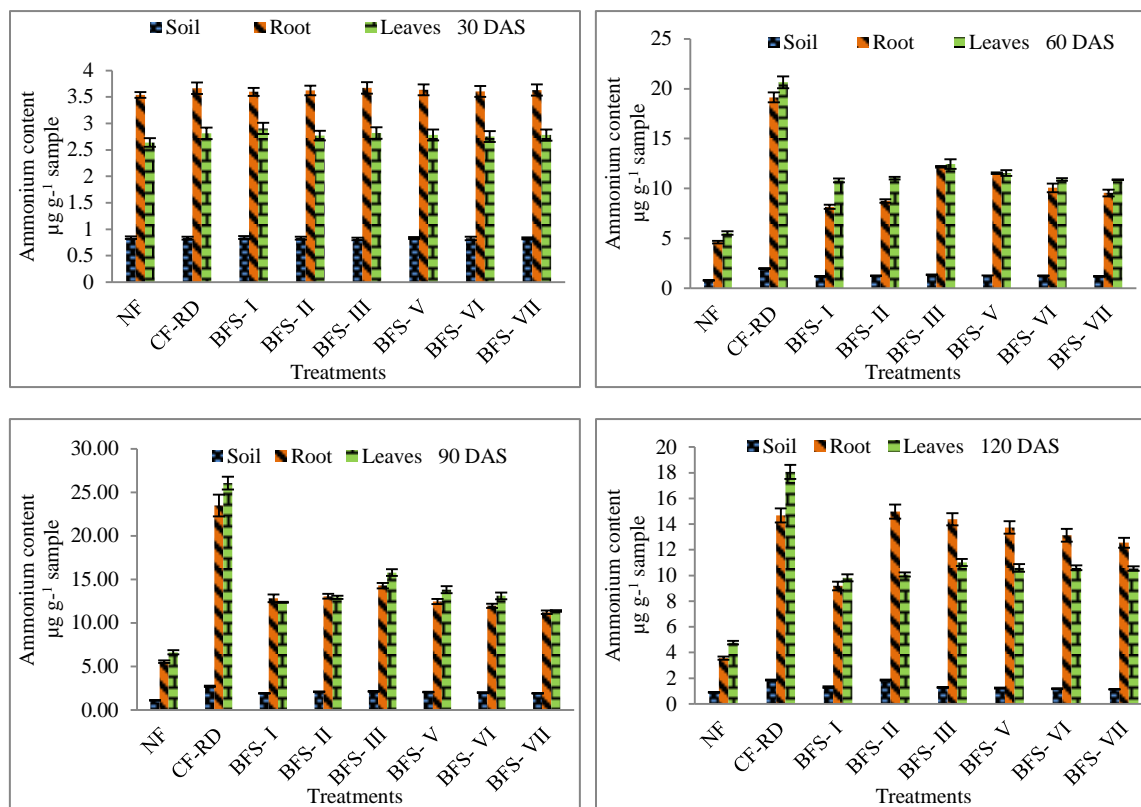


Figure 4.2.3 Levels of ammonium ($\mu\text{g g}^{-1}$) in soil of *T. aestivum* L. at 30, 60, 90 and 120 days after sowing in different treatments. (Other details are described in Table 4.2.1)

4.2.5 Level of Phosphate Content in Rhizospheric Soil, Roots and Leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Biofertilizers in Seed Coated Form

The application of BFS-III increased soil phosphate was 82.57, 2.01, 6.61, 16.63 and 23.52% and 83.65, 17.09, 6.33, 15.84 and 21.23% over NF, BFS-I, BFS-V, BFS-VI and

BFS-VII at 60 and 90 DAS respectively. Application of BFS-II also increased soil phosphate was 70.61, 4 and 8.19% over NF, BFS-I and BFS-VII (Figure 4.2.4).

The root phosphate increased by 61.75, 16.97, 25.48, 8.48, 17.34 and 23.33% at 60 DAS, 62.58, 19.97, 12.70, 2.28, 4.27 and 9.47% at 90 DAS and 54.99, 21.22, 11.80, 7.43, 14.32 and 18% 120 DAS with the application of BFS-III over NF, BFS-I, BFS-V, BFS-VI and BFS-VII. The percentage increase of phosphate in root of the plant applied with BFS-II was 49.87 and 7.26% and 43.19 and 9.4% respectively on 90 and 120 DAS over NF and BFS-I (Figure 4.2.4).

Further, phosphate content in wheat leaves applied with BFS-III increased 64.93, 12.59, 7.51, 3.10, 6.26 and 12.54% at 60 DAS respectively over NF, BFS-I, BFS-II, BFS-V, BFS-VI and BFS-VII. Similarly at 90 and 120 DAS nitrate content was recorded over NF, BFS-I, BFS-V, BFS-VI and BFS-VII on application of BFS-III (Figure 4.2.4).

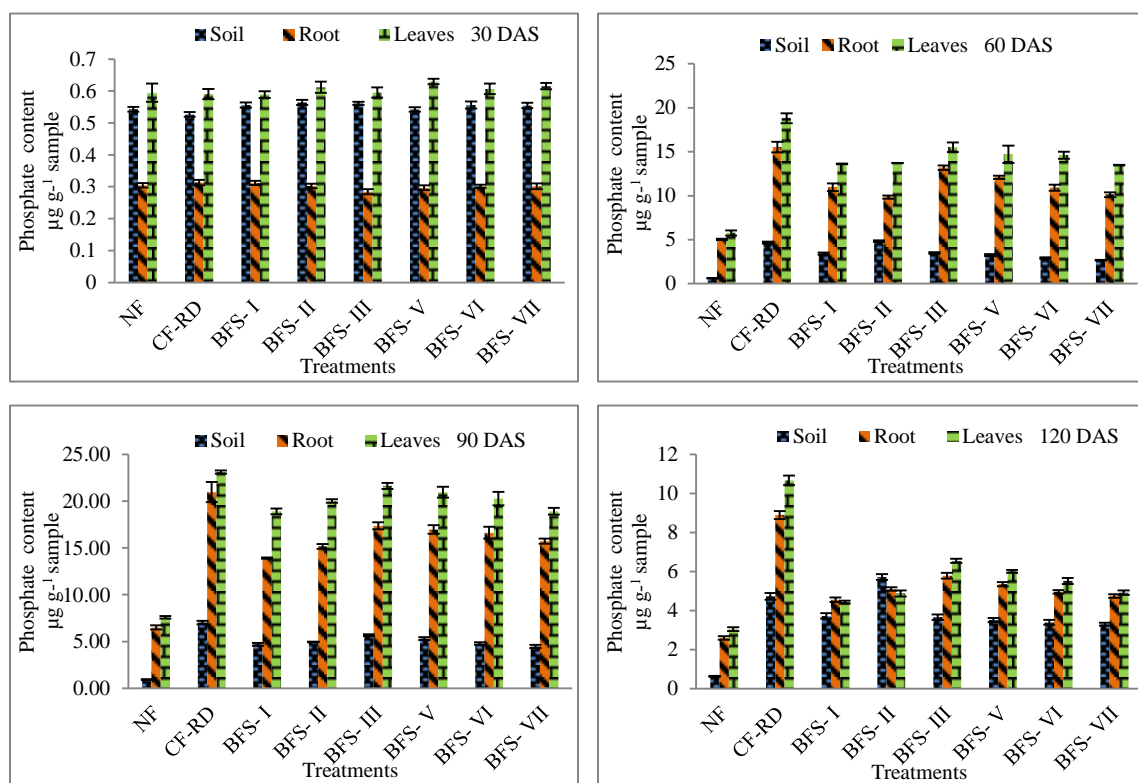


Figure 4.2.4 The seed coated with biofertilizers significantly effect on the nutrient availability of phosphate content in the soil roots and leaves at on 30, 60, 90, and 120 days after shown (DAS) the seeds. The nutrient transfer from soil to roots and root to leaves were significantly recorded with the application of BFS-III followed by BFS-II and BF-V. (Other details are described in Table. 4.2.1)

4.2.6 Yield and Productivity of Wheat (*T. aestivum* L.) Plant Applied with Free Form of Biofertilizers in seed coated form

The percent increased significantly by 43.34, 19.93, 17.57, 6, 13.89 and 27.80 % in grain yield was recorded on application of BFS-III over NF, BFS-I, BFS-II, BFS-VI and BFS-VII (Figure 4.2.5). Application of BFS-II was increased by 36 and 2.35% over NF and BF-I after harvesting crop

Wheat straw yield increased on application of BFS-III was 60.62, 3.34, 2.72, 1.34, 2.85 and 3.01% over NF, BFS-I, BFS-II, BFS-VI and BFS-VII after harvesting the crop. BFS-II was increased by 71.41 and 0.61% over NF and BF-I after harvesting crop.

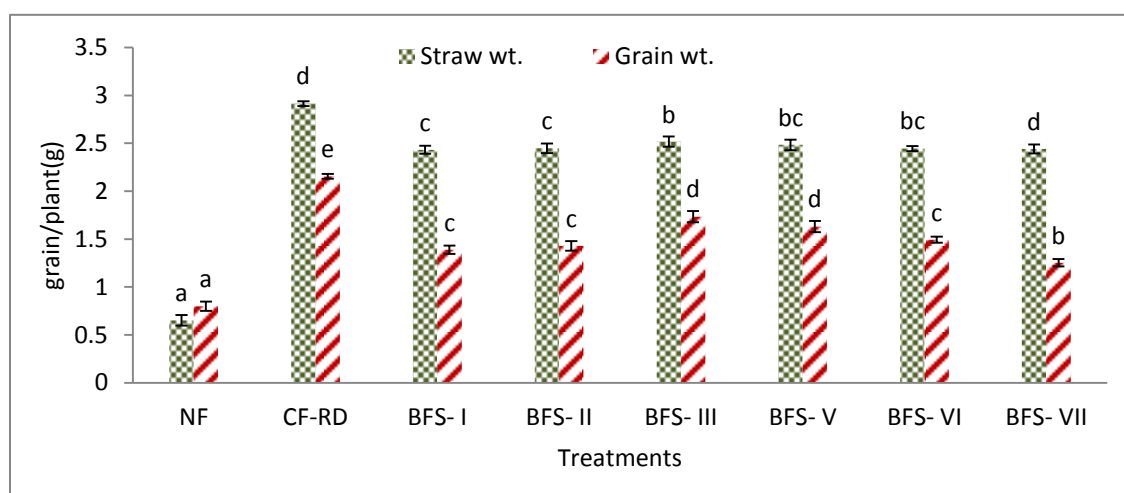


Figure 4.2.5 Effect of enhanced dose of biofertilizers on yield of wheat *T. aestivum* L. at after harvesting. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significantly differences between the treatments at 5% level. (Other details are described in Table. 4.2.1)

4.3. Growth and Productivity of Wheat (*T. aestivum* L. cv. PBW-502) Applied with Charcoal Based Biofertilizers (*A. chroococcum* and *B. subtilis*) in Enhanced Dose and Seed Coated with Biofertilizers and Organic Matrix form on 30, 60, 90 and 120 days after sowing (DAS)

4.3.1 Growth Parameters of Wheat Applied with Seed Coated Biofertilizers and Organic Matrix, Recorded on 30, 60, 90 and 120 (DAS)

Mode of application of biofertilizers (*A. chroococum* and *B. subtilis*) significantly effect on growth of the plant. The increased fresh and dry weight of roots and shoots, numbers of leaves and roots significantly recorded over no fertilizers, free form of biofertilizers on 60 and 120 days after sowing (DAS). The optimized dose of biofertilizers (*A. chroococcum*- 1.80 kg ha⁻¹ and *B. subtilis*- 1.80 kg ha⁻¹) was increased the plant height over the no fertilizer and free form of biofertilizers. The plant height of BF-3(SV) application was 62.13, 14.55, 10.70, 4.38 and 16.01% and 35.31, 2.36, 2.07, 4.01 and 9.35% on 30 and 90 DAS over NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) respectively (Table 4.3.1).

On application of BF-3(SV) the number of plant leaves recorded significantly by 60.54, 50.04, 7.89, 31.57, 50 and 47.36%, and 53.45, 37.19, 13.96, 25.54, 34.89 and 41.87% over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) at 60 and 120 DAS respectively. The number of tillers increased by 62.55, 50, 12.73, 25.09, 50.19 and 37.45% at 60 DAS over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Table 4.3.1).

The optimized dose of biofertilizers was increased the fresh weight of wheat plant. The percentage of fresh weight of shoot was increased by 51.47, 21.69, 17.28, 11.03, 12.50 and 22.43% at 30 DAS and 47.50, 21.68, 15.83, 4.99, 10.84 and 14.55% at 120 DAS respectively over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) on application of biofertilizers with vermicompost in seed coated form BF-3(SV) (Table 4.3.1)

The charcoal based biofertilizers has significantly found the dry weight of plant production. Dry weight of plant shoot of BF-3(SV) contains 63.89, 4.17, 2.78, 1.39, 5.56 and 8.33% at 60 DAS and 75, 3.23, 1.61, 1.61, 4.03 and 6.45% at 90 DAS and 42.02, 5.32, 2.66, 3.19, 5.85 and 6.91% at 120 DAS respectively by the application of BF-3(SV) over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) was significantly recorded (Table 4.3.1).

Table 4.3.1 Effect of Coated Seed with Organic Matrix and Biofertilizers on Plant Growth of Wheat (*T. aestivum* L. cv. PBW-502) Shoot on 30, 60, 90 and 120 DAS

	30 DAS	60 DAS	90 DAS	120 DAS
Shoot Height				
NF	21.60±1.42 ^a	46.90±1.73 ^a	50.93±1.22 ^{ab}	56.10±1.80 ^a
CFC R/D	21.53±2.30 ^a	64.13±2.41 ^{bc}	81.23±2.41 ^d	87.57±1.75 ^d
BF-1 (SV)	48.73±0.64 ^{bc}	68.63±1.40 ^{bc}	76.87±0.78 ^{bc}	78.57±0.81 ^{bc}
BF-2 (SV)	50.93±0.90 ^c	72.23±0.23 ^{cd}	77.10±0.79 ^{bc}	79.03±0.91 ^{bc}
BF-3 (SV)	57.03±0.45 ^{cd}	74.97±0.68 ^{cd}	78.73±0.57 ^{bc}	81.27±1.20 ^{cd}
BF-5 (SV)	57.37±1.88 ^{cd}	75.40±0.53 ^d	77.60±1.31 ^{bc}	80.23±1.17 ^{cd}
BF-6 (SV)	54.53±0.90 ^{cd}	73.83±0.31 ^{cd}	75.57±0.95 ^{bc}	78.90±1.06 ^{bc}
BF-7 (SV)	47.90±0.36 ^{bc}	67.17±1.26 ^{bc}	71.37±1.10 ^{bc}	76.13±1.64 ^{bc}
Number of Leaves				
NF	3.67±0.58 ^a	5.00±1.00 ^a	5.67±0.58 ^a	6.67±0.58 ^a
CFC R/D	3.67±0.58 ^a	10.67±1.15 ^a	15.67±2.08 ^a	16.67±3.06 ^a
BF-1 (SV)	3.67±0.58 ^a	6.33±1.53 ^b	6.33±0.58 ^b	9.00±3.46 ^c
BF-2 (SV)	6.67±1.15 ^c	11.67±3.51 ^e	11.67±2.89 ^e	12.33±2.08 ^f
BF-3 (SV)	6.33±0.58 ^c	12.67±3.06 ^f	14.00±2.65 ^f	14.33±1.53 ^g
BF-5 (SV)	6.00±2.00 ^c	8.67±1.15 ^{cd}	9.67±1.53 ^d	10.67±2.08 ^e
BF-6 (SV)	4.67±1.15 ^b	6.33±1.53 ^b	9.67±2.52 ^d	9.33±1.15 ^{cd}
BF-7 (SV)	4.33±1.53 ^b	6.67±1.53 ^b	8.67±3.06 ^{cd}	8.33±1.15 ^{bcd}
Number of Tillers				
NF	1.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a
CFC R/D	1.00±0.00 ^a	2.00±0.00 ^b	2.33±0.58 ^b	3.00±0.00 ^d
BF-1 (SV)	1.00±0.00 ^a	1.33±0.58 ^a	1.00±0.00 ^a	1.33±0.58 ^a
BF-2 (SV)	2.00±0.00 ^b	2.33±0.58 ^a	2.33±0.58 ^b	2.33±0.58 ^b
BF-3 (SV)	2.00±0.00 ^b	2.67±0.58 ^b	2.67±0.58 ^b	2.67±0.58 ^{bc}
BF-5 (SV)	2.00±0.00 ^b	2.00±0.00 ^b	2.00±0.00 ^b	2.00±0.00 ^b
BF-6 (SV)	1.67±0.58 ^{ab}	1.33±0.58 ^a	2.00±0.00 ^b	1.67±0.58 ^{ab}
BF-7 (SV)	1.33±0.58 ^a	1.67±0.58 ^{ab}	1.33±0.58 ^{ab}	1.67±0.58 ^{ab}
Fresh weight of Shoot				
NF	1.32±0.07 ^a	2.09±0.08 ^a	2.44±0.07 ^a	3.68±0.18 ^a
CF R/D	1.27±0.01 ^a	3.86±0.13 ^a	6.73±0.42 ^d	11.18±0.20 ^a
BF-1 (SV)	2.13±0.10 ^b	2.87±0.13 ^{ab}	3.76±0.11 ^{bc}	5.49±0.15 ^{bc}
BF-2 (SV)	2.25±0.12 ^b	3.11±0.06 ^c	3.87±0.07 ^{bc}	5.90±0.04 ^{bc}
BF-3 (SV)	2.72±0.13 ^b	3.95±0.05 ^{cd}	4.45±0.11 ^c	7.01±0.13 ^e
BF-5 (SV)	2.42±0.30 ^b	3.45±0.10 ^c	4.40±0.14 ^c	6.66±0.08 ^{de}
BF-6 (SV)	2.38±0.02 ^b	3.39±0.06 ^c	4.15±0.15 ^c	6.25±0.16 ^d
BF-7 (SV)	2.11±0.11 ^a	3.19±0.06 ^c	3.91±0.17 ^{bc}	5.99±0.15 ^{bc}
Dry weight of Shoot				
NF	0.11±0.02 ^a	0.26±0.03 ^a	0.31±0.01 ^a	1.09±0.05 ^a
CFC R/D	0.11±0.01 ^a	0.72±0.01 ^{df}	1.60±0.02 ^f	4.00±0.17 ^h

BF-1 (SV)	0.58±0.01 ^{bcd}	0.69±0.01 ^{bcd}	1.20±0.02 ^a	1.78±0.02 ^d
BF-2 (SV)	0.65±0.05 ^{de}	0.70±0.01 ^{cd}	1.22±0.02 ^d	1.83±0.02 ^{ef}
BF-3 (SV)	0.70±0.02 ^f	0.72±0.01 ^{df}	1.24±0.01 ^e	1.88±0.01 ^g
BF-5 (SV)	0.65±0.02 ^{de}	0.71±0.01 ^{cd}	1.22±0.01 ^d	1.82±0.04 ^e
BF-6 (SV)	0.60±0.01 ^d	0.68±0.02 ^{bcd}	1.19±0.01 ^{bc}	1.77±0.02 ^{cd}
BF-7 (SV)	0.53±0.01 ^{bcd}	0.66±0.03 ^{bcd}	1.16±0.01 ^{bc}	1.75±0.02 ^{bcd}

The enhance doses of biofertilizers in seed coated form significantly increased the growth of plant and biomass. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significantly differences between the treatments at 5% level. [Where NF; No fertilizer, CFC-R/D; Recommended dose of urea and DAP, BF-1(SV); single dose of biofertilizers in seed coated form, BF-2(SV); twofold of biofertilizers in seed coated form, BF-3(SV); threefold of biofertilizers in seed coated form, BF-5(SV); fivefold of biofertilizers in seed coated form, BF-6(SV); sixfold of biofertilizers in seed coated form and BF-7(SV); sevenfold of biofertilizers in seed coated form]

The root length of wheat plant increased on application of BF-3(SV) was 57.11, 9.97, 5.50, 8.31, 8.31 and 28.35% and 45.95, 15.84, 12.40, 8.95 and 29.82% on 60 and 120 DAS over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Table 4.3.2)

Number of plant roots recorded significantly by on application of BF-3(SV) was 56.85, 22.77, 15.95, 20.45, 22.61 and 31.83%, at 120 DAS respectively over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) (Table 4.3.2)

Application of BF-3(SV) increased dry weight of the plant parts significantly ($p < 0.05$) over minimum and higher doses of biofertilizers on 30, 60, 90 and 120 DAS. About 30-70% increase in fresh weight of root and 25-62 % increase in dry weight of root was observed in 120 days after sowing by application of BF-3(SV) (Table 4.3.2).

The percentage of fresh weight of roots increased by on application of BF-3(SV) was 63.01, 17.81, 10.96, 23.29, 26.03 and 30.14% at 60 DAS and 74.79, 15.13, 2.52, 18.49, 20.17 and 25.21 at 120 DAS over NF, BF-1(SV), BF-2(SV) BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Table 4.3.2).

The percentage of dry weight of roots increased by 62.50, 18.75, 12.50, 25, 27.08 and 31.25% at 120 DAS respectively on application of BFS-III over NF, BF-1(SV), BF-2(SV) BF-5(SV), BF-6(SV) and BF-7(SV) respectively. On application of BF-2(SV) was increased by 50, 6.25, 12.50, 14.58 and 18.75 % at 120 DAS over NF and BF-1(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Table 4.3.2)

Table 4.3.2 Effect of Application of *A. chroococcum* and *B. subtilis* and Organic Matrix as Seed Coated Form, on Plant Growth of Wheat (*T aestivum* L. cv. PBW-502) Roots at 30, 60, 90 and 120 DAS

	Root Height			
NF	3.77±0.25 ^a	4.13±0.32 ^a	4.97±0.45 ^a	5.80±0.53 ^a
CFC R/D	3.80±0.30 ^a	9.27±0.38 ^a	11.73±0.86 ^a	11.97±0.61 ^a
BF-1 (SV)	7.63±0.32 ^a	8.67±0.29 ^{cd}	8.80±0.26 ^c	9.03±0.21 ^c
BF-2 (SV)	7.97±0.15 ^{cd}	9.10±0.10 ^{cd}	9.43±0.40 ^{cd}	9.40±0.36 ^c
BF-3 (SV)	9.10±0.10 ^e	9.63±0.23 ^{cd}	10.30±0.20 ^e	10.73±0.21 ^e
BF-5 (SV)	9.23±0.21 ^e	9.80±0.20 ^d	10.27±0.21 ^e	10.03±0.80 ^{de}
BF-6 (SV)	8.27±0.25 ^d	8.83±0.42 ^{cd}	9.77±0.25 ^d	9.77±0.46 ^d
BF-7 (SV)	6.37±0.42 ^b	6.90±0.90 ^b	7.13±0.71 ^{bc}	7.53±0.45 ^b
	Number of Roots			
NF	4.33±0.58 ^a	5.00±1.00 ^a	5.67±0.58 ^a	6.33±0.58 ^a
CFC R/D	4.67±0.58 ^a	10.00±1.00 ^a	11.33±1.15 ^{de}	12.33±1.53 ^{cde}
BF-1 (SV)	6.67±0.58 ^{bc}	9.67±1.53 ^{bcd}	11.33±0.58 ^{de}	11.33±0.58 ^{cd}
BF-2 (SV)	8.00±1.00 ^d	10.67±1.15 ^d	11.67±1.53 ^{de}	12.33±0.58 ^{cde}
BF-3 (SV)	8.67±0.58 ^d	11.00±1.00 ^d	13.33±1.53 ^e	14.67±1.53 ^f
BF-5 (SV)	8.00±1.00 ^d	10.00±0.00 ^d	11.00±1.00 ^{de}	11.67±0.58 ^{cd}
BF-6 (SV)	8.67±0.58 ^d	10.67±1.53 ^d	11.33±1.53 ^{de}	11.33±1.53 ^{cd}
BF-7 (SV)	6.33±0.58 ^{bc}	9.00±1.00 ^{bcd}	9.67±0.58 ^{bcd}	10.00±0.00 ^{bc}
	Fresh weight of Root			
NF	0.11±0.01 ^a	0.19±0.01 ^a	0.27±0.01 ^a	0.30±0.01 ^a
CFC R/D	0.11±0.01 ^a	0.40±0.02 ^a	0.59±0.00 ^f	0.61±0.02 ^a
BF-1 (SV)	0.17±0.02 ^b	0.28±0.01 ^{bc}	0.32±0.02 ^{bc}	0.34±0.01 ^{bc}
BF-2 (SV)	0.21±0.01 ^c	0.30±0.01 ^c	0.35±0.01 ^a	0.35±0.00 ^{cd}
BF-3 (SV)	0.24±0.01 ^d	0.34±0.03 ^e	0.38±0.01 ^e	0.39±0.01 ^f
BF-5 (SV)	0.24±0.02 ^d	0.31±0.02 ^d	0.36±0.02 ^d	0.37±0.02 ^e
BF-6 (SV)	0.22±0.01 ^c	0.28±0.01 ^{bc}	0.34±0.02 ^{bc}	0.35±0.01 ^{cd}
BF-7 (SV)	0.18±0.02 ^b	0.29±0.01 ^{bc}	0.33±0.01 ^{bc}	0.33±0.01 ^b
	Dry weight of Root			
NF	0.04±0.01 ^a	0.06±0.00 ^a	0.08±0.01 ^a	0.08±0.01 ^a
CFC R/D	0.04±0.00 ^a	0.13±0.01 ^{cd}	0.15±0.00 ^e	0.17±0.01 ^{de}
BF-1 (SV)	0.06±0.01 ^b	0.12±0.01 ^{bcd}	0.12±0.00 ^{bc}	0.13±0.01 ^{bcd}
BF-2 (SV)	0.08±0.01 ^{cd}	0.13±0.01 ^{cd}	0.13±0.00 ^{bcd}	0.14±0.01 ^{bcd}
BF-3 (SV)	0.09±0.01 ^e	0.15±0.00 ^a	0.14±0.00 ^{cd}	0.15±0.00 ^{cd}
BF-5 (SV)	0.08±0.01 ^{cd}	0.13±0.01 ^{cd}	0.13±0.00 ^{bcd}	0.14±0.00 ^{bcd}
BF-6 (SV)	0.08±0.01 ^{cd}	0.12±0.00 ^{bcd}	0.13±0.00 ^{bcd}	0.14±0.00 ^{bcd}
BF-7 (SV)	0.06±0.00 ^b	0.12±0.01 ^{bcd}	0.13±0.00 ^{bcd}	0.13±0.00 ^{bcd}

All the values are means of three replicates with two determinations (n=6) ±S.D.) (Other details are described in Table. 4.3.1)

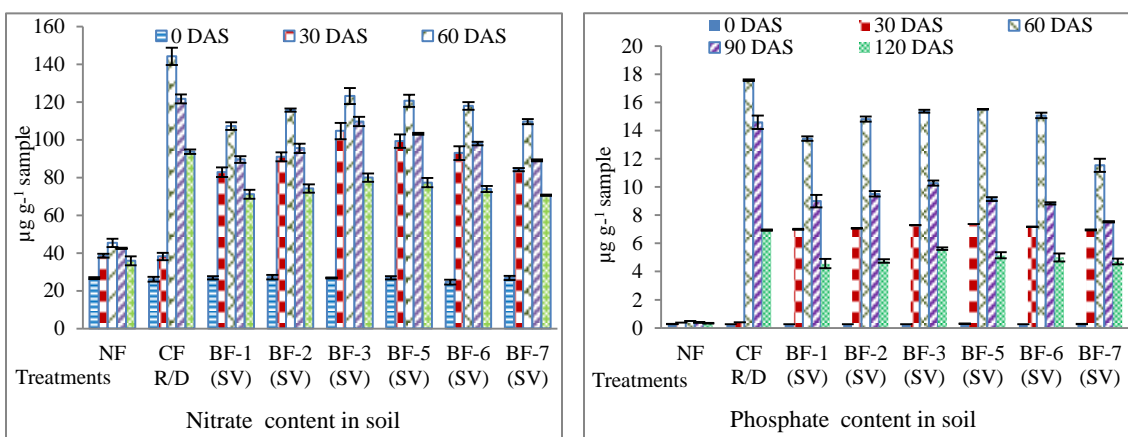
4.3.2 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Soil of Wheat (*T. aestivum* L.) Plant Applied with Seed Coated with Biofertilizers and Organic Matrix

The soil nitrate content was increased by 64.12, 10, 4.12, 9.41, 11.18 and 7.23%, and 63.83, 2.13, 3.19, 1.06, 2.13 and 10.64% at 60 and 120 DAS respectively by the application of BF-3(SV) over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively.

The percentage increase of nitrite content in the soil when applied with BF-3(SV) was 61.32, 18.33, 12.93, 5.88, 10.59 and 18.78 %, 55.10, 10.94, 7.07, 3.25, 7.51 and 11.68% on 90 and 120 days after sowing over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.1).

The ammonium content of soil increased by 71.49, 11.88, 6.34, 4.16, 4.95 and 11.49% and 68.40, 24, 12.4, 7.20, 12 and 18% on 60 and 120 days after sowing over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.1).

Phosphate content in soil increased on application of (BF-3(SV) was 94.14, 19.01, 15.81, 8.53, 11.19 and 16.34% on 90 days after sowing respectively over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) (Figure 4.3.1).



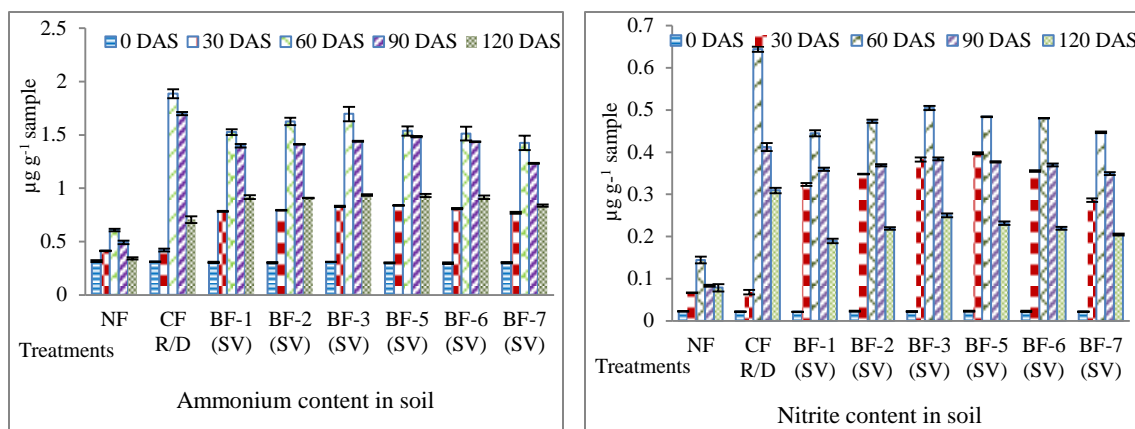


Figure 4.3.1 Seed coated with biofertilizers and organic matrix significantly increased nutrient availability in the soil on 30, 60, 90, and 120 days after shown (DAS) the seeds. The nutrient transfer from soil to roots and root to leaves were significantly recorded with the application of BF-3(SV) followed by BF-2 (SV) and BF-5(SV). All the values are means of three replicates with two determinations ($n=6 \pm \text{S.E}$) (Other details are described in Table. 4.3.1)



Plate:4.3.1 Experimental site at Environmental field Station, Babasaheb Bhimrao Ambedkar University, Lucknow

4.3.3 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Roots of Wheat (*T. aestivum* L.) Plant Applied with Seed Coated with Biofertilizers and Organic Matrix

Nitrate content increased in wheat roots when applied with biofertilizers in seed coated form; (BF-3(SV))by 73.98, 19.90, 16.33, 7.65, 11.73 and 17.35% on 90 DAS and 66.67, 17.54, 9.65, 9, 14.91 and 16.67% on 120 DAS over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.2)

Nitrite content in wheat roots applied with enhanced dose (1.8 kg ha^{-1}) of biofertilizers (BF-3(SV) increased by 59.12, 12.88, 9.75, 3.58, 7.22 and 11.09% and

52.56, 13.64, 6.94, 5.41, 8.74 and 10.22% on 60 and 120 days after sowing respectively over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) (Figure 4.3.2).

Significant increase in ammonium content in roots on application of BF3-(SV) was 78.85, 11.54, 3.85, 7.69, 17.31 on 30 DAS and 71.43, 20, 11.43, 11.1 and 25.71% on 120 DAS respectively over NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) (Figure 4.3.2).

In roots, phosphate content was observed in BF3-(SV) by 97.27, 17.52, 11.74, 13.02, 16.24 and 26.21% at 120 DAS over the NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.2).

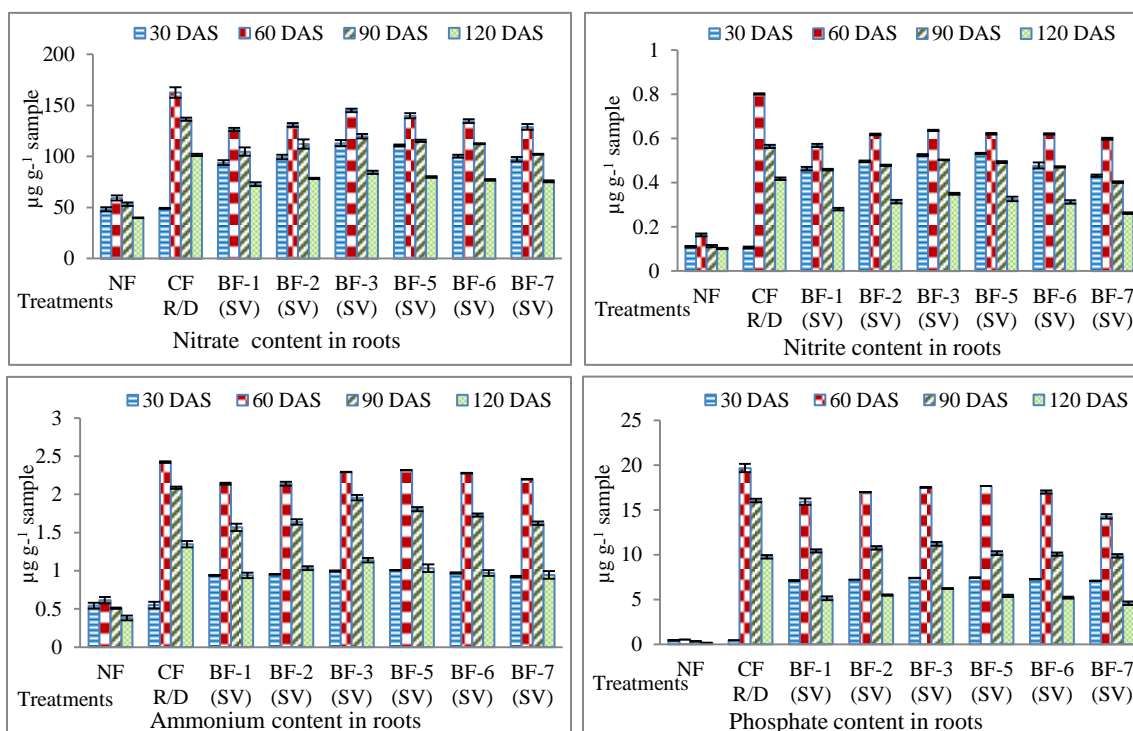


Figure 4.3.2 The seed coated with biofertilizers and organic matrix increased nutrient availability in the roots on 30, 60, 90, and 120 days after sown (DAS) the seeds. All the values are means of three replicates with two determinations ($n=6$) \pm S.E) (Other details are described in Table. 4.3.1)

4.3.4 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Leaves of Wheat (*T. aestivum* L.) Plant Applied with Seed Coated with Biofertilizers and Organic Matrix

Nitrate content in leaves on application of BF-3(SV) increased by 63.59, 8.72, 2.56, 8.21, 9.74 on 60DAS and 14.87%, 68.18, 9.09, 5.30, 3.79, 6.82, 20.45% on 90DAS and 67.26, 32.74, 16.81, 6.19, 9.73 and 14.16% on 120 days after sowing respectively over NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) (Figure 4.3.3).

The nitrite content in leaves increased by 57.37, 10.25, 5.46, 2.66, 5.95, 12.65% and 49.99, 12.06, 7.45, 4.80, 11.24 and 17.30% respectively in 60 and 120 days old plants with the application of BF3-(SV) over NF, BF-1(SV), BF-2(SV), BF-5(SV), BF-6(SV) and BF-7(SV) respectively. The application of BF5-(SV) increases phosphate content by 45.19, 7.25, 2.65, 6.43, 12.50% over NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) at 120 DAS respectively (Figure 4.3.3).

Application with BF3-(SV) was recorded significantly ammonium content at different level by 74.07, 9.26, 1.85, 5.56 and 12.96% at 60 DAS, 73.33, 15.56, 2.22, 6.67, 17.78% at 90 DAS and 67.86, 21.43, 10.71, 14.29 and 21.43% at 120 DAS on application of BF3-(SV) over NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.3).

The percentage of phosphate content in the leaves increased by 95.45, 11.44, 7.33, 10.85, 12.90 and 25.95% and 49, 8, 4, 2, 4 and 10% respectively at 60 and 120 days old plants with the application of BF3-(SV) over that in NF, BF-1(SV), BF-2(SV), BF-6(SV) and BF-7(SV) respectively (Figure 4.3.3).

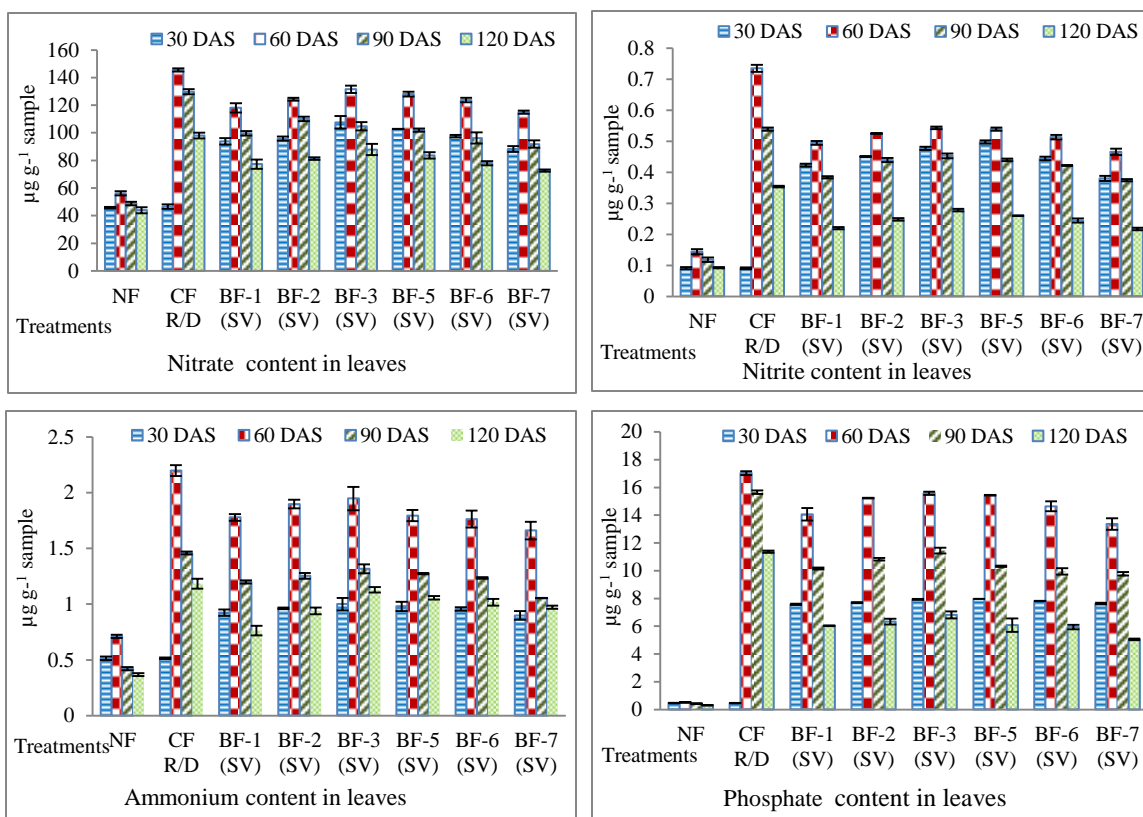


Figure 4.3.3 Seed coated with biofertilizers organic matrix increased nutrient availability in the leaves on 30, 60, 90, and 120 days after sown (DAS) the seeds. (Other details are described in Table. 4.3.1)

4.3.5 Grain and Straw Yield Production of Wheat on Application of Biofertilizers in Seed Coated Form with Organic Matrix

Organic matrix and biofertilizers applied as in seed coated form increased the grain yield of *T. aestivum* L. The percentage increases of the grain yield significantly with the application of BF-3(SV) over the application of NF, BF-1(SV), BF-2 (SV), BF-5(SV), BF-6(SV) and BF-7(SV) after the harvest of crop was 48.71, 17.72, 8.45, 8.83, 10.94 and 24.35% respectively at 120 DAS (Figure 4.3.4). Applied with seed coated form on BF-5(SV) increased the percentage was 39.88, 8.89, 2.11 and 15.52% respectively after harvesting over application on NF, BF-1(SV), BF-6 (SV), and BF-7(SV).

The straw production also increased by the application on BF-3(SV) was 50.33, 21.68, 15.85, 4.91, 10.83 and 14.59% over NF, BF-1(SV), BF-2 (SV), BF-5(SV), BF-6(SV) and BF-7(SV) and by BF-2(SV) was 34.47 and 5.83% over NF and BF-1(SV) respectively at 120 DAS (Figure 4.3.4).

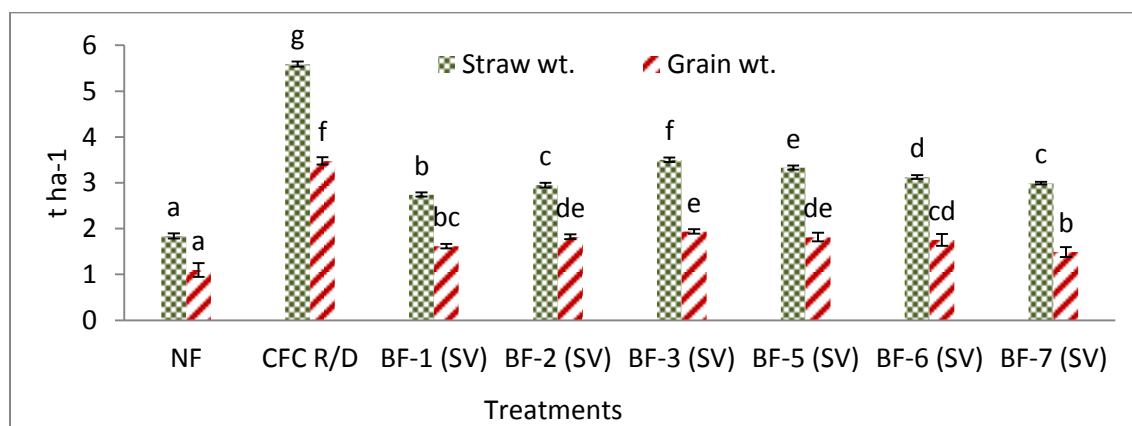


Figure: 4.3.4. Effect of seed coated organic matrix and biofertilizers on yield of wheat *T. aestivum* L. at after harvesting. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significantly differences between the treatments at 5% level. (Other details are described in Table 4.3.1).

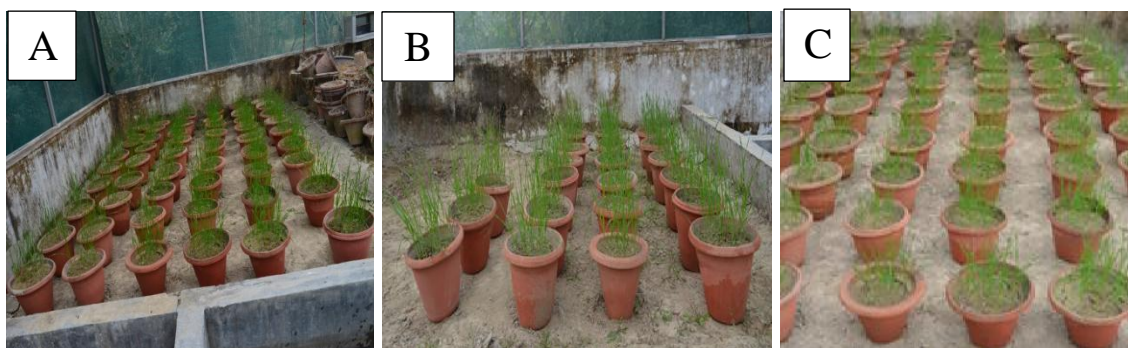


Plate: 4.3.2 Effect of enhanced dose of biofertilizers (*A. chroococcum* and *B. subtilis*) in seed coated form for dose optimization during wheat crop cultivation in net house

4.4. Characterization of Organic Matrix and Immobilized Form of *A. chroococum* and *B. subtilis* with Various Organic Matrix as Super Granular Biofertilizer

The microbial biofertilizers were immobilized in organic matrix to prepare super granular biofertilizer. To assess the nutritional potential of the organic matrix with or without chemical/microbial fertilizers its physical and chemical properties were determined using various relevant techniques. The objective of these studies was to certain the additional structural and nutritional availability provided by these granules to commercial microbial fertilizers in immobilized forms.

4.4.1. Scanning Electron Microscope Energy Dispersive X-ray (SEM EDX) analysis

The elemental properties of different components of organic matrix i.e. clay soil, cow-dung, neem leaf powder and urea and DAP; charcoal based biofertilizers immobilized in clay soil and neem leaf powder in combination with organic matrix such as cow dung, vermicompost, and Farm Yard Manure (FYM) separately with different organic binders i.e. *acacia* gum, molasses and jaggery in form of granules. Organic matrix immobilized granular biofertilizer are shown in (Figure 4.4.1-4.4.12).



Plate: 4.4.1. - Consortium of biofertilizers (*A. chroococum* and *B.s subtilis*) with enhance doses, immobilized in organic matrix i.e. cow dung, vermicompost, FYM (combination with clay soil+ neem leaf) with different organic binders i.e. *acacia* gum, molasses and jaggery

The elemental properties of prepared super granular biofertilizer shows that out of these elements C, Mg and Zr are not present in the chemical fertilizers; urea and DAP whereas C in range of 18-58, O- 54.35-70.81, Mg- 00.28-1.80, Ca- 0.44-5.02, Al- 0.28-2.38, F- 3.68-7.52, Si- 1.91-7.42, P- 0.50-3.54, S- 0.07-0.10, K- 0.09-0.30, Zr- 1.13-2.20 are present in various organic matrix immobilized fertilizers.

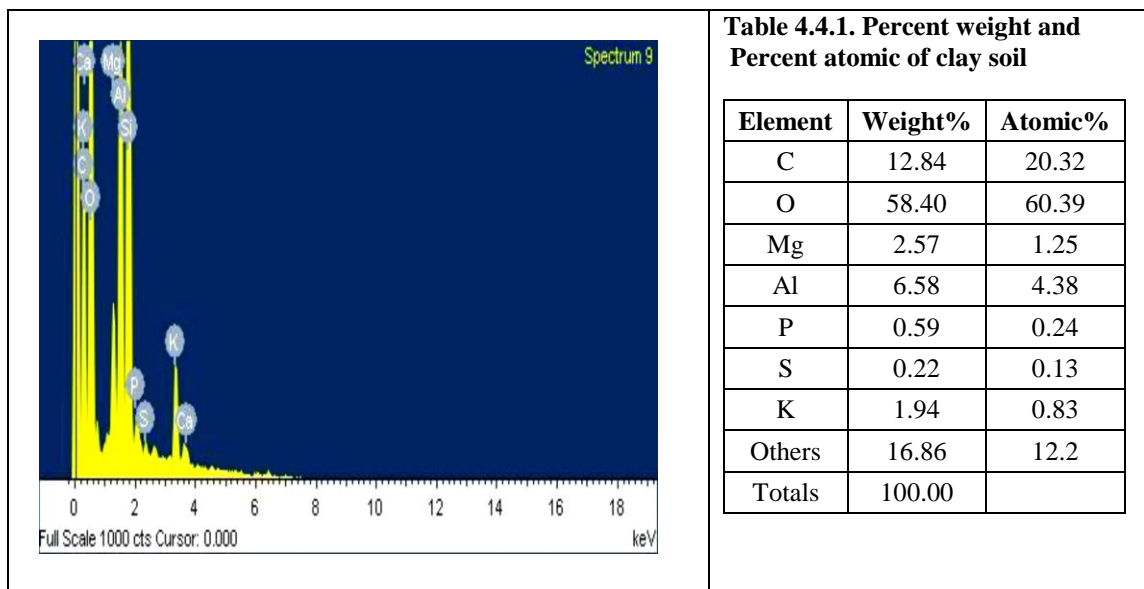


Figure 4.4.1. SEM EDX analysis of clay soil

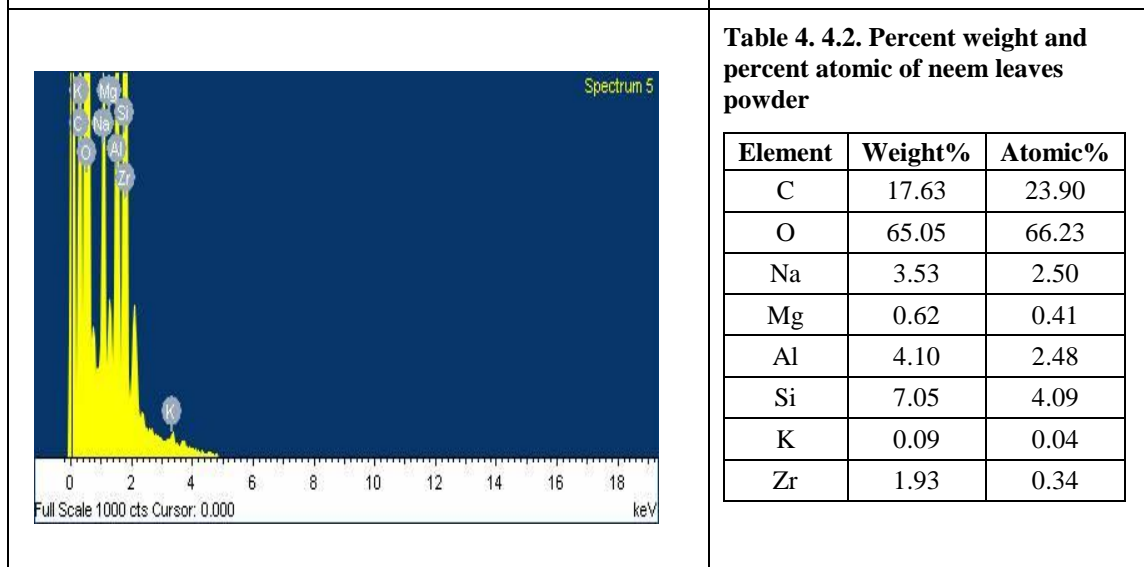
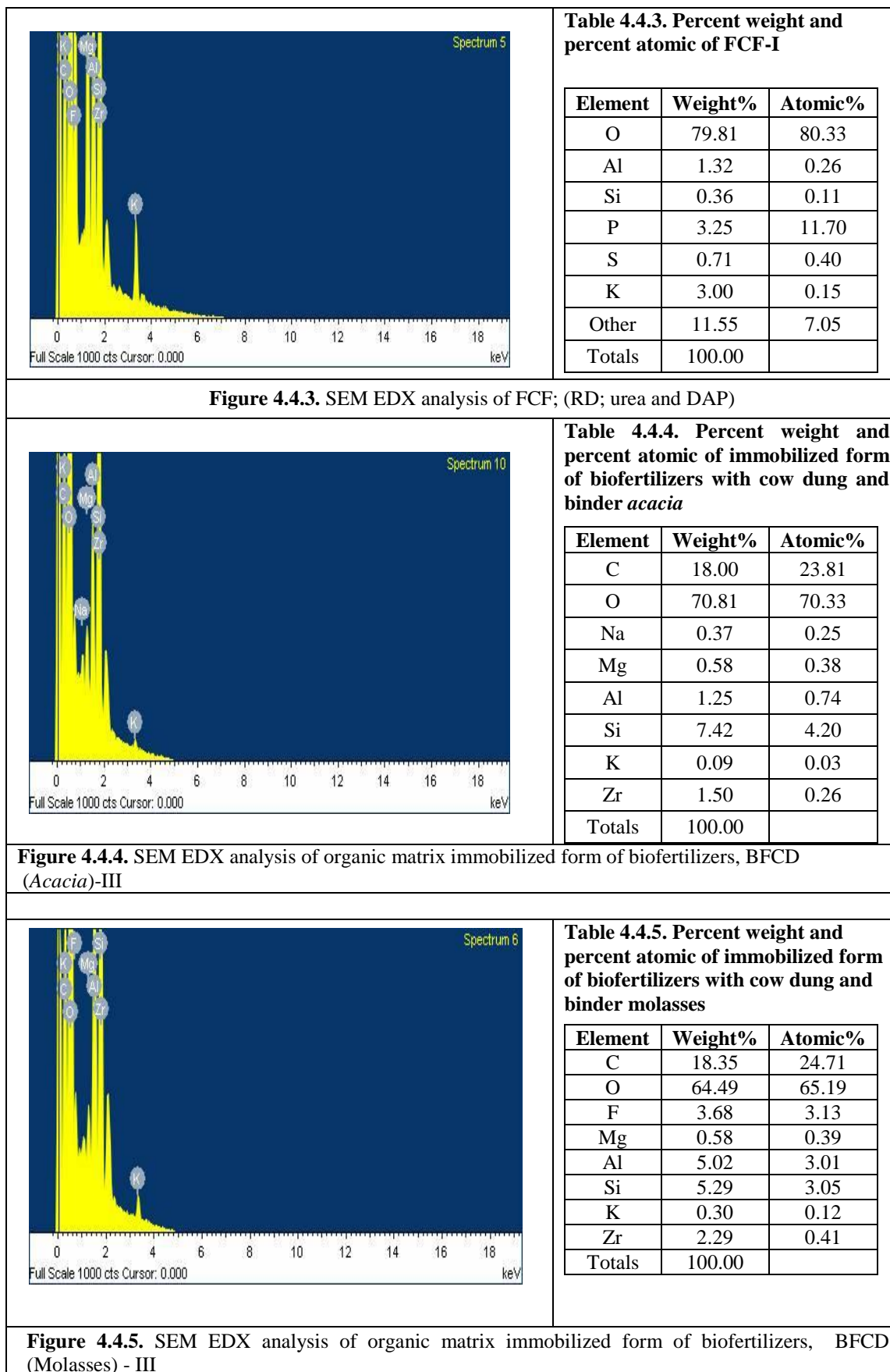
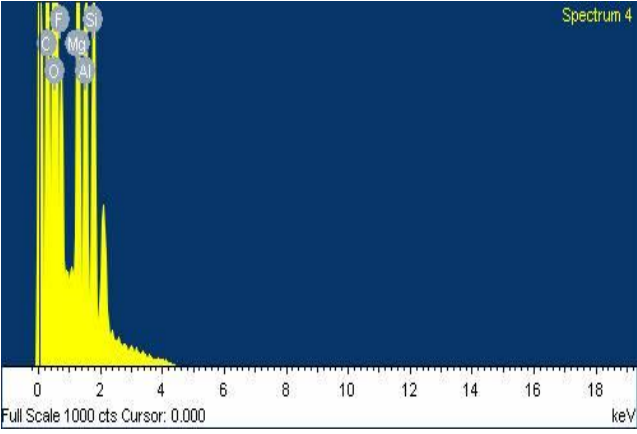
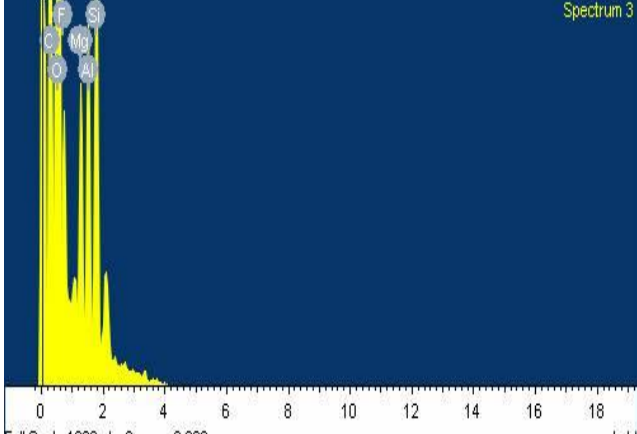
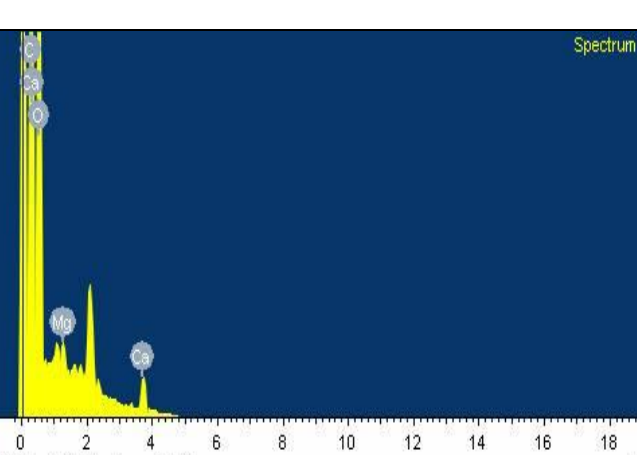
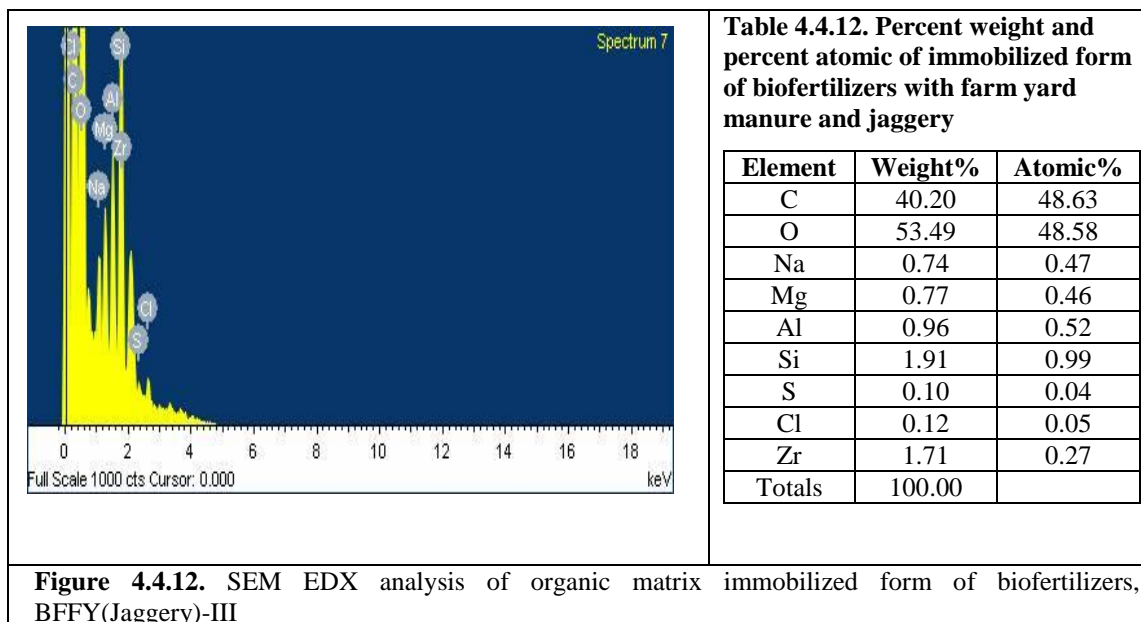


Figure 4.4.2. SEM EDX analysis of neem leaves powder



 <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.6. Percent weight and percent atomic of immobilized form of biofertilizers with cow dung and binder jaggery</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>26.60</td> <td>33.80</td> </tr> <tr> <td>O</td> <td>60.24</td> <td>57.47</td> </tr> <tr> <td>F</td> <td>4.54</td> <td>3.65</td> </tr> <tr> <td>Mg</td> <td>4.07</td> <td>2.56</td> </tr> <tr> <td>Al</td> <td>2.38</td> <td>1.34</td> </tr> <tr> <td>Si</td> <td>2.17</td> <td>1.18</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	26.60	33.80	O	60.24	57.47	F	4.54	3.65	Mg	4.07	2.56	Al	2.38	1.34	Si	2.17	1.18	Totals	100.00	
Element	Weight%	Atomic%																							
C	26.60	33.80																							
O	60.24	57.47																							
F	4.54	3.65																							
Mg	4.07	2.56																							
Al	2.38	1.34																							
Si	2.17	1.18																							
Totals	100.00																								
<p>Figure 4.4.6. SEM EDX analysis of organic matrix immobilized form of biofertilizers BFGD (Jaggery)-III</p>																									
 <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.7. Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure and binder acacia</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>29.47</td> <td>36.94</td> </tr> <tr> <td>O</td> <td>58.22</td> <td>54.78</td> </tr> <tr> <td>F</td> <td>5.82</td> <td>4.62</td> </tr> <tr> <td>Mg</td> <td>1.80</td> <td>1.12</td> </tr> <tr> <td>Al</td> <td>1.91</td> <td>1.06</td> </tr> <tr> <td>Si</td> <td>2.77</td> <td>1.49</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	29.47	36.94	O	58.22	54.78	F	5.82	4.62	Mg	1.80	1.12	Al	1.91	1.06	Si	2.77	1.49	Totals	100.00	
Element	Weight%	Atomic%																							
C	29.47	36.94																							
O	58.22	54.78																							
F	5.82	4.62																							
Mg	1.80	1.12																							
Al	1.91	1.06																							
Si	2.77	1.49																							
Totals	100.00																								
<p>Figure 4.4.7. SEM EDX analysis of organic matrix immobilized form of biofertilizers EV(Acacia)-III</p>																									
 <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.8. Percent weight and percent atomic of immobilized form of biofertilizers with vermicompost and binder molasses</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>42.80</td> <td>50.08</td> </tr> <tr> <td>O</td> <td>56.47</td> <td>49.60</td> </tr> <tr> <td>Mg</td> <td>0.28</td> <td>0.16</td> </tr> <tr> <td>Ca</td> <td>0.45</td> <td>0.16</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	42.80	50.08	O	56.47	49.60	Mg	0.28	0.16	Ca	0.45	0.16	Totals	100.00							
Element	Weight%	Atomic%																							
C	42.80	50.08																							
O	56.47	49.60																							
Mg	0.28	0.16																							
Ca	0.45	0.16																							
Totals	100.00																								
<p>Figure 4.4.8. SEM EDX analysis of organic matrix immobilized form of biofertilizers EV(Molasses)-III</p>																									

<p>Spectrum 8</p> <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.9. Percent weight and percent atomic of immobilized form of biofertilizers with vermicompost and binder jaggery</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>58.00</td> <td>65.54</td> </tr> <tr> <td>O</td> <td>39.73</td> <td>33.71</td> </tr> <tr> <td>Mg</td> <td>0.29</td> <td>0.16</td> </tr> <tr> <td>Al</td> <td>0.28</td> <td>0.14</td> </tr> <tr> <td>Si</td> <td>0.50</td> <td>0.24</td> </tr> <tr> <td>S</td> <td>0.07</td> <td>0.03</td> </tr> <tr> <td>Zr</td> <td>1.13</td> <td>0.17</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	58.00	65.54	O	39.73	33.71	Mg	0.29	0.16	Al	0.28	0.14	Si	0.50	0.24	S	0.07	0.03	Zr	1.13	0.17	Totals	100.00				
Element	Weight%	Atomic%																													
C	58.00	65.54																													
O	39.73	33.71																													
Mg	0.29	0.16																													
Al	0.28	0.14																													
Si	0.50	0.24																													
S	0.07	0.03																													
Zr	1.13	0.17																													
Totals	100.00																														
<p>Figure 4.4.9. SEM EDX analysis of organic matrix immobilized form of biofertilizers, EV(Jaggery)-III</p>																															
<p>Spectrum 9</p> <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.10. Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure and binder acacia</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>29.01</td> <td>36.72</td> </tr> <tr> <td>O</td> <td>61.92</td> <td>58.84</td> </tr> <tr> <td>Mg</td> <td>0.92</td> <td>0.57</td> </tr> <tr> <td>Al</td> <td>2.88</td> <td>1.62</td> </tr> <tr> <td>Si</td> <td>3.54</td> <td>1.91</td> </tr> <tr> <td>K</td> <td>0.20</td> <td>0.08</td> </tr> <tr> <td>Zr</td> <td>1.53</td> <td>0.26</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	29.01	36.72	O	61.92	58.84	Mg	0.92	0.57	Al	2.88	1.62	Si	3.54	1.91	K	0.20	0.08	Zr	1.53	0.26	Totals	100.00				
Element	Weight%	Atomic%																													
C	29.01	36.72																													
O	61.92	58.84																													
Mg	0.92	0.57																													
Al	2.88	1.62																													
Si	3.54	1.91																													
K	0.20	0.08																													
Zr	1.53	0.26																													
Totals	100.00																														
<p>Figure 4.4.10. SEM EDX analysis of organic matrix immobilized form of biofertilizers, BFFY(Acacia)-III</p>																															
<p>Spectrum 1</p> <p>Full Scale 1000 cts Cursor: 0.000 keV</p>	<p>Table 4.4.11. Percent weight and percent atomic of immobilized form of biofertilizers with farm yard manure use molasses as binder</p> <table border="1"> <thead> <tr> <th>Element</th> <th>Weight%</th> <th>Atomic%</th> </tr> </thead> <tbody> <tr> <td>C</td> <td>29.31</td> <td>37.30</td> </tr> <tr> <td>O</td> <td>54.35</td> <td>51.93</td> </tr> <tr> <td>F</td> <td>7.52</td> <td>6.05</td> </tr> <tr> <td>Mg</td> <td>0.83</td> <td>0.52</td> </tr> <tr> <td>Al</td> <td>2.04</td> <td>1.15</td> </tr> <tr> <td>Si</td> <td>3.13</td> <td>1.71</td> </tr> <tr> <td>P</td> <td>2.38</td> <td>1.17</td> </tr> <tr> <td>Ca</td> <td>0.44</td> <td>0.17</td> </tr> <tr> <td>Totals</td> <td>100.00</td> <td></td> </tr> </tbody> </table>	Element	Weight%	Atomic%	C	29.31	37.30	O	54.35	51.93	F	7.52	6.05	Mg	0.83	0.52	Al	2.04	1.15	Si	3.13	1.71	P	2.38	1.17	Ca	0.44	0.17	Totals	100.00	
Element	Weight%	Atomic%																													
C	29.31	37.30																													
O	54.35	51.93																													
F	7.52	6.05																													
Mg	0.83	0.52																													
Al	2.04	1.15																													
Si	3.13	1.71																													
P	2.38	1.17																													
Ca	0.44	0.17																													
Totals	100.00																														



4.4.2 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis

FTIR analysis of the organic matrix and organic matrix based granular biofertilizer was performed to evaluate the importance of various functional groups associated with the nutritional makeup of these granules. The FTIR spectra of organic matrix based granules showed strong absorption bands in the range of $2850-3000\text{ cm}^{-1}$ and $3200-3500\text{ cm}^{-1}$. The occurrence of peak band at 3375 cm^{-1} depicts the N-H bonded secondary amines in the organic matrix. The occurrence of peaks in the band region of $500-540$ is due to S-S (disulphide) weak group in granule mixtures of ECF-I, ECF-II and ECFBF. The absorption peak at $3300-3400$ are related to stretching vibration of structural OH and NH groups of granules consisting of organic matrix based urea granules, potassium dihydrogen and ammonium dihydrogen phosphate.

The qualitative analysis of super granules, using SEM EDX detection and FTIR analysis indicate that the granular biofertilizer contain higher carbon, potassium and phosphorous per unit weight as compared to commercial chemical fertilizers (Figure 4.4.13-4.4.18 and Table – 4.4.1).

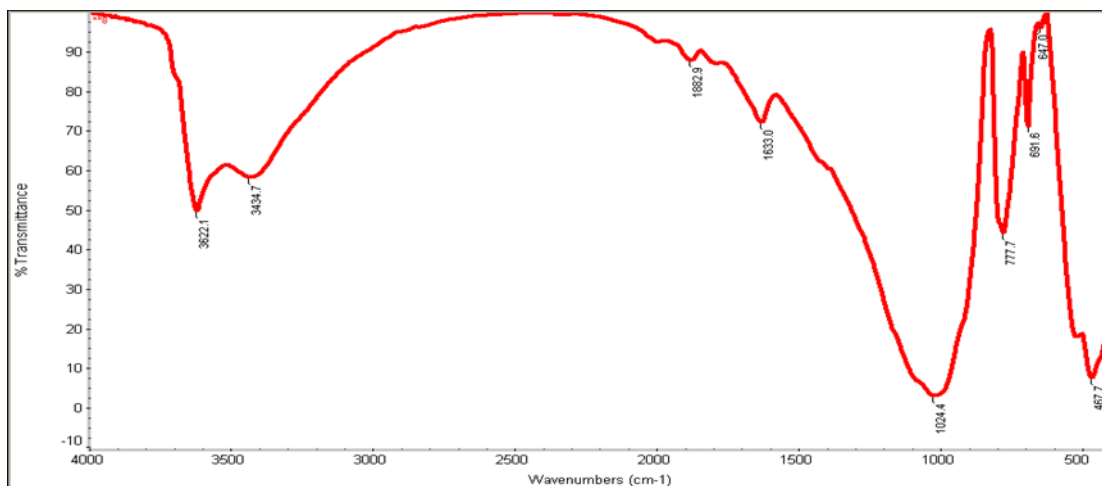


Figure 4.4.13 FTIR transmittance spectra of clay soil

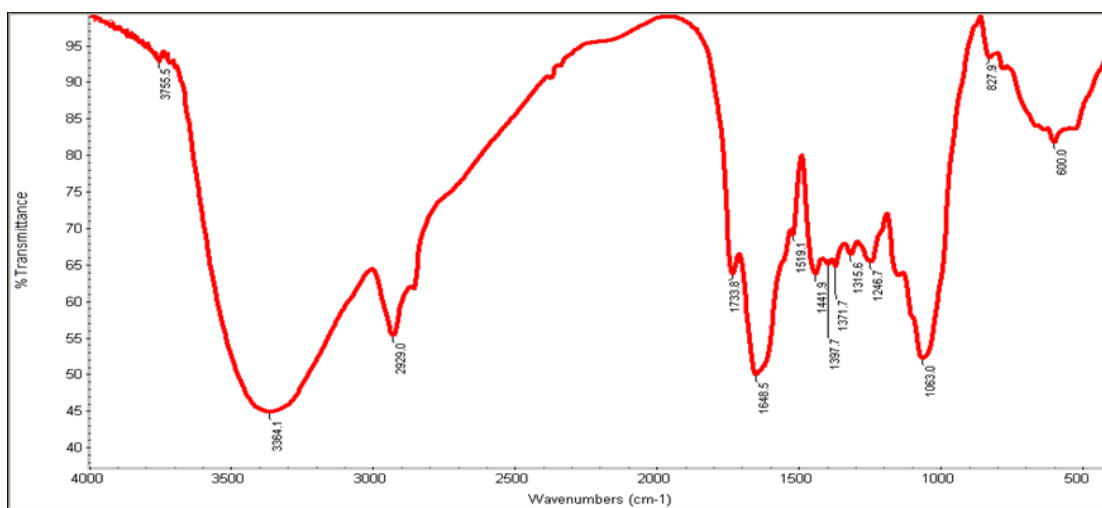


Figure 4.4.14 FTIR transmittance spectra of neem leaves powder

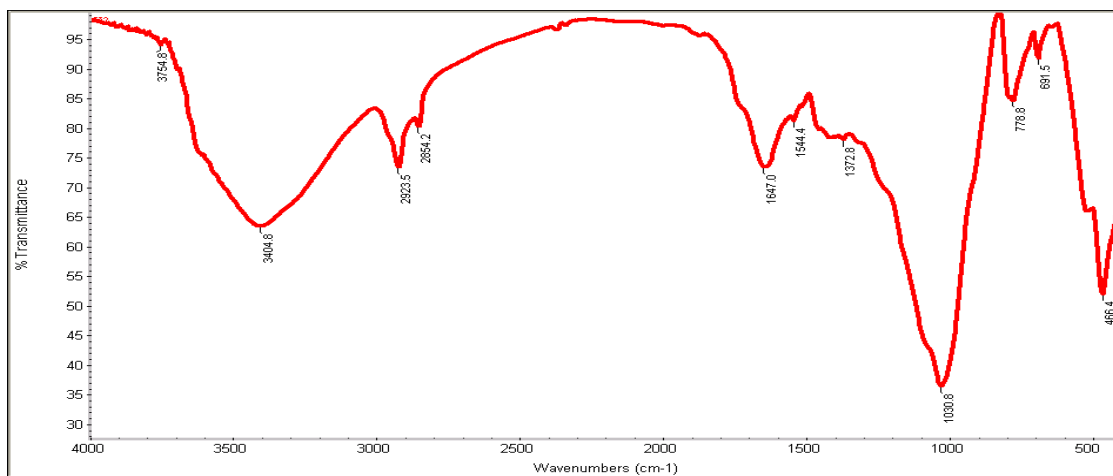


Figure 4.4.15 FTIR transmittance spectra of free form of charcoal based biofertilizer

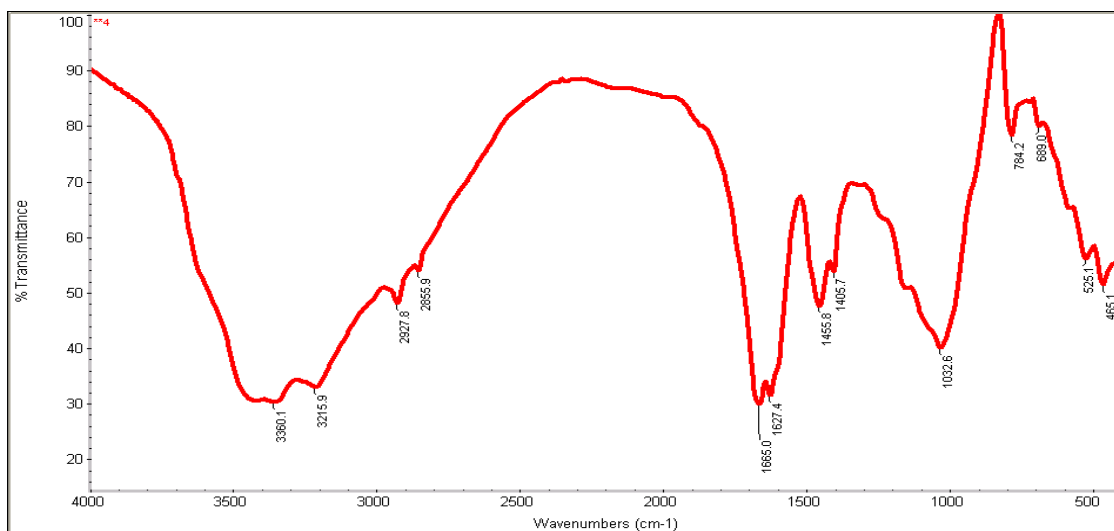


Figure 4.4.16 FTIR transmittance spectra of free form of urea and DAP

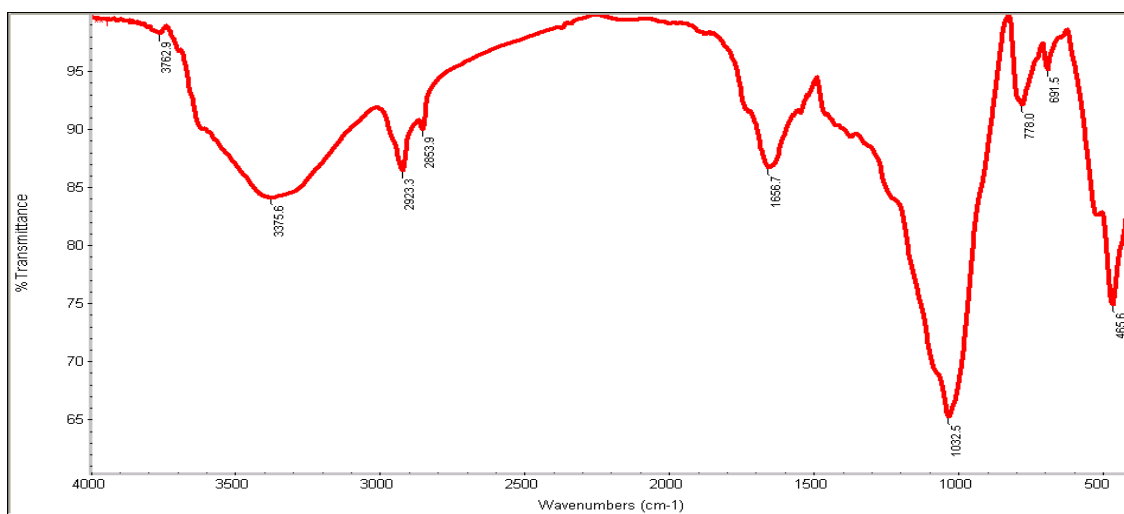


Figure 4.4.17 FTIR transmittance spectra of organic matrix immobilized form of Biofertilizers with cow-dung

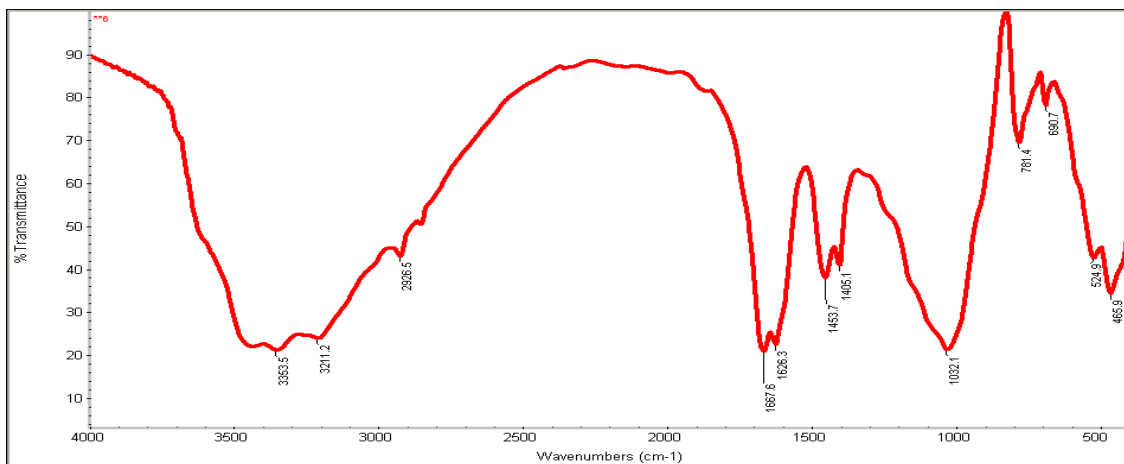


Figure 4.4.18 FTIR transmittance spectra of organic matrix immobilized form of biofertilizers with vermicompost

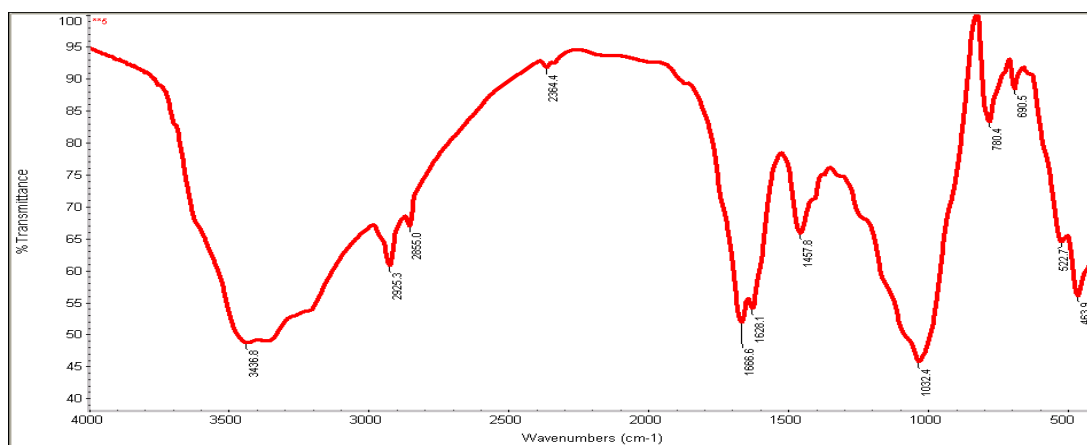


Figure 4.4.19 FTIR transmittance spectra of organic matrix immobilized form of Biofertilizers with farm yard manure

Table 4.4.13 Fourier Transform Infrared Spectrophotometer (FTIR) Analysis of Organic Matrix (clay soil, neem leaves), Conventional Chemical Fertilizer (urea and DAP) and Organic Matrix Immobilized Granular Biofertilizers (cow dung, vermicompost and farm yard manure)

Stretching frequency range (cm ⁻¹)		CS	NL	FBF-III	FCF-I	BFCDC N-III	EV-III	BFFY CN-III
500-540	S-S	-	-	-	+	-	+	+
500-600	C-Br		+	-	-	-	-	-
550-690	C-Br	+	+	-	-	-	-	-
600-700	C-H	+	+	+	+	+	+	+
600-800	C-Cl		+	+	+	+	+	+
550-850	C-Cl	+	+	+	+	-	+	+
660-900	N-H	+	+	+	+	+	+	+
690-900	C-H	+	+	+	+	+	+	+
780-850	C=C	+	+	-	+	+	+	+
880-995	=C-H, =CH ₂	+		-	-	-	-	-
970-1250	C-O	+	+	+	+	+	+	+
1000-1250	C-N	+	+	+	+	+	+	+
1000-1400	C-F	+	+	+	+	+	+	+
1350-1470	CH ₃ , CH ₂ , CH	-	+	+	+	-	+	+
1395-1440	O-H	-	+	+	+	-	+	-
1400-1450	C=O	-	+	-	+	-	+	-
1500-1560	NH ₂	-	+	+	-	-	-	-
1630-1680	C=C	+	+	+	+	+	+	+
1630-1695	C=O	+	+	+	+	+	+	+
1720-1740	C=O	-	+	-	-	-	-	-
2280-2440	P-H	-	-	-	+	-	-	-

2850-3000	CH ₃ ,CH ₂ , CH	-	+	+	+	+	+	+
2500-3300	O-H	+	+	+	+	+	+	+
3200-3550	O-H	+	+	+	+	+	+	+
3300-3400	N-H	-	+	-	+	+	+	-
3400-3500	N-H	+	-	+		-	+	+
3400-3650	O-H	+		+	-	+	+	+
3580-3650	O-H	+	-	-	-	-	-	-

(Where, CL- clay soil, NL- neem leaf, FBF- Free form of charcoal based consortium of biofertilizers (*A. chroococum* and *B. subtilis*), FCF-I- Free form of recommended dose of urea; 150 kg ha⁻¹ and DAP; 75 kg ha⁻¹), BFCDCN-III- immobilized form of organic matrix (combination of clay soil+ cow dung+ neem leaf) and charcoal based consortium of biofertilizers (*A. chroococum* and *B.s subtilis*), EV-III- immobilized form of organic matrix(combination of clay soil+ vermicompost+ neem leaf) and charcoal based consortium of biofertilizers (*A. chroococum* and *B. subtilis*), BFFYCN-III- immobilized form of (combination of clay soil+ farm yard manure+ neem leaf) and charcoal based consortium of biofertilizer (*A. chroococum* and *B. subtilis*).

4.4.3 Microbial Growth and Biochemical Properties in Organic Matrix Immobilized Super Granules

Microbial growth (total bacterial count; *Azotobacter* and *Bacillus*) counted in organic matrix immobilized with biofertilizers i.e. *A. chroococum* and *B. subtilis* were measured using colony forming count (cfu) technique. The study indicate that organic matrix contain 15 to 20 cfu g⁻¹ bacteria g⁻¹ of granules, whereas after addition of *Azotobacter* and *Bacillus* the bacterial count increased by two to four fold in various organic matrix immobilized biofertilizers. Eight to ten cfu g⁻¹ granule material *Azotobacter* and *Bacillus* was present in organic matrix without any amendment of microbes which increased by about four fold for *Azotobacter* and *Bacillus* count when vermicompost used as organic carrier for making super granules. The addition of commercial preparation of *Azotobacter* and *Bacillus* increased their population very significantly (upto about four folds) which was highest in the presence of organic matrix. It appears that presence of organic nutrients in the granules increased the population of bacteria in general and *Azotobacter* and *Bacillus* in particular (Table 4.4.14).

In this study various combinations of super granules were prepared in which the super granule of BFVCN contained highest growth of bacterial population by 78.28, 13.67 and 7.30% over soil (control), BFCDCN and BFFYCN. On super granule of

BFFYCN increased percentage by 76.96, 7.27 and 16.36 over soil (control), BFCDCN and BFC. BFCDCN was also increased the percentage by 74.73% over soil (control). Amendment of organic matrix as carrier in granules significantly enhanced the population of the microbial growth.

Nitrate content in this granules also recorded significantly by BFVCN was 72.22, 3.98 and 6.21 over soil (control), BFCDCN and BFFYCN. Similarly phosphate content by BFVCN was 64.49, 14.83 and 24.49% over soil (control), BFCDCN and BFFYCN on freshly prepared granule. The all biofertilizer immobilized organic matrix in form of super granules contain high level of nutrients content in it.

Table 4.4.14 Bacterial Colonies in Organic Matrix Immobilized Super Granules, Soil and Biochemical Properties

Treatments (granules)	10^{-4} (cfu g ⁻¹) of granules	Nitrate conc. µg/g of granules	Phosphate conc. µg/g of granules
Soil(control)	38.66±3.05 ^a	22.42±1.31 ^a	0.157±0.02 ^a
BFCDCN	153.66±9.86 ^{bc}	75.71±0.41 ⁱ	0.379±0.02 ^e
BFCDN	144.66±4.93 ^{bc}	46.52±2.12 ^g	0.337±0.01 ^{bcd}
BFCDC	148.66±8.73 ^{bc}	54.18±0.27 ^e	0.353±0.02 ^{bcd}
BFCD	150.33±21.36 ^{bc}	28.73±0.39 ^c	0.317±0.02 ^b
BFVCN	178.00±17.57 ^c	80.73±1.41 ^k	0.445±0.00 ^f
BFVN	158.00±25.23 ^{bc}	50.12±0.55 ^h	0.360±0.01 ^{cde}
BFVC	161.00±13.11 ^{bc}	60.52±0.41 ^f	0.382±0.02 ^e
BFV	174.33±24.37 ^{bc}	34.07±1.59 ^d	0.336±0.00 ^{bcd}
BFFYCN	165.00±44.30 ^{bc}	77.51±0.78 ^j	0.366±0.03 ^{de}
BFFYN	154.00±8.18 ^{bc}	48.94±0.53 ^h	0.349±0.02 ^{bcd}
BFFYC	157.33±29.02 ^{bc}	59.10±0.26 ^f	0.370±0.01 ^{de}
BFFY	163.00±7.93 ^{bc}	30.36±0.27 ^c	0.333±0.01 ^{bcd}
BFNC	131.33±8.73 ^b	23.33±1.24 ^a	0.318±0.01 ^b
BFC	138.66±51.47 ^{bc}	26.24±1.74 ^b	0.328±0.01 ^{bc}

Note: The biofertilizers immobilized with organic matrix (cow dung, vermicompost and FYM, clay soil, neem leaves powder and organic binder *acacia* gum) in granular form. Counted microbial growth (*Azotobacter* and *Bacillus*) used colony forming count (cfu) technique. The vermicompost immobilized biofertilizer shows the highest microbial growth in compare with FYM and cow dung immobilized biofertilizers. One way ANOVA is performed to compare the means of different treatments at p<0.05. Values by different letters are significantly differences between the treatments at 5% level. (Other details are described in Table. 4.5.1)

4.4.4. Microbial Growth of Super Granules at Storage Condition and Different Temperature

Microbial growth was counted in organic matrix immobilized with biofertilizers (*A. chroococcum* and *B. subtilis*) were measured using colony forming count (cfu) technique at storage condition. The super granules were stored in different temperature to check the growth of microbial population at 30 days of time intervals. In super granules which contains nutrients for growth of microbial population significantly. For formulation of super granules used various organic carriers such as cow dung, vermicompost, FYM, clay soil, neem leaf powder and organic binders *acacia* gum, molasses and jaggery. The immobilized form of fertilizer used *acacia* gum as binders showed the best formulation at storage condition in comparisons with molasses and jaggery.

The percentage increases of microbial growth by prepared granules significantly at storage in different temperature. At 4⁰C the granule of BFVJ-III increased percentage by 4.41% over BFVS-III. BFVM-III increased percentage was 12.16% over BFVS-III. But at 34⁰C temperature BFVJ-III increased percentage by 31.03 and 13.79% over BFVS-III and BFVM-III respectively. After 30 days of prepared granules at 45⁰C temperature increased percentage of microbial growth by 24.59 and 13.93 respectively on BFVJ-III over BFVS-III and BFVM-III.

After 90 days of prepared granules at 4⁰C temperature increased percentage of microbial growth by 7.25 and 5.64 on BFVJ-III over BFVS-III and BFVM-III respectively. At 34⁰C temperature BFVJ-III was increased percentage by 14.16% over BFVS-III. At 45⁰C temperature increased percentage of microbial growth by 3.90 and 3.22 respectively on BFVJ-III over BFVS-III and BFVM-III. Similarly other granules also increased the microbial growth during the storage condition.

Table-4.4.15 Microbial Counting in Various Organic Matrix Based Granular Biofertilizer at Storage Condition and Temperature

Treatments	cfu /g ⁻¹ at 30 DAY			cfu / g ⁻¹ at 90 DAY		
	Temperature					
	4 ⁰ C	34 ⁰ C	45 ⁰ C	4 ⁰ C	34 ⁰ C	45 ⁰ C
BFVS-II	53±8 ^{bcdefg}	67±4 ^{bc}	80±9 ^{abc}	108±4 ^{cd}	97±3 ^{abcde}	114±7 ^{bcd}
BFFYS-II	56±10 ^{cdef}	63±7 ^b	77±5 ^{ab}	102±6 ^{bcd}	91±9 ^{abc}	105±4 ^a
BFCDS-II	41±8 ^{abc}	49±6 ^a	68±6 ^a	104±7 ^{bcd}	90±6 ^{ab}	112±7 ^{bcd}
BFVS-III	65±8 ^{efgh}	80±7 ^{def}	92±8 ^{cdef}	115±5 ^{efg}	103±5 ^{cdef}	123±7 ^{fghi}
BFFYS-III	56±6 ^{bcdefg}	73±6 ^{bcde}	87±4 ^{bcde}	114±5 ^{efg}	103±7 ^{def}	118±4 ^{def}
BFCDS-III	43±6 ^{abc}	69±8 ^{bcd}	84±5 ^{bcd}	109±6 ^{cd}	97±4 ^{abcde}	116±4 ^{cde}
BFVS-V	40±6 ^{ab}	85±7 ^{efg}	100±9 ^{efg}	116±7 ^{efgh}	108±7 ^{efg}	116±7 ^{cde}
BFVM-II	65±12 ^{fgh}	78±9 ^{cdef}	84±9 ^{bcd}	97±7 ^a	89±8 ^a	110±7 ^{bcd}
BFFYM-II	55±8 ^{bcdefg}	70±8 ^{bcd}	87±9 ^{bcde}	104±9 ^{bcd}	94±5 ^{abcd}	113±7 ^{bcd}
BFCDM-II	35±7 ^a	76±4 ^{cdef}	92±3 ^{cdef}	111±6 ^{efg}	102±5 ^{cdef}	118±5 ^{def}
BFVM-III	74±9 ^h	100±8 ^{hi}	105±6 ^{ghi}	117±6 ^{fghi}	120±10 ^{hi}	124±6 ^{ghij}
BFFYM-III	59±9 ^{defg}	89±6 ^{fgh}	102±4 ^{fg}	115±7 ^{efg}	110±8 ^{fgh}	122±6 ^{fghi}
BFCDM-III	54±8 ^{bcdefg}	96±8 ^{ghi}	104±7 ^{fgh}	120±6 ^{hi}	113±6 ^{fghi}	119±4 ^{efgh}
BFVM-IV	51±9 ^{bcdef}	106±7 ^{ij}	116±7 ^{hij}	121±4 ⁱ	124±7 ⁱ	136±7 ^j
BFVJ-II	53±9 ^{bcdef}	87±8 ^{fg}	93±5 ^{defg}	108±5 ^{cd}	101±5 ^{bcdef}	119±3 ^{efgh}
BFFYJ-II	42±5 ^{abc}	103±7 ^{ij}	97±7 ^{defg}	112±5 ^{efg}	103±8 ^{cdef}	114±5 ^{bcd}
BFCDJ-II	41±8 ^{abc}	82±6 ^{def}	88±4 ^{bcde}	103±5 ^{bcd}	97±5 ^{abcde}	110±5 ^{bcd}
BFVJ-III	68±6 ^{gh}	116±9 ^j	122±6 ^j	124±7 ^{ij}	120±5 ^{hi}	128±6 ^{ij}
BFFYJ-III	50±6 ^{abcde}	105±7 ^{ij}	121±9 ^j	117±4 ^{fghi}	118±5 ^{ghi}	120±5 ^{fghi}
BFCDJ-III	45±7 ^{abcd}	106±8 ^{ij}	117±10 ^{ij}	119±9 ^{ghi}	105±7 ^{def}	124±7 ^{ghij}
BFVJ-IV	47±8 ^{abcd}	114±9 ^j	116±7 ^{hij}	129±3 ^k	124±7 ⁱ	136±6 ^j

Note: Microbial counting in various organic matrix based granular biofertilizers. Counted microbial growth (*Azotobacter* and *Bacillus*) used colony forming count (cfu) technique (cfu count at *10⁻⁴ Dilution Factor). Bacterial colony in growth culture was determined by serial dilutions of the suspension. [Where **BFVS-II**; Twofold of biofertilizers immobilized in vermicompost used saresh as binder, **BFFYS-II**; Twofold of biofertilizers immobilized in FYM used saresh as binder, **BFCDS-II**; Twofold of biofertilizers immobilized in cow dung, used saresh as binder, **BFVS-III**; Threefold of biofertilizers immobilized in vermicompost used saresh as binder, **BFFYS-III**; Threefold of biofertilizers immobilized in FYM used saresh as binder, **BFCDS-III**; Threefold of biofertilizers immobilized in cow dung used saresh as binder, **BFVS-V**; Fivefold of biofertilizers immobilized in vermicompost used saresh as binder, **BFVM-II**; Twofold of biofertilizers immobilized in vermicompost molasses, **BFFYM-II**; Twofold of biofertilizers immobilized in FYM used molasses as binder, **BFCDM-II**; Twofold of biofertilizers immobilized in cow dung used molasses as binder, **BFVM-III**; Threefold of biofertilizers immobilized in vermicompost used molasses as binder, **BFFYM-III**; Threefold of biofertilizers immobilized in FYM used molasses as binder, **BFCDM-III**; Threefold of biofertilizers immobilized in cow dung used molasses as binder, **BFVM-IV**; Fourfold of biofertilizers immobilized in vermicompost used molasses as binder, **BFVJ-II**; Twofold of biofertilizers immobilized in vermicompost used jaggery as binder, **BFFYJ-II**; Twofold of biofertilizers immobilized in FYM used jaggery as binder, **BFCDJ-II**; Twofold of biofertilizers immobilized in cow dung used jaggery as binder, **BFVJ-III**; Threefold of biofertilizers immobilized in vermicompost used jaggery as binder, **BFFYJ-III**; Threefold of biofertilizers immobilized in FYM used jaggery as binder, **BFCDJ-III**; Threefold of biofertilizers immobilized in cow dung used jaggery as binder, **BFVJ-IV**; Fourfold of biofertilizers immobilized in vermicompost used jaggery as binder]

4.5. Growth and Productivity of Wheat (*T. aestivum* L. cv. PBW-502) Applied with Charcoal Based Biofertilizers (*A. chroococcum* and *B. subtilis*) Immobilized with Different Organic Matrix with Enhanced Dose (triple dose) on 30, 60, 90 and 120 days After Sowing (DAS)

4.5.1 Growth Parameters of Wheat Applied with Optimised Dose of Biofertilizers Immobilized with Organic Matrix analysed on 30, 60, 90 and 120 (DAS)

The optimized dose of biofertilizers (*A. chroococcum*- 1.80 kg ha⁻¹ and *B. subtilis*- 1.80 kg ha⁻¹) has increases the plant growth over free form of biofertilizers and seed coated form of biofertilizers when applied as immobilized form with organic matrix. The plant height of BFVCN application was 29.15, 7.05, 20.97, 5.43, 11.49, 8.29, 14.92, 5.51, 2.81, 9.23, 2.35, 10.08, 7.57, 13.08, 9.63 and 4.28% at 90 DAS and 30.82, 10.86, 20.29, 8.09, 12.44, 12.16, 18.61, 9.92, 7.04, 16.51, 5.29, 11.01, 9.55, 17.13, 15.60 and 8.83% at 120 DAS over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Table 4.5.1).

On application of BFVCN the number of plant leaves recorded significantly by 66.67, 13.73, 36.27, 8.82, 26.47, 24.51, 39.22, 11.76, 12.75, 22.55, 1.96, 10.78, 9.80, 18.63, 17.65, and 31.37% at 90 DAS over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Table 4.5.1).

Number of tillers was increased by 60, 6.67, 33.33, 6.67, 13.33, 6.67, 13.33, 20, 6.67 and 20% at 60 DAS and 71.43, 14.29, 38.10, 4.76, 4.76, , 14.29, , 23.81, 19.05, 14.29 and 19.05% at 120 DAS over NF, CF-RD, FBF, BFCDCN, BFCD, BFVN, BFV, BFFYN, BFFYC and BFFY respectively on application of BFVCN (Table 4.5.1).

The optimized dose of biofertilizers was increased the fresh weight of wheat plant. The percentage of fresh weight of shoot was increased by 70.94, 22.41, 47.37, 19.38, 28.61, 33.17, 38, 26.88, 31.64, 35.12, 4.49, 27.58, 32.18, 35.97, 39.35 and 44.57% at 60 DAS and 73.53, 14.21, 50.90, 7.41, 27.49, 31.41, 36.67, 18.43, 28.55, 31.71, 4.01, 28.14, 30.64, 34.21, 45.11 and 49.85% at 120 DAS respectively over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC on application of the BFVCN (Table 4.5.1).

The charcoal based biofertilizers has significantly found the dry weight of plant production. Dry weight of plant shoot increased by 71.60, 20.26, 46.52, 14.68, 18.10,

24, 31.24, 9.16, 15.86, 23.26, 5.92, 14.15, 19.62, 26.47, 39.78 and 45.14% at 60 DAS and 69.68, 14.38, 51.29, 16.76, 20.21, 23.69, 29.97, 17.21, 21.04, 23.63, 11.57, 20.86, 24.91, 26.92, 44.38 and 49.79% at 120 DAS respectively on application of BFVCN was significantly recorded over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFC, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Table 4.5.1).

Table 4.5.1 Effect of Organic Matrix Immobilized Biofertilizers (*A. chroococcum* and *B. subtilis*) on Plant Growth of Wheat (*T. aestivum* L. cv. PBW-502) Shoot on 30, 60, 90 and 120 DAS

Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Root Height				
NF	2.33±0.27 ^a	3.30±0.42 ^a	4.82±0.62 ^a	5.85±0.54 ^a
CF-RD	2.45±0.37 ^a	5.63±0.31 ^b	7.80±0.52 ^b	8.52±0.33 ^b
FB-3	2.52±0.34 ^a	6.95±0.58 ^{cd}	8.12±0.36 ^{bc}	8.75±0.19 ^{bc}
BFCDCN	2.32±0.29 ^a	7.50±0.52 ^{cde}	9.30±1.00 ^{defg}	10.07±0.73 ^{defg}
BFCDN	2.25±0.25 ^a	7.37±0.24 ^{cde}	8.45±0.31 ^{cdefg}	9.65±0.62 ^{defg}
BFCDC	2.47±0.22 ^a	7.02±0.42 ^{cde}	8.17±0.80 ^{cdef}	9.53±0.34 ^{cde}
BFC	2.42±0.28 ^a	6.88±0.32 ^{bc}	10.38±0.51 ^{cdef}	9.12±0.61 ^{cd}
BFVNC	2.42±0.39 ^a	7.97±0.55 ^{de}	10.62±0.54 ⁱ	10.72±0.78 ^h
BFVN	2.50±0.30 ^a	7.85±0.48 ^{cde}	9.48±0.69 ^{hi}	9.92±0.56 ^{efg}
BFVC	2.53±0.32 ^a	7.48±0.51 ^{de}	8.97±0.18 ^{cde}	9.62±0.33 ^{defg}
BFV	2.55±0.48 ^a	7.17±0.87 ^{cde}	8.88±0.68 ^{defg}	9.28±0.31 ^{cdef}
BFFYCN	2.43±0.27 ^a	7.85±0.81 ^{cde}	9.43±0.67 ^{ghi}	10.13±0.63 ^{fg}
BFFYN	2.53±0.37 ^a	7.75±0.34 ^{cd}	9.27±0.39 ^{efg}	9.77±0.30 ^{def}
BFFYC	2.40±0.25 ^a	7.53±0.53 ^{cd}	8.75±0.67 ^{defg}	9.52±0.25 ^{cde}
BFFY	2.43±0.30 ^a	7.35±0.65 ^{cde}	8.55±0.42 ^{cdefg}	9.23±0.33 ^{cd}
BFCN	2.63±0.46 ^a	7.28±0.55 ^c	9.10±0.44 ^{ef}	9.39±0.41 ^{cd}
BFC	2.53±0.33 ^a	7.2±0.60 ^c	9.47±0.72 ^{efgh}	9.75±0.45 ^{defg}
No. of Roots				
NF	3.67±0.82 ^a	7.17±1.17 ^a	8.83±0.75 ^a	10.00±0.89 ^a
CF-RD	4.00±0.89 ^a	15.00±0.89 ^{cd}	19.67±1.63 ^{efg}	22.83±2.04 ^{def}
FB-3	3.67±0.52 ^a	11.83±1.33 ^{cd}	13.67±1.21 ^{bc}	17.67±1.63 ^{bcde}
BFCDCN	3.50±0.55 ^a	13.67±2.94 ^{def}	19.00±3.29 ^{efg}	20.33±3.27 ^{de}
BFCDN	4.00±0.63 ^a	10.50±2.17 ^{bcd}	17.33±1.97 ^{efg}	18.50±2.17 ^{cde}
BFCDC	3.61±0.49 ^a	9.33±1.75 ^{bc}	16.83±2.14 ^{def}	18.50±1.87 ^{cde}
BFC	3.50±0.55 ^a	8.50±1.05 ^a	15.17±0.75 ^{de}	16.67±1.63 ^{bcd}
BFVNC	3.83±0.75 ^a	18.83±1.33 ^{ghi}	25.67±2.66 ⁱ	30.17±2.99 ^{fgh}
BFVN	3.50±1.38 ^a	14.17±1.83 ^{efg}	23.17±2.04 ^{hi}	28.00±1.41 ^{fg}
BFVC	3.83±0.75 ^a	11.50±2.95 ^a	21.33±1.97 ^{ghi}	24.50±2.35 ^{efg}
BFV	4.28±0.77 ^a	9.94±2.34 ^{bc}	19.67±1.37 ^{ghi}	18.17±1.83 ^{cde}
BFFYCN	3.50±0.55 ^a	15.17±2.99 ^{fgh}	20.33±3.14 ^{ghi}	21.50±1.76 ^{de}

BFFYN	3.39±0.49 ^a	12.50±2.26 ^{cde}	18.83±1.72 ^{fg}	18.50±1.38 ^{cde}
BFFYC	3.67±1.51 ^a	11.00±1.79 ^{cd}	18.67±2.16 ^{fg}	18.33±1.63 ^{cde}
BFFY	3.44±0.50 ^a	9.50±1.52 ^{bc}	17.33±1.97 ^{ef}	17.50±1.76 ^{bcd}
BFCN	3.50±0.84 ^a	13.33±1.51 ^{def}	18.33±1.63 ^{fg}	22.33±1.75 ^{def}
BFC	3.67±0.52 ^a	10.67±1.21 ^{bcd}	14.67±1.51 ^{cde}	20.33±2.42 ^{de}
Fresh wt. of Root				
NF	0.14±0.04 ^a	0.22±0.02 ^a	0.29±0.02 ^a	0.35±0.02 ^a
CF-RD	0.17±0.01 ^a	0.45±0.04 ^{def}	0.60±0.02 ^{ef}	0.68±0.03 ^{ef}
FB-3	0.13±0.01 ^a	0.29±0.01 ^{ab}	0.33±0.03 ^{bcd}	0.39±0.01 ^{bc}
BFCDCN	0.14±0.01 ^a	0.43±0.05 ^{de}	0.56±0.03 ^{def}	0.65±0.05 ^{ef}
BFCDN	0.13±0.01 ^a	0.38±0.02 ^{bcd}	0.54±0.03 ^{def}	0.61±0.04 ^{def}
BFCDC	0.13±0.01 ^a	0.36±0.03 ^{bc}	0.53±0.03 ^{def}	0.60±0.03 ^{def}
BFCD	0.13±0.01 ^a	0.35±0.03 ^{bc}	0.51±0.03 ^{de}	0.58±0.03 ^{de}
BFVNC	0.16±0.01 ^a	0.58±0.02 ^{fg}	0.69±0.02 ^a	0.76±0.03 ^{gh}
BFVN	0.14±0.01 ^a	0.52±0.01 ^{defg}	0.63±0.05 ^a	0.72±0.02 ^{fg}
BFVC	0.14±0.01 ^a	0.49±0.01 ^{def}	0.61±0.04 ^{ef}	0.69±0.03 ^{ef}
BFV	0.14±0.01 ^a	0.47±0.04 ^{def}	0.57±0.03 ^{de}	0.67±0.03 ^{ef}
BFFYCN	0.14±0.01 ^a	0.52±0.02 ^{defg}	0.64±0.03 ^{efg}	0.72±0.03 ^{fg}
BFFYN	0.14±0.01 ^a	0.52±0.01 ^{defg}	0.62±0.04 ^{efg}	0.69±0.01 ^{ef}
BFFYC	0.14±0.01 ^a	0.51±0.01 ^{defg}	0.59±0.02 ^a	0.67±0.03 ^{ef}
BFFY	0.14±0.01 ^a	0.48±0.03 ^{def}	0.57±0.02 ^a	0.61±0.02 ^{def}
BFCN	0.14±0.01 ^a	0.31±0.02 ^{bc}	0.37±0.02 ^{bcd}	0.42±0.01 ^{bc}
BFC	0.13±0.01 ^a	0.28±0.03 ^{ab}	0.34±0.02 ^{bcd}	0.39±0.03 ^b
Dry wt. of Root				
NF	0.04±0.01 ^a	0.06±0.01 ^a	0.07±0.01 ^a	0.08±0.01 ^a
CF-RD	0.04±0.01 ^a	0.12±0.01 ^{ef}	0.15±0.01 ^{fg}	0.15±0.01 ^{ef}
FB-3	0.03±0.00 ^a	0.07±0.01 ^{bcd}	0.08±0.01 ^{bc}	0.11±0.01 ^{cde}
BFCDCN	0.04±0.00 ^a	0.10±0.01 ^{dc}	0.15±0.01 ^{fg}	0.14±0.02 ^{def}
BFCDN	0.04±0.01 ^a	0.09±0.01 ^d	0.14±0.01 ^{fg}	0.13±0.01 ^{de}
BFCDC	0.04±0.00 ^a	0.09±0.00 ^d	0.12±0.01 ^{de}	0.13±0.00 ^{de}
BFCD	0.03±0.00 ^a	0.09±0.00 ^d	0.11±0.01 ^{de}	0.11±0.01 ^{cde}
BFVNC	0.04±0.01 ^a	0.15±0.01 ^g	0.17±0.01 ^{gf}	0.18±0.01 ^{hi}
BFVN	0.03±0.01 ^a	0.13±0.01 ^f	0.16±0.01 ^g	0.16±0.01 ^{efg}
BFVC	0.03±0.00 ^a	0.10±0.01 ^{de}	0.16±0.01 ^g	0.14±0.01 ^a
BFV	0.04±0.01 ^a	0.10±0.01 ^{de}	0.15±0.01 ^{fg}	0.13±0.01 ^{de}
BFFYCN	0.04±0.01 ^a	0.13±0.02 ^f	0.16±0.01 ^g	0.16±0.01 ^{efg}
BFFYN	0.03±0.01 ^a	0.10±0.01 ^{de}	0.16±0.00 ^g	0.15±0.01 ^{ef}
BFFYC	0.04±0.00 ^a	0.10±0.01 ^{de}	0.15±0.01 ^{fg}	0.14±0.01 ^{def}
BFFY	0.03±0.01 ^a	0.09±0.01 ^d	0.14±0.01 ^{fg}	0.14±0.01 ^{def}
BFCN	0.03±0.00 ^a	0.08±0.01 ^{cd}	0.09±0.01 ^c	0.11±0.01 ^{cde}
BFC	0.03±0.00 ^a	0.07±0.01 ^{bcd}	0.08±0.01 ^{bc}	0.09±0.01 ^{bc}

[Data are represented by mean of six individual measurement ± SD. One way analysis of variance was used to compare the means. Values followed by different letters are significantly different at P < 0.05.]

The root length of wheat plant increased on application of BFVCN was 58.58, 29.29, 12.76, 5.86, 7.53, 11.92, 13.60, 1.46, 6.07, 10.04, 1.46, 2.72, 5.44 and 7.74% on 60 DAS and 54.63, 26.53, 23.55, 12.39, 20.41, 23.08, 2.20, 10.68, 15.54, 16.33, 11.15, 12.72, 17.58 and 19.47% 120 DAS over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC and BFFY respectively (Table 4.5.2)

Number of plant roots recorded significantly by on application of BFVCN was 61.95, 20.35, 27.43, 44.25, 50.44 and 33.63% and 66.85, 24.31, 2.60, 38.67, 38.67 and 38.67%, at 60 and 120 DAS respectively over NF, CF-RD, BFCDCN, BFCDN, BFCDC and BFFYN (Table 4.5.2).

The percentage of fresh weight of roots increased on application of BFVCN was 63.05, 22.30, 50.82, 26.05, 39.41, 19.60 and 17.29% and 54.13, 11.11, 49.21, 14.43, 23.48, 12.46 and 19.82% at 60 and 120 DAS and 74.79, 15.13, 2.52, 18.49, 20.17 and 25.21 at 120 DAS over NF, CF-RD, FB-3, BFCDCN, BFCD, BFV and BFFY respectively (Table 4.5.2).

The percentage of dry weight of roots increased by 60.60, 21.24, 55.42, 14.05, 29.75 and 33.25%, 60.75, 14.60, 54.75, 3.66, 6.62 and 10.14% and 54.96, 15.14, 42.10, 10.36, 21.16 and 27.65% at 60, 90 and 120 DAS respectively on application of BFVCN over NF, CF-RD, FB-3, BFVN, BFVC, and BFV (Table 4.5.2).

Table 4.5.2 - Effect of Organic Matrix Immobilized Biofertilizer on Growth of Wheat (*T. aestivum* L. cv. PBW-502) Shoots on 30, 60, 90 and 120 DAS

Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Plant Height				
NF	21.38±1.92 ^a	37.22±3.44 ^a	44.15±3.34 ^a	48.18±1.91 ^a
CF-RD	21.48±2.05 ^a	50.60±1.41 ^{de}	57.93±1.48 ^{cde}	62.08±2.17 ^{def}
FB-3	20.05±1.82 ^a	43.59±1.54 ^{bcd}	49.25±1.44 ^{bc}	55.52±2.31 ^{bcd}
BFCDCN	20.02±1.45 ^a	50.85±2.30 ^{de}	58.93±1.19 ^{ef}	64.02±2.52 ^{defg}
BFCDN	20.92±1.07 ^a	47.02±0.90 ^{bcd}	55.16±2.50 ^{de}	60.99±1.64 ^a
BFCDC	21.95±1.57 ^{aa}	47.60±1.32 ^{bcd}	57.15±1.59 ^{cde}	61.18±1.07 ^{def}
BFCD	21.60±1.99 ^a	45.63±1.03 ^{bcd}	53.02±2.46 ^{cde}	56.69±0.83 ^{bcd}
BFVNC	20.07±2.01 ^a	54.15±1.35 ^a	62.32±2.63 ^{efg}	69.65±4.46 ^{efg}
BFVN	21.97±1.75 ^a	47.50±1.17 ^{bcd}	58.88±4.18 ^{ef}	62.74±0.99 ^{def}
BFVC	22.20±1.31 ^b	48.57±0.90 ^{cd}	60.57±3.60 ^{efg}	64.75±1.71 ^{defg}

BFV	22.17±1.66 ^b	46.05±1.21 ^{bcd}	56.57±2.05 ^{de}	58.15±1.59 ^{cde}
BFFYCN	20.33±1.99 ^a	52.82±2.47 ^{de}	60.85±2.37 ^a	65.97±2.48 ^{defg}
BFFYN	19.95±1.21 ^a	47.25±1.16 ^{bcd}	56.03±1.95 ^{de}	61.98±1.17 ^{def}
BFFYC	20.58±1.40 ^a	47.47±1.16 ^{bcd}	57.60±1.62 ^{de}	63.00±2.24 ^{def}
BFFY	20.90±2.07 ^a	45.47±1.65 ^{bcd}	54.17±1.71 ^{cde}	57.72±1.46 ^{bcdde}
BFCN	20.73±1.49 ^a	49.67±2.28 ^{cd}	56.32±1.37 ^{de}	58.78±2.76 ^{cde}
BFC	21.75±1.85 ^a	55.02±1.87 ^{def}	59.65±2.06 ^{ef}	63.50±1.49 ^a
No. of leaves				
NF	3.33±0.52 ^a	4.33±0.52 ^a	5.67±0.82 ^a	5.67±0.52 ^a
CF-RD	3.17±0.41 ^a	10.00±2.10 ^e	14.67±2.42 ^{def}	15.50±3.45 ^{cd}
FB-3	3.17±0.41 ^a	7.17±2.14 ^{bc}	10.83±2.23 ^a	10.50±2.95 ^{bc}
BFCDCN	3.33±0.52 ^a	9.33±1.21 ^{de}	15.50±1.52 ^{efg}	17.67±1.51 ^{def}
BFCDN	3.17±0.41 ^a	6.83±1.83 ^b	12.50±2.66 ^{cd}	15.00±2.00 ^{cd}
BFCDC	3.17±0.41 ^a	8.33±1.51 ^{cd}	12.83±1.83 ^{cd}	15.67±2.07 ^{cde}
BFCD	3.00±0.00 ^a	6.83±1.83 ^b	10.33±1.37 ^{bc}	14.17±1.33 ^{cd}
BFVNC	3.17±0.41 ^a	11.50±2.81 ^{fg}	17.00±3.74 ^{gh}	21.67±5.13 ^h
BFVN	3.17±0.41 ^a	8.67±3.93 ^{cd}	15.00±1.26 ^{ef}	19.67±2.73 ^a
BFVC	3.00±0.00 ^a	8.67±3.01 ^{cd}	14.83±1.60 ^{def}	19.33±2.50 ^a
BFV	3.00±0.00 ^a	6.83±2.14 ^b	13.17±1.60 ^{de}	15.83±1.60 ^{cde}
BFFYCN	3.00±0.00 ^a	10.50±1.22 ^{ef}	16.67±1.21 ^{fgh}	19.67±2.94 ^{fg}
BFFYN	3.17±0.41 ^a	8.67±0.82 ^{cd}	15.17±1.83 ^{ef}	16.83±2.04 ^{de}
BFFYC	3.17±0.41 ^a	8.83±2.04 ^{cd}	15.33±1.63 ^{ef}	19.33±1.63 ^{fg}
BFFY	3.00±0.00 ^a	8.33±1.51 ^{cd}	13.83±1.60 ^{de}	17.67±1.86 ^{def}
BFCN	3.17±0.41 ^a	8.50±2.35 ^{cd}	14.00±3.58 ^{de}	15.50±3.21 ^{cde}
BFC	3.17±0.41 ^a	7.50±2.51 ^{bc}	11.67±2.58 ^{bc}	11.67±3.14 ^{bcd}
No. of tillers				
NF	1.00±0.00 ^a	1.00±0.00 ^a	1.17±0.41 ^a	1.00±0.00 ^a
CF-RD	1.00±0.00 ^a	2.33±0.52 ^b	3.17±0.41 ^a	3.00±0.63 ^{cd}
FB-3	1.00±0.00 ^a	1.67±0.52 ^a	2.33±0.52 ^{bc}	2.17±0.41 ^b
BFCDCN	1.00±0.00 ^a	2.50±0.55 ^{bc}	3.67±0.82 ^d	3.50±0.55 ^{cde}
BFCDN	1.00±0.00 ^a	2.33±0.52 ^b	3.00±0.00 ^{cd}	3.33±0.52 ^{cd}
BFCDC	1.00±0.00 ^a	2.50±0.55 ^{bc}	3.00±0.00 ^{cd}	3.33±0.52 ^{cd}
BFCD	1.00±0.00 ^a	2.17±0.41 ^b	3.00±0.00 ^{cd}	3.00±0.00 ^{cd}
EVNC	1.00±0.00 ^a	2.50±0.55 ^{bc}	3.50±0.55 ^{cde}	3.50±0.55 ^{cde}
BFVN	1.00±0.00 ^a	2.33±0.52 ^b	2.83±0.41 ^{bc}	3.00±0.00 ^{cd}
BFVC	1.00±0.00 ^a	2.50±0.55 ^{bc}	3.00±0.00 ^{cd}	3.00±0.00 ^{cd}
BFV	1.00±0.00 ^a	2.17±0.41 ^b	2.83±0.41 ^{bc}	2.67±0.52 ^{bc}
BFFYCN	1.00±0.00 ^a	2.50±0.55 ^{bc}	3.50±0.55 ^{cde}	3.50±0.55 ^{cd}
BFFYN	1.00±0.00 ^a	2.00±0.63 ^b	2.83±0.41 ^{bc}	2.83±0.41 ^{bc}
BFFYC	1.00±0.00 ^a	2.33±0.52 ^b	3.00±0.00 ^{cd}	3.00±0.00 ^{cd}
BFFY	1.00±0.00 ^a	2.00±0.00 ^b	2.83±0.41 ^{bc}	2.83±0.41 ^{bc}
BFCN	1.00±0.00 ^a	2.00±0.00 ^b	2.17±0.41 ^b	2.17±0.41 ^b
BFC	1.00±0.00 ^a	2.00±0.00 ^b	2.00±0.00 ^b	2.17±0.41 ^b

Fresh wt. of Shoot				
NF	0.18±0.01 ^a	1.11±0.04 ^a	1.35±0.13 ^a	3.23±0.28 ^a
CF-RD	0.17±0.01 ^a	2.97±0.24 ^{cd}	4.18±0.23 ^{ef}	10.46±0.84 ^{fgh}
FB-3	0.16±0.01 ^a	2.01±0.11 ^{bc}	2.68±0.20 ^{bc}	5.99±0.51 ^{bcd}
BFCDCN	0.17±0.01 ^a	3.08±0.13 ^{fg}	4.62±0.18 ^{ef}	11.29±0.59 ^{gh}
BFCDN	0.15±0.01 ^a	2.73±0.15 ^{ef}	3.92±0.04 ^{def}	8.84±0.24 ^{ef}
BFCDC	0.17±0.01 ^a	2.56±0.14 ^{de}	3.67±0.09 ^{de}	8.37±0.14 ^{de}
BFCD	0.16±0.02 ^a	2.37±0.07 ^{cd}	3.31±0.06 ^d	7.72±0.14 ^{cd}
BFVNC	0.18±0.01 ^a	3.83±0.26 ^{gh}	5.55±0.51 ^{fg}	12.20±1.07 ^h
BFVN	0.17±0.01 ^a	2.80±0.12 ^{fgh}	4.14±0.25 ^{ef}	9.95±0.42 ^{fg}
BFVC	0.16±0.01 ^a	2.62±0.19 ^{efg}	3.81±0.12 ^{de}	8.71±0.26 ^{def}
BFV	0.17±0.01 ^a	2.48±0.15 ^{de}	3.62±0.13 ^{de}	8.33±0.17 ^{de}
BFFYCN	0.16±0.01 ^a	3.65±0.18 ^g	5.23±0.37 ^f	11.71±0.44 ^{gh}
BFFYN	0.16±0.01 ^a	2.77±0.12 ^{ef}	4.03±0.15 ^{ef}	8.76±0.24 ^{def}
BFFYC	0.17±0.01 ^a	2.60±0.19 ^{def}	3.69±0.10 ^{de}	8.46±0.09 ^{de}
BFFY	0.18±0.01 ^a	2.45±0.17 ^{cde}	3.35±0.18 ^d	8.02±0.26 ^{de}
BFCN	0.17±0.01 ^a	2.32±0.15 ^{cd}	3.07±0.18 ^d	6.70±0.53 ^{bcd}
BFC	0.16±0.01 ^a	2.12±0.10 ^{bc}	2.90±0.22 ^{bc}	6.12±0.11 ^{bcd}
Dry wt. of Shoot				
NF	0.04±0.00 ^a	0.27±0.02 ^a	0.34±0.03 ^a	1.11±0.15 ^a
CF-RD	0.04±0.00 ^a	0.76±0.05 ^{cde}	1.01±0.08 ^{abc}	3.13±0.20 ^{de}
FB-3	0.04±0.00 ^a	0.51±0.04 ^{bcd}	0.67±0.08 ^{ab}	1.78±0.17 ^{bc}
BFCDCN	0.07±0.03 ^{bc}	0.81±0.05 ^f	1.30±0.06 ^{bc}	3.04±0.12 ^{de}
BFCDN	0.07±0.02 ^{bc}	0.78±0.05 ^{de}	1.22±0.06 ^{bc}	2.92±0.06 ^{cde}
BFCDC	0.06±0.04 ^b	0.72±0.03 ^{de}	1.15±0.03 ^{bc}	2.79±0.06 ^{cde}
BFCD	0.06±0.03 ^b	0.65±0.04 ^{cd}	1.02±0.12 ^{abc}	2.56±0.10 ^{cde}
BFVNC	0.05±0.00 ^{ab}	0.95±0.07 ⁱ	1.37±0.15 ^{bcd}	3.66±0.16 ^{ef}
BFVN	0.06±0.04 ^b	0.86±0.05 ^h	1.32±0.06 ^{bcd}	3.03±0.14 ^{de}
BFVC	0.06±0.03 ^b	0.80±0.10 ^f	1.24±0.10 ^{bcd}	2.89±0.07 ^{cde}
BFV	0.06±0.04 ^b	0.73±0.09 ^{ed}	1.18±0.03 ^{bcd}	2.79±0.06 ^{cde}
BFFYCN	0.06±0.04 ^b	0.90±0.05 ^a	1.33±0.08 ^{bcd}	3.23±0.14 ^{de}
BFFYN	0.07±0.02 ^b	0.82±0.04 ^f	1.26±0.02 ^{bcd}	2.89±0.06 ^{cde}
BFFYC	0.06±0.04 ^b	0.76±0.09 ^{cd}	1.20±0.05 ^{bcd}	2.75±0.05 ^{cde}
BFFY	0.06±0.01 ^b	0.70±0.02 ^c	1.11±0.03 ^{bcd}	2.67±0.05 ^{cde}
BFCN	0.04±0.00 ^a	0.57±0.04 ^{bcd}	0.77±0.07 ^{ab}	2.03±0.11 ^{cd}
BFC	0.04±0.00 ^a	0.52±0.04 ^{bcd}	0.71±0.06 ^{ab}	1.84±0.08 ^{bc}

[Data are represented by mean of six individual measurement ± SD. One way analysis of variance was used to compare the means. Values followed by different letters are significantly different at P < 0.05.] (Other details are described in Table. 5.3.1)

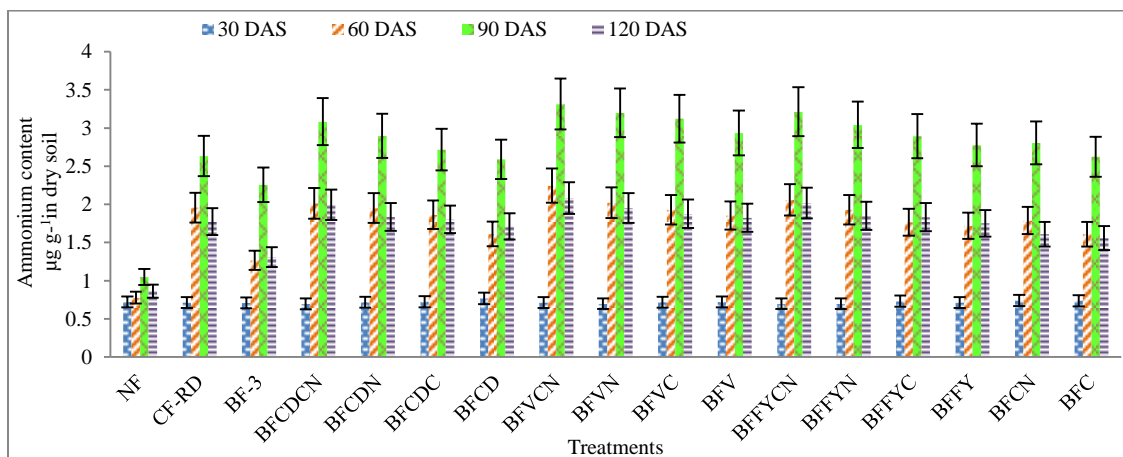
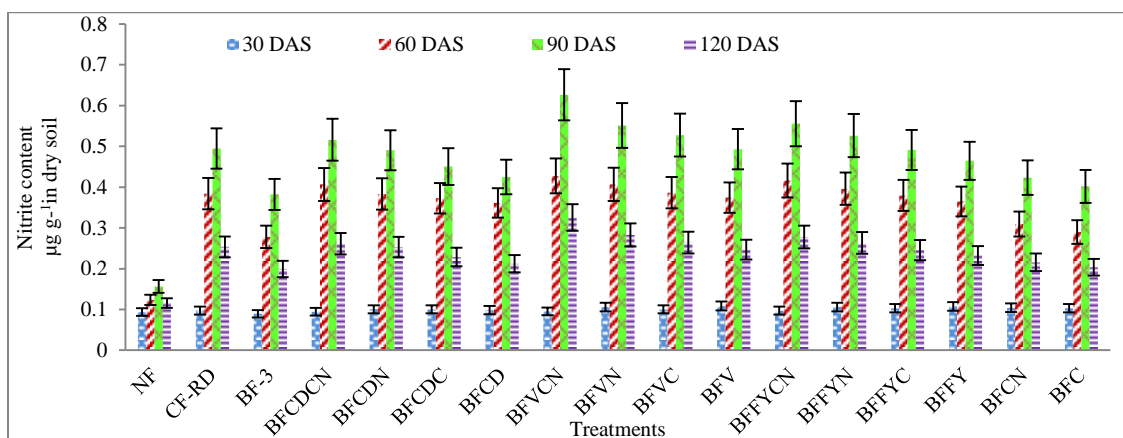
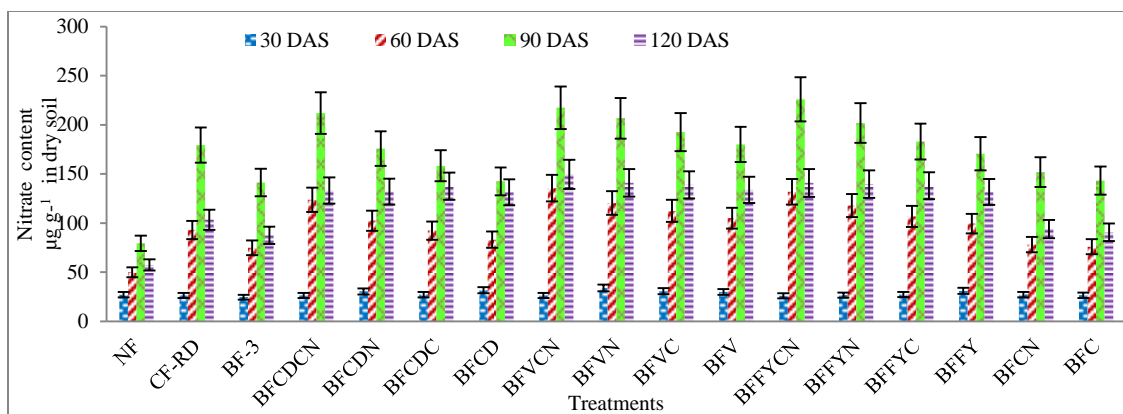
4.5.2 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Rhizospheric Soil, of Wheat (*T. aestivum* L.) Plant Applied with Immobilized form of Biofertilizers

Nitrate content assimilation was recorded highest by the application of BFVCN by 61.69, 30.90, 41.45, 10.98, 11.69, 8.03, 12.13, 5.76, 7.13, 10.67, 5.88, 6.57, 7.78, 11.95, 37.23 and 39.44% at 120 DAS on application of BFVCN over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVCN, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN, BFC respectively. Soil nitrate content were also increased on application of BFCDCN and BFFYCN over FB-3, BFCDCN, BFCDC, BFCD and BFFY at 60, 90 and 120 DAS (Figure 4.5.1).

The immobilized biofertilizers with organic matrix increases the percentage of nitrite content in the soil. Soil nitrite was 63.64, 24.24, 39.39, 21.21, 24.24, 30.30, 36.36, 15.15, 21.21, 24.24, 15.15, 21.21, 24.24, 30.30, 33.33 and 39.39% recorded at 120 DAS respectively on application of BFVCN over NF, CF-RD, FBF, BFCDCN, BFCDN, BFCDC, BFCD, BFVCN, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Figure 4.5.1).

The increased ammonium content in soil was highest recorded by the application of BFVCN by 68.28, 20.54, 31.72, 6.95, 12.39, 17.82, 21.75, 3.32, 5.74, 11.18, 3.02, 8.16, 12.69, 16.01, 15.41, 20.85% at 90 DAS respectively over NF, CF-RD, FBF, BFCDCN, BFCDN, BFCDC, BFCD, BFVCN, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC. Similarly increased soil ammonium content also recorded at 60 and 120 DAS (Figure 4.5.1).

Soil phosphate content in soil was recorded significantly by 90.48, 31.31, 46.18, 11.98, 19.48, 24.96, 29.73, 8.23, 15.58, 18.47, 9.38, 17.46, 19.77, 26.26, 36.80 and 39.39% at 120 DAS respectively over NF, CF-RD, FBF, BFCDCN, BFCDN, BFCDC, BFCD, BFVCN, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC (Figure 4.5.1).



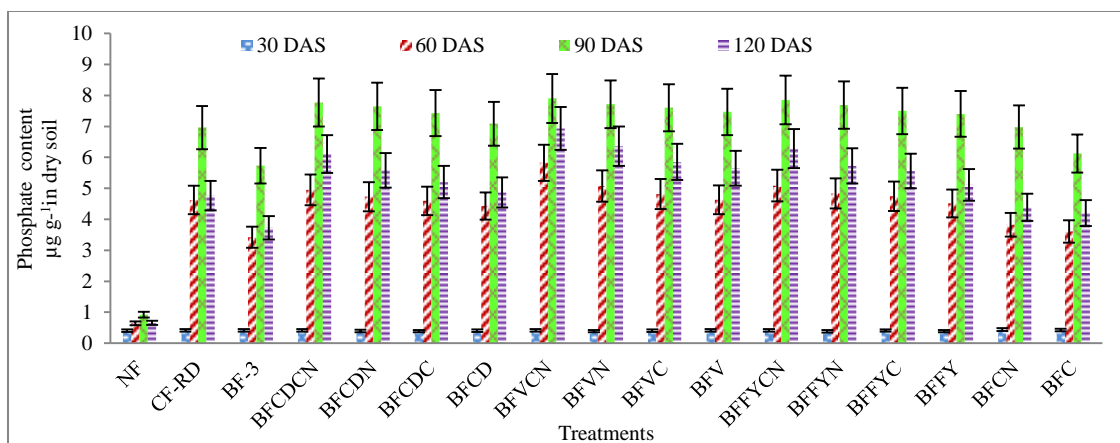


Figure 4.5.1 The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the soil on 30, 60, 90, and 120 days after sowing (DAS). All the values are means of three replicates with two determinations ($n=6$) \pm S.E). (Other details are described in Table. 4.5.1)

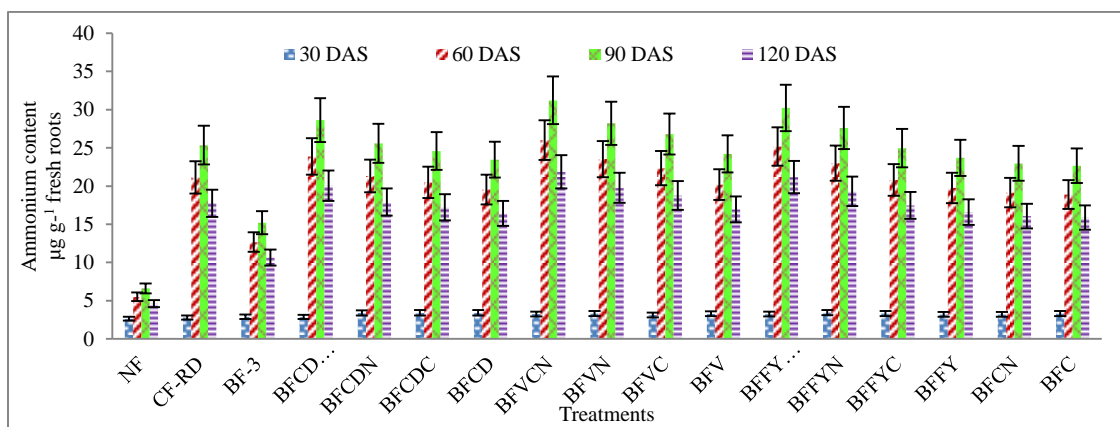
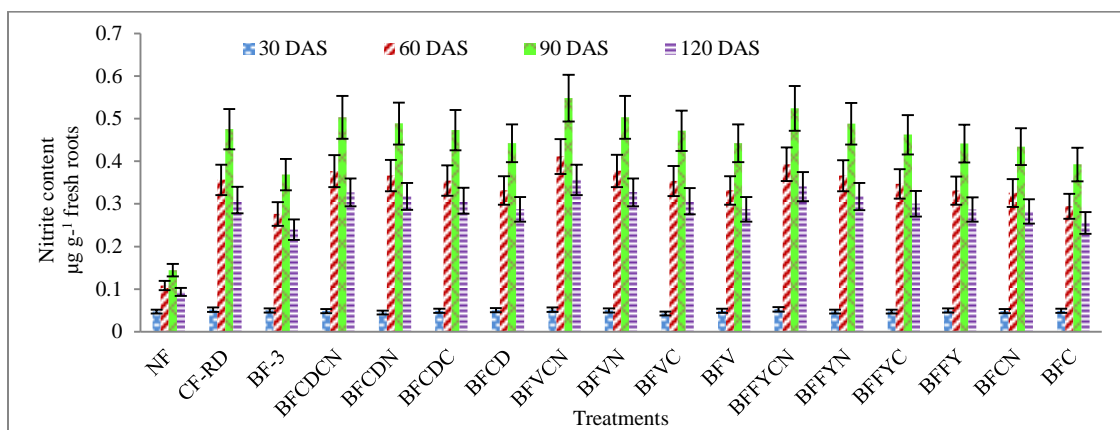
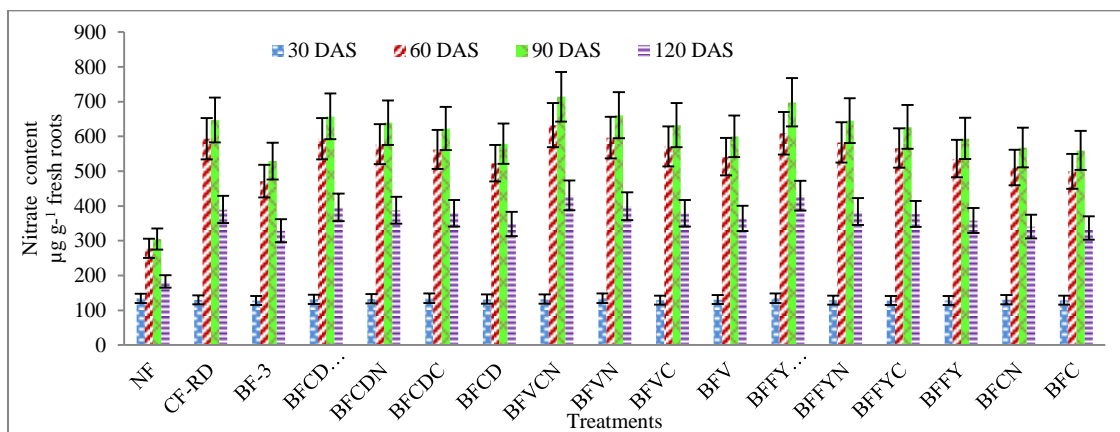
4.5.3 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Roots of Wheat (*T. aestivum* L.) Plant Applied with Immobilized form of Biofertilizers

Nitrate content in plant roots was recorded highest on application of (BFVCN) during cultivation of wheat crop. Increased nitrate percentage in roots by BFVCN was 56.03, 6.15, 25.50, 6.15 and 3.73% at 60 DAS over NF, CF-RD, FB-3, BFCDCN and BFFVCN respectively (Figure 4.5.2) providing nutrient availability to the plants for better plant growth.

Application of biofertilizers immobilized with organic matrix increased the nitrite content in plants roots by BFVCN was 74.55, 12.73, 32.73, 9.09, 10.91, 14.55, 20, 9.09, 14.55, 20, 5.45, 10.91, 16.36, 20, 21.82 and 29.09% at 90 DAS respectively recorded significantly over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFVCN, BFFYN, BFFYC, BFFY, BFCN and BFC (Figure 4.5.2).

Similarly the root ammonium content was recorded significantly and it increases at different level by 78.83, 18.80, 51.30, 8.29, 18.06, 21.29, 24.91, 9.61, 14.15, 22.45, 3.20, 11.59, 20.04, 24.11, 26.45 and 27.44% at 90 DAS respectively over NF, CF -RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFVCN, BFFYN, BFFYC, BFFY, BFCN and BFC. The immobilized formulations enhanced the ammonium content in soil. The nutrient uptake by plant roots significantly and it translocate into the plants leaves as well (Figure 4.5.2).

In roots, application of BFVCN was increased phosphate percentage by 75.14, 15.17, 39.56, 11.12, 13.50, 19.46, 24.78, 11.04, 21.29, 25.34, 8.74, 16.28, 20.89, 26.05, 32.80 and 32.17% at 120 DAS respectively over NF, CF-RD, BF-3, BFCDCN, BFCDN, BFCDC, BFCN, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC , the various combinations of organic matrix immobilized biofertilizer. The application of BFVCN also increased phosphate content at different intervals (Figure 4.5.2).



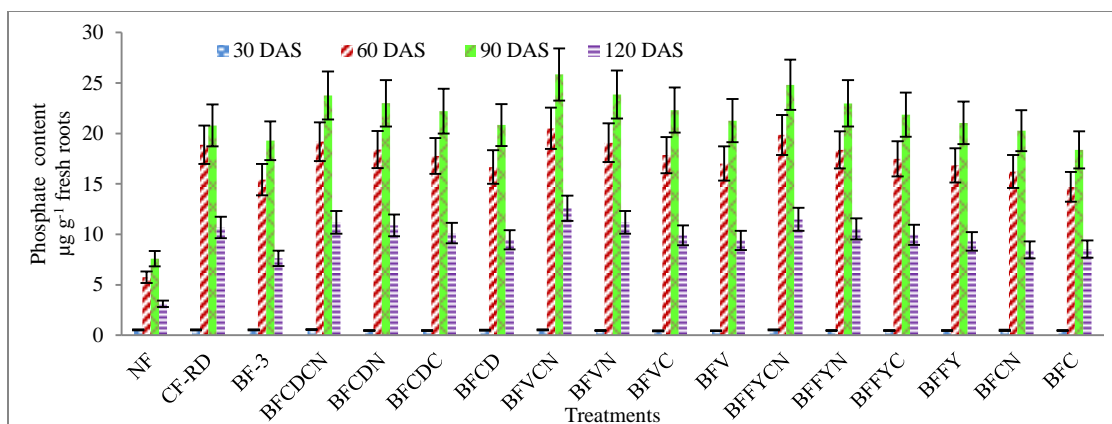


Figure 4.5.2 The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the roots on 30, 60, 90, and 120 days after sowing (DAS). All the values are means of three replicates with two determinations ($n=6 \pm S.E$). (Other details are described in Table. 4.5.1)

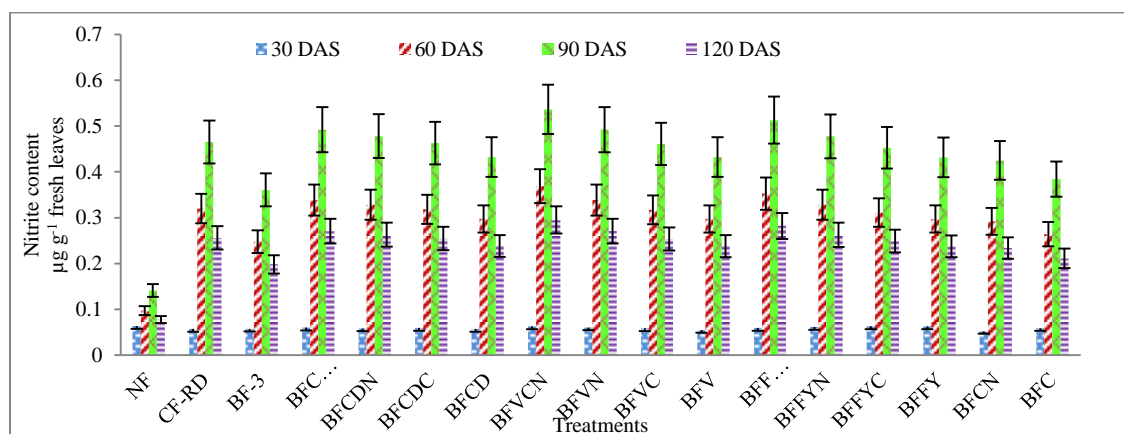
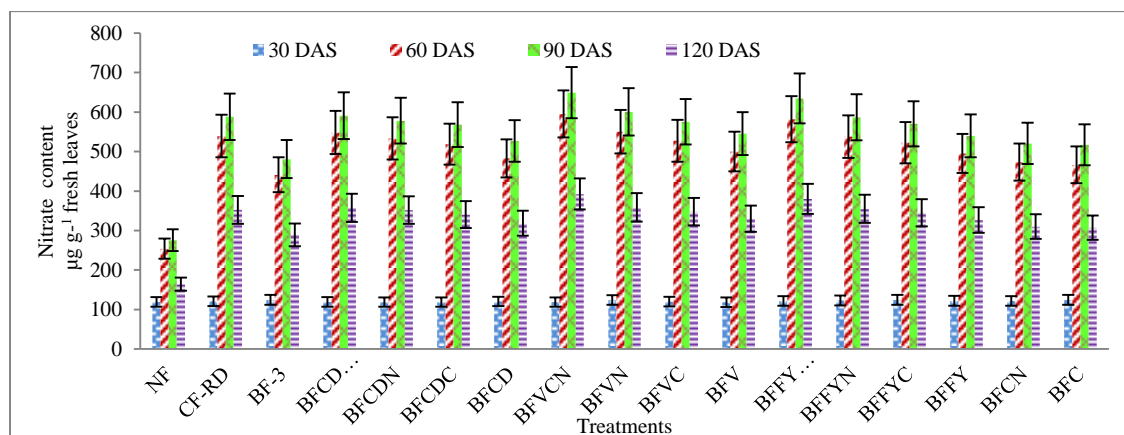
4.5.4 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in leaves of Wheat (*T. aestivum* L.) Plant Applied with Enhance Doses of Free form of Biofertilizers

The biofertilizers in the form of super granules contains different organic carrier in which immobilized biofertilizers with vermicompost as a carrier (BFVCN) was provide nitrate content in soil from where it assimilate in the wheat leaves and increased percentage by 58.17, 10.23, 26.35, 9.01, 10.40, 13.24, 18.85, 8.69, 11.38, 15.94, 3.24, 11.38, 9.58, 12.13 and 16.80% at 120 DAS over the others organic combination respectively NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC. Similarly the increased amount of nitrate content in plant leaves was also recorded at the different intervals of 30, 60 and 90 DAS (Figure 4.5.3).

Nitrite content in plant leaves were significantly increased by the application of BFVCN followed by BFFYCN (FYM) as a carrier) and BFCD with cow dung as a carrier over controls NF, CCF (RD), FBF, at different days of intervals. Application of super granule of BFVCN was increased 74.07, 12.96, 33.33, 9.26, 11.11, 14.81, 20.37, 9.26, 14.81, 20.37, 5.56, 11.11, 16.67 and 20.37% at 90 DAS over the application of NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Figure 4.5.3).

The ammonium content in leaves of wheat plants significantly assimilate there and it was highest recorded by the application of BFVCN was 79.22, 14.21, 46.28, 12.20, 12.69, 18.05, 20.10, 9.46, 12.50, 19.41, 5.47 and 12.46% at 90 DAS respectively over NF, CF-RD, FB-3, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC and BFFY (Figure 4.5.3).

The phosphate content in rhizospheric soil was uptakes by the plant leaves recorded highest by the application of BFVCN was 71.92, 7.94, 24.79, 6.45, 10.27, 13.30, 18.74, 6.93, 12.94, 17.03, 3.20, 10.36, 14.70 and 17.90% at 90 DAS over NF, CF-RD, FBF, BFCDCN, BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC .The increased phosphate content percentage also recorded at 30, 60 and 120 DAS (Figure 4.5.3).



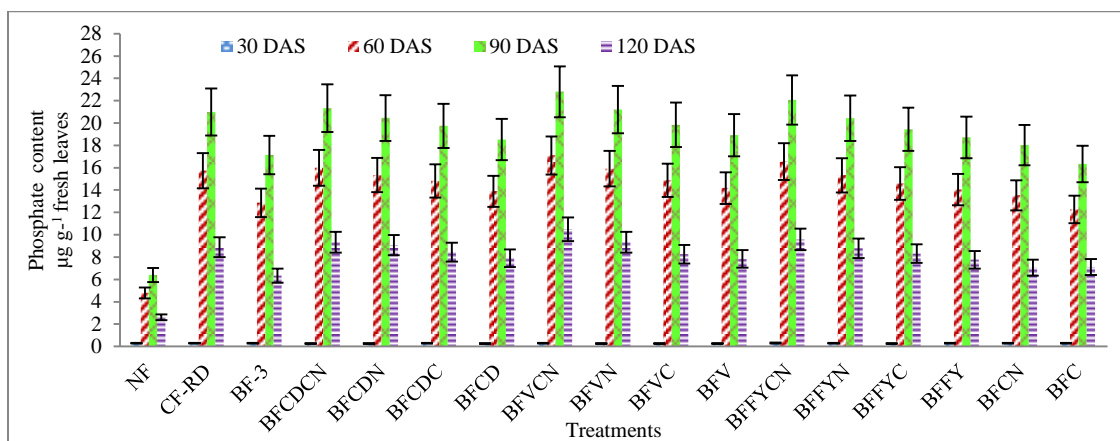
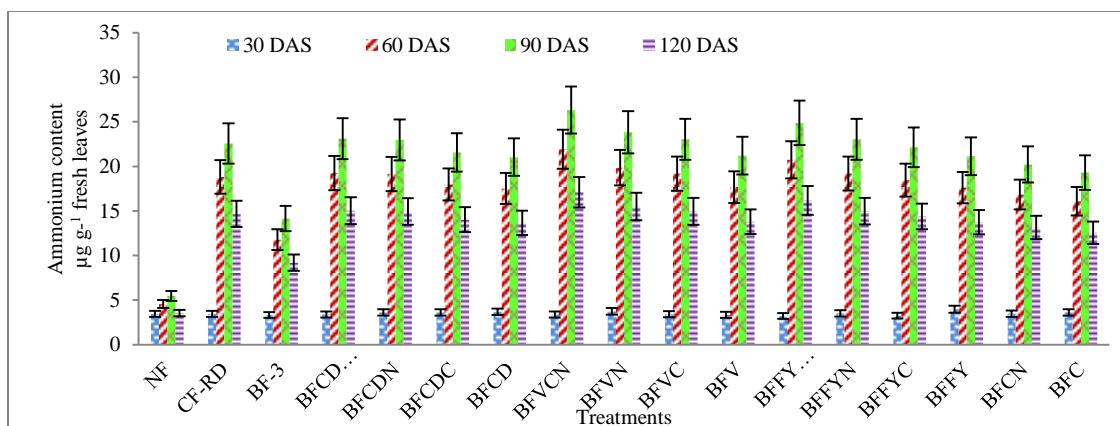


Figure 4.5.3 The effect of enhance doses of biofertilizers on nitrate, nitrite, ammonium and phosphate content in the leaves on 30, 60, 90, and 120 days after sowing (DAS). All the values are means of three replicates with two determinations ($n=6 \pm S.E$). (Other details are described in Table. 4.5.1)

4.5.5 Yield and Productivity of Wheat (*T. aestivum* L.) Plant Applied with Free Form of Biofertilizers

Application of commercial free form of charcoal based biofertilizers (*A. chroococcum* and *B. subtilis*) increased the yield percentage. Enhance doses of biofertilizers increased percentage grain yield by 54.47, 12.14, 46.45, 3.99, 27.02, 28.88, 35.11, 12.22, 26.67, 30.20, 3.37, 20.49, 28.10, 32.18, 39.94 and 44.93% on application of BFVCN over NF, CF-RD, FB-3, BFCDCN, BFCDCN, BFCDC, BFCDC, BFVN, BFVC, BFV, BFFVCN, BFFYN, BFFYC, BFFY, BFCN and BFC after harvesting at 120 DAS (Fig. 4.5.4).

The percentage of straw yield was increased by 73.53, 14.21, 50.90, 17.07, 27.49, 31.41, 36.67, 18.43, 28.55, 31.71, 9.94, 28.14, 30.64, 34.21, 45.11 and 49.85% on after harvesting with the application on BFVCN over NF, CF-RD, FB-3, BFCDCN,

BFCDN, BFCDC, BFCD, BFVN, BFVC, BFV, BFFYCN, BFFYN, BFFYC, BFFY, BFCN and BFC respectively (Figure 4.5.4).

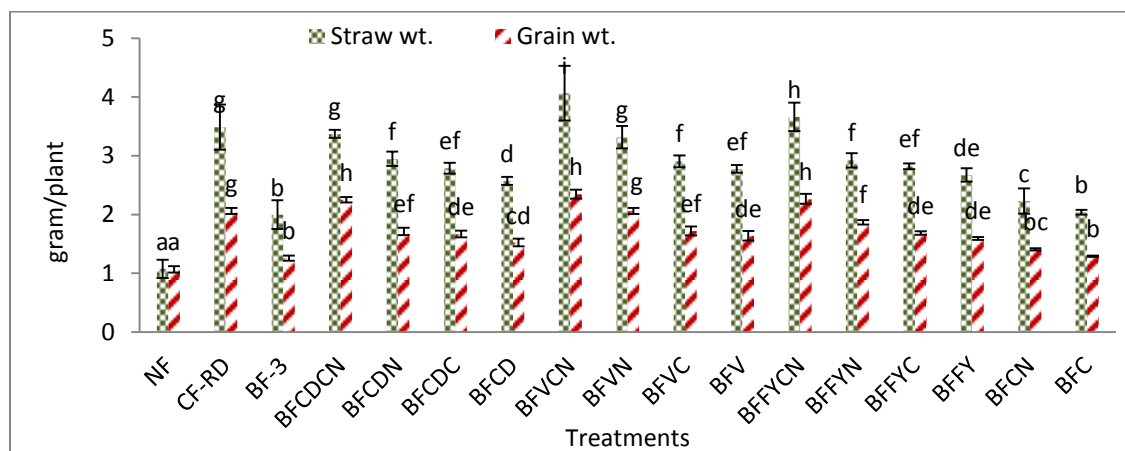


Figure 4.5.4 The application of enhance doses of immobilized form of biofertilizers on soil significantly recorded yield and straw weight. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values followed by different letters are significantly differences between the treatments. (Other details are described in Table. 4.5.1)



Plate: 4.5.1 Experimental site at Mohanlalganj, Lucknow during cultivation of wheat crop

4.6. Growth and productivity of wheat (*T. aestivum* L. cv. PBW-502) applied with charcoal based Immobilized biofertilizers (*A. chroococcum* and *B. subtilis*) in enhanced doses (triple dose) with different organic carrier and binders on 30, 60, 90 and 120 days after sowing (DAS)

4.6.1 Growth Parameters of wheat applied with charcoal based Immobilized biofertilizers in enhanced doses (triple dose) with different organic carrier and binders on 30, 60, 90 and 120 days after sowing (DAS)

The optimized dose of biofertilizers (i.e. *A. chroococcum*- 1.80 kg ha⁻¹ and *B. subtilis*- 1.80 kg ha⁻¹) immobilized with organic matrix using highly nutritive organic binders significantly affects the growth of wheat plants. Application of IVJ increased the fresh and dry weight of shoots and roots, height of shoot and length of roots, numbers of leaves, tillers and roots significantly recorded over the controls of no fertilizers (NF) and free form of chemical fertilizers (CF) on 60, 90 and 120 DAS (Table 4.6.1).

The application with IVJ has increases the plant height by 42.84, 11.05%, 42.83, 9.15% and 40.31, 6.00% over NF and CF at 60, 90 and 120 DAS. IVM increased the plant height was 41.20, 8.50%, 42.54, 6.68% and 39.20, 4.25% and by the application of IVA was increased 40.36, 7.20% 41.81, 5.49% and 38.39, 2.98% at 60, 90 and 120 DAS over NF and CF respectively (Table 4.6.1).

The percentages of fresh weight of shoot was increased by 53.20, 9.49%, 72.95 and 10.12% at 60 and 120 DAS with the application of IVJ over NF and CF. Same as IVM and IVA by 66.53, 7.25% 72.72 and 9.37%, and 65.93, 5.60%, 72.10 and 7.29% over NF and CF at 90 and 120 DAS. And also IVJ was increased by .83 and 3.14% over the application of immobilized biofertilizers with vermicompost using binder as molasses IVM and immobilized biofertilizers with vermicompost using binder as acacia gum IVA respectively at 120 DAS (Table 4.6.1).

The dry weight of plant production was recorded by 67.02, 8.51% and 72.88, 11.72% at 90 and 120 DAS respectively and 66.66, 7.52% and 72.64, 10.94% and 66.12, 6.01% and 72.04, 8.97% by the application of IVM and IVA over the NF and CF respectively at 90 and 120 DAS. The biofertilizers immobilized with organic matrix using various nutritive binders found significantly differential between them. IVJ was

recorded by .88, 5.30% and 1.06 and 2.65% over IVM and IVA at 60 and 90 DAS respectively (Table 4.6.1).

Table 4.6.1 Effect of Immobilized Biofertilizers on Growth of Wheat (*T. aestivum* L. cv. PBW-502) Shoots on 30, 60, 90 and 120 (DAS)

		30 DAS	60 DAS	90 DAS	120 DAS
Plant Height	NF	24.93±0.48	39.04±1.79ns	50.52±1.29ns	54.97±1.36ns
	CF	24.80±1.14	60.75±1.91*	82.05±2.78*	86.57±0.93*
	IVA	24.68±0.77	65.47±1.59*	86.82±1.29*	89.23±0.81**
	IVM	24.71±0.95	66.40±1.58**	87.93±1.30**	90.42±1.13**
	IVJ	24.35±0.65	68.30±0.80**	88.37±1.55**	92.10±1.61**
Number of leaves	NF	3.67±0.52	6.33±0.52ns	7.50±1.05	7.17±0.75
	CF	3.50±0.55	11.33±1.21*	17.00±1.10*	18.83±0.98*
	IVA	3.17±0.41	11.50±1.52**	22.17±2.99**	22.00±1.41**
	IVM	3.83±0.41	11.83±0.75**	22.33±1.86**	22.67±1.97**
	IVJ	3.67±0.52	12.50±1.22**	24.67±1.63**	24.33±1.37**
Number of tillers	NF		1.00±0.00	1.17±0.41	1.17±0.41
	CF		2.50±0.55**	3.33±0.52*	3.50±0.55**
	IVA		2.17±0.41*	3.67±0.52**	3.50±0.55**
	IVM		2.50±0.55**	3.67±0.52**	3.67±0.52**
	IVJ		3.00±0.00**	3.83±0.41**	3.83±0.41**
Fresh wt. of shoot	NF	1.16±0.02	2.12±0.03	2.49±0.08ns	3.90±0.07ns
	CF	1.16±0.02	4.10±0.09*	6.90±0.04*	12.96±0.40*
	IVA	1.15±0.01	4.27±0.08*	7.31±0.17**	13.98±0.17**
	IVM	1.15±0.01	4.48±0.24*	7.44±0.23**	14.30±0.13**
	IVJ	1.15±0.02	4.53±0.32**	7.54±0.20**	14.42±0.20**
Dry wt. of shoot	NF	0.35±0.01	0.53±0.01ns	0.62±0.02ns	2.15±0.04ns
	CF	0.35±0.01	1.02±0.02*	1.72±0.01*	7.00±0.09*
	IVA	0.34±0.00	1.07±0.02*	1.83±0.04**	7.69±0.09**
	IVM	0.35±0.00	1.12±0.06**	1.86±0.06**	7.86±0.07**
	IVJ	0.35±0.01	1.13±0.08**	1.88±0.05**	7.93±0.11**

All the values are means of three replicates with two determination (n=6) ±SD. **Where, NF: No Fertilizers; CF; Chemical fertilizers, IVA; Immobilized form of biofertilizers with vermicompost used acacia gum as binder, IVM; Immobilized form of biofertilizers with vermicompost used molasses as binder, IVJ; Immobilized form of biofertilizers with vermicompost used jaggery as binder. (One way ANOVA) ns= Not significant; *= $P < 0.05$, **= $P < 0.01$. Treatment symbols are defined in the text.**

Application of IVJ increased the root length of the wheat plant by 63.63, 9.29%, 58.47, 12.28% and 50.88, 5.92% over NF and CF at 60, 90 and 120 DAS. Application on IVM increases the root length by 60.53, 1.56%, 57.86, 11% and 49.10, 4.08% over NF and CF at 60, 90 and 120 DAS respectively. The numbers of roots was significantly recorded by the application of IVJ at 60, 90 and 120 DAS was 71.52, 43.80, 65.86, 25.87, 68.54 and 23.69% over NF and CF respectively. Similarly the significant increased number of leaves, roots and tillers were counted at regular intervals during crop cultivation (Table 4.6.2).

Biofertilizers immobilized with organic matrix using organic binder increases the root dry biomass. The percentages of increased dry root weight recorded by 63.94, 8.19% and 55.84, 8.97% at 60 and 120 DAS on application of IVJ over NF and CF. Same as IVM and IVA by 62.71, 5.08%, 53.24, 7.79% and 61.40, 1.75%, 52.63 and 6.57% over NF and CF at 90 and 120 DAS. The dry weight of root biomass was also increases by applied the SGBs used nutritive binders. The percentage of dry weight of root was highest recorded by immobilized biofertilizers with organic matrix used jaggery as a binder (IVJ) was 60, 6.66% and 55 and 15% over NF and CF at 60 and 120 DAS (Table 4.6.2).

Table 4.6.2 Effect of Immobilized Biofertilizers on Growth of Wheat (*T. aestivum* L. cv. PBW-502) Roots on 30, 60, 90 and 120 (DAS)

		30 DAS	60 DAS	90 DAS	120 DAS
Root length	NF	2.42±0.17	3.52±0.23	4.90±0.45	5.98±0.45
	CF	2.40±0.18	8.78±0.17*	10.35±0.49*	11.27±0.54*
	IVA	2.38±0.19	8.85±0.39*	11.35±0.42*	11.55±0.42**
	IVM	2.42±0.28**	8.92±0.55*	11.63±0.42**	11.75±0.15**
	IVJ	2.46±0.23**	9.68±0.63**	11.80±0.74**	11.98±0.44**
Number of roots	NF	4.17±0.41	6.50±1.05ns	9.67±0.52ns	10.17±0.75ns
	CF	4.17±0.75	12.83±0.98*	21.00±0.89*	24.67±1.21*
	IVA	4.00±0.63	22.17±2.40**	27.00±0.89**	29.83±1.33**
	IVM	4.50±0.55	22.33±2.25**	28.00±1.10**	30.50±1.64**
	IVJ	4.00±0.89	22.83±2.14**	28.33±1.21**	32.33±2.25**
Fresh wt. of root	NF	0.18±0.02	0.22±0.02ns	0.30±0.02ns	0.36±0.02ns
	CF	0.18±0.01	0.56±0.03*	0.62±0.03*	0.71±0.03*
	IVA	0.18±0.01	0.57±0.02*	0.67±0.02**	0.76±0.01**

	IVM	0.17±0.02	0.59±0.01**	0.69±0.01**	0.77±0.03**
	IVJ	0.18±0.02	0.61±0.03**	0.70±0.02**	0.78±0.01**
Dry wt. of roots	NF	0.04±0.00	0.06±0.01ns	0.08±0.01ns	0.09±0.00ns
	CF	0.04±0.00	0.14±0.01*	0.16±0.01*	0.17±0.01*
	IVA	0.04±0.00	0.14±0.00*	0.17±0.01**	0.19±0.00**
	IVM	0.04±0.00	0.15±0.00**	0.17±0.00**	0.19±0.01**
	IVJ	0.04±0.00	0.15±0.01**	0.18±0.01**	0.20±0.00**

All the values are means of three replicates with two determinations (n=6) ±SD, (one way ANOVA). ns= Not significant; *= $P < 0.05$, **= $P < 0.01$. (Other details are described in Table. 4.6.1)

4.6.2 Root Transverse Section Examine under Scanning Electron Microscope

It is observed that on application of IVJ was the maximum surface area, root strength and enlarged cortex over NF, CF, IVA and IVM respectively (Figure 4.6.1). Biofertilizers immobilized with organic matrix used organic binders which increases the fresh and dry weight of root. The percentages of fresh and dry root weight was recorded significantly at 60 and 120 DAS (Table 4.6.1 and 4.6.2).on application of IVJ over NF, CF, IVA and IVM respectively. Immobilized biofertilizers with organic matrix used jaggery as a binder significantly increased the root biomass. The SEM analysis of transverse section of wheat roots showed the well differentiated vascular tissues.

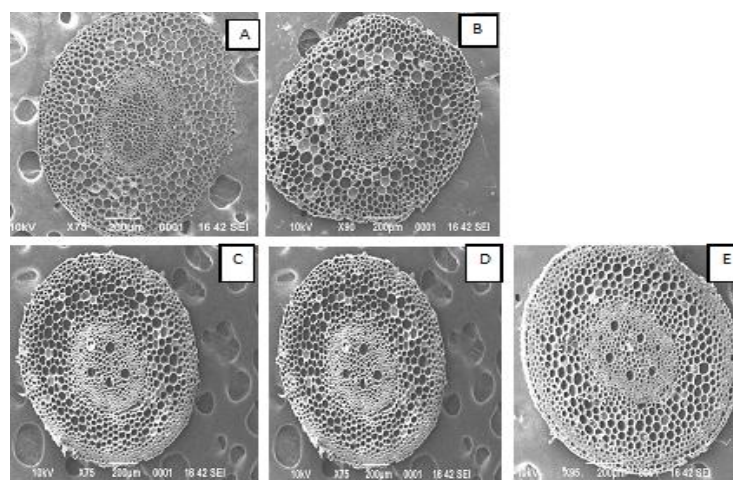


Figure 4.6.1 (A-E) - Transverse section of root examine under Scanning Electron Microscope

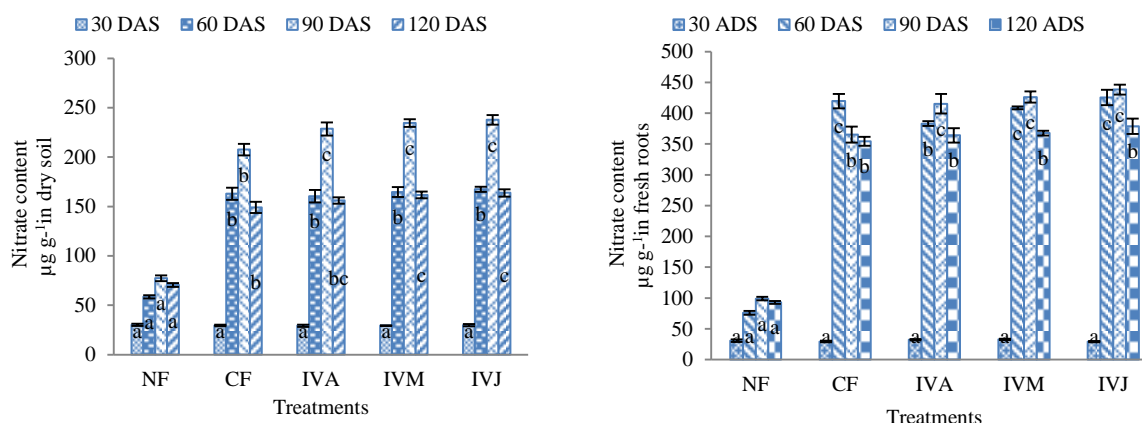
Note: The transverse section of root shows the well differentiated vascular tissues, A; NF, B; FCF- (R/D; Recommended dose of Urea and DAP), and by amending the C; EVC-III immobilized form of organic matrix (combination of clay soil+ vermicompost+ neem leaf) and charcoal based consortium of biofertilizers (*A. chroococum* and *B. subtilis*) with binder acacia and D; EVC-III immobilized form of organic matrix (combination of clay soil+ vermicompost+ neem leaves powder) with binder molasses and charcoal based consortium of biofertilizers (*A. chroococum* and *B. subtilis*), E; EVC-III immobilized form of organic matrix (combination of clay soil+ vermicompost+ neem leaves powder) and charcoal based consortium of biofertilizers (*A. chroococum* and *B. subtilis*) with binder jaggery.

4.6.3 Level of Nitrate Content Assimilation in the Soil and Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

On applications of biofertilizers in immobilized form with organic matrix used organic binders enhanced the assimilation rate of nitrate content in the soil. Nitrate content was recorded highest by the application of IVJ by 64.99, 2.64, 1.64, 4.13%, 67.44, 12.64, 1.37, 3.80% and 56.94, 8.91, 1.20, 4.67% at 60, 90 and 120 DAS over the NF, CF, IVM and IVA respectively. Application on IVM was recorded by 64.40, 1%, 66.99, 11.42% and 56.41, 7.80% over NF and CF at 60, 90 and 120 DAS respectively and IVA by 66.15, 9.18% and 55.24, 4.44% over NF and CF at 90 and 120 DAS respectively (Figure 4.6.2).

The immobilized form of biofertilizers with organic matrix increases the percentages of nitrate content in the root. The nitrate content in the root was increases by 82.15, 1.37%, 77.45, 16.70% and 75.48, 6.39% over the NF and CF at 60, 90 and 120 DAS respectively. Application on IVM and IVA nitrate content increased by 76.81, 14.34%, 74.75, 3.59% and 76.20, 12.09%, 74.49, 2.62% at 90 and 120 DAS over NF and CF respectively (Figure 4.6.2).

The application IVJ has increases nitrate content significantly at different levels by 83.94, 2.86, 4.27, 9.49% and 87.16, 9.51, 1.37 and 6.09% over NF, CF, IVM and IVA at 60 and 120 DAS respectively. The increased nitrate content in leaves was also recorded by the application of IVM by 86.98, 8.24% and 86.33, 3.64% at 90 DAS over the control of NF and CF respectively (Figure 4.6.2). Correlation between average soil nitrate and average plant nitrate content in different formulation was linearly significant at 120 DAS ($R^2 = 0.660$).



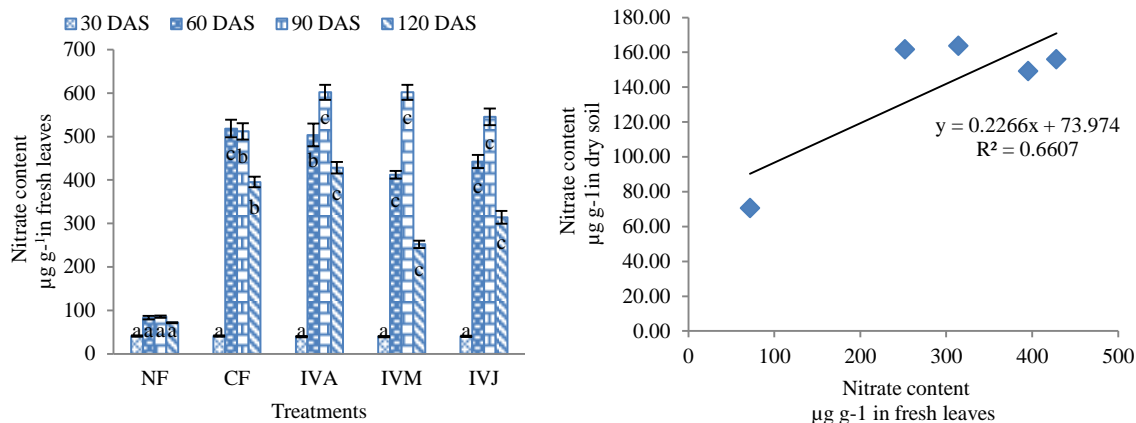


Figure 4.6.2 Levels of Nitrate ($\mu\text{g g}^{-1}$) in dry soil, roots and leaves of *T. aestivum* L. at 30, 60, 90 and 120. All the values are means of three replicates with two determinations ($n=6$) \pm SE (one way ANOVA). Values followed by different letters show significant differences between the treatments at $p<0.05$. (Other details are described in Table. 4.6.1)

4.6.4 Level of Nitrite Content Assimilation in the Soil and Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

Application of SGBs significantly increases the nitrite content in soil. By applied with IVJ were increases by 75, 10.41%, 69.04, 26.19%, application of IVM by 74.46, 8.51%, 67.5, 22.5% over the NF and CF at 90 and 120 DAS respectively. Application of IVA was 72.09 and 64.86% over NF at 90 and 120 DAS respectively (Figure 4.6.3).

Nitrite content assimilation in roots was recorded significantly and it increases at different level by the application of immobilized form of biofertilizers; (IVJ) was 68.33, 10%, IVM by 67.24, 6.89% and IVA BY 66.66, 5.26% at 120 DAS over over no fertilizer (NF) and conventional free form of chemical fertilizer (CF) respectively (Figure 4.6.3).

Nitrite content assimilation in plant leaves significantly increases their percentage levels applied with IVJ by 64.55, 12.65, 5.06 and 6.32% over the NF, CF, IVM and IVA at 120 DAS. Same as by the application of IVM and IVA at 120 DAS was recorded by 62.66, 8% and 62.6 and 6.75% over NF and CF respectively (Figure 4.6.3). By the application of different formulation was applied in experimental field and find the correlation between average soil nitrite and average plant nitrite content at 120 DAS was linearly significant ($R^2 = 0.952$).

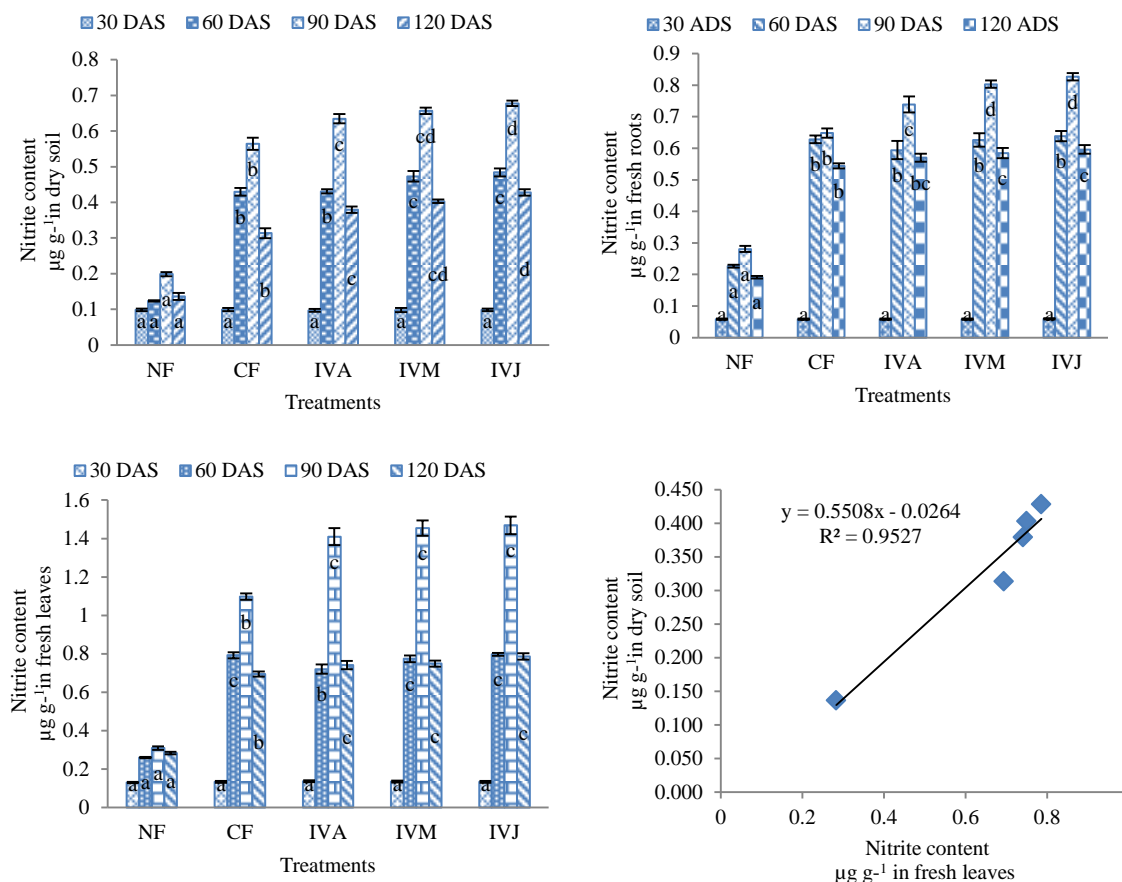


Figure 4.6.3 Levels of nitrite ($\mu\text{g g}^{-1}$) in dry soil, roots and leaves of *T. aestivum* L. at 30, 60, 90 and 120 DAS in different treatments. All the values are means of three replicates with two determinations ($n=6$) \pm SE (one way ANOVA). Values followed by different letters show significant differences between the treatments at $p<0.05$. (Other details are described in Table. 4.6.1)

4.6.5 Level of Ammonium Content Assimilation in the Soil and Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

The soil ammonium content increased by application on (IVJ) was 72.84, 10.54%, 71.46, 11.95% and 62.82, 14.52% at 60, 90 and 120 DAS over the no fertilizer (NF) and free form of chemical fertilizers (CF) respectively. On Application of (IVM) and (IVA) recorded ammonium content by 71.76, 6.97% and 62.33, 13.41% and 70.07, 1.40% and 61.16, 10.71% over of NF and CF at the 60 and 120 DAS respectively (Figure 4.6.4).

Ammonium content in the roots of wheat plant recorded significantly, it increases by the application of IVJ was 68.44, 54, 3.02, 6.04%, 77.36, 17.86, 3.77, 9% and 71, 20.88, 2.09, and 9.25% over the NF CF, IVM and IVA at 60, 90 and 120 DAS respectively. Application of (IVM) and (IVA) also increases their concentration level

in root by 76.60, 15.08% and 70.38, 19.19% and 75.12, 9.73% and 68.04 and 12.81% at 90 and 120 DAS respectively over NF and CF (Figure 4.6.4).

In leaves, the assimilation of ammonium content was recorded different levels by applied with SGBs i.e. immobilized biofertilizers with organic matrix used jaggery as a binder; (IVJ) by 78.54, 5.62, 4.01, 15.75%, 79.42, 21.35, 6.12, 9.78% and 82.47, 24.70, 1.75 and 15.30% over the NF CF, IVM, and IVA at 60, 90 and 120 DAS respectively. Similarly by the application of IVM and IVA increased percentage by 78.08, 16.22%, 82.16, 23.36% and 77.19, 12.82% and 79.31 and 11.10% over the NF and CF at 90 and 120 DAS respectively (Figure 2c). Correlation between average soil ammonium and average plant leaves ammonium content by the application of SGBs based different formulation was found linearly significant at 120 DAS ($R^2 = 0.983$) (Figure 4.6.4).

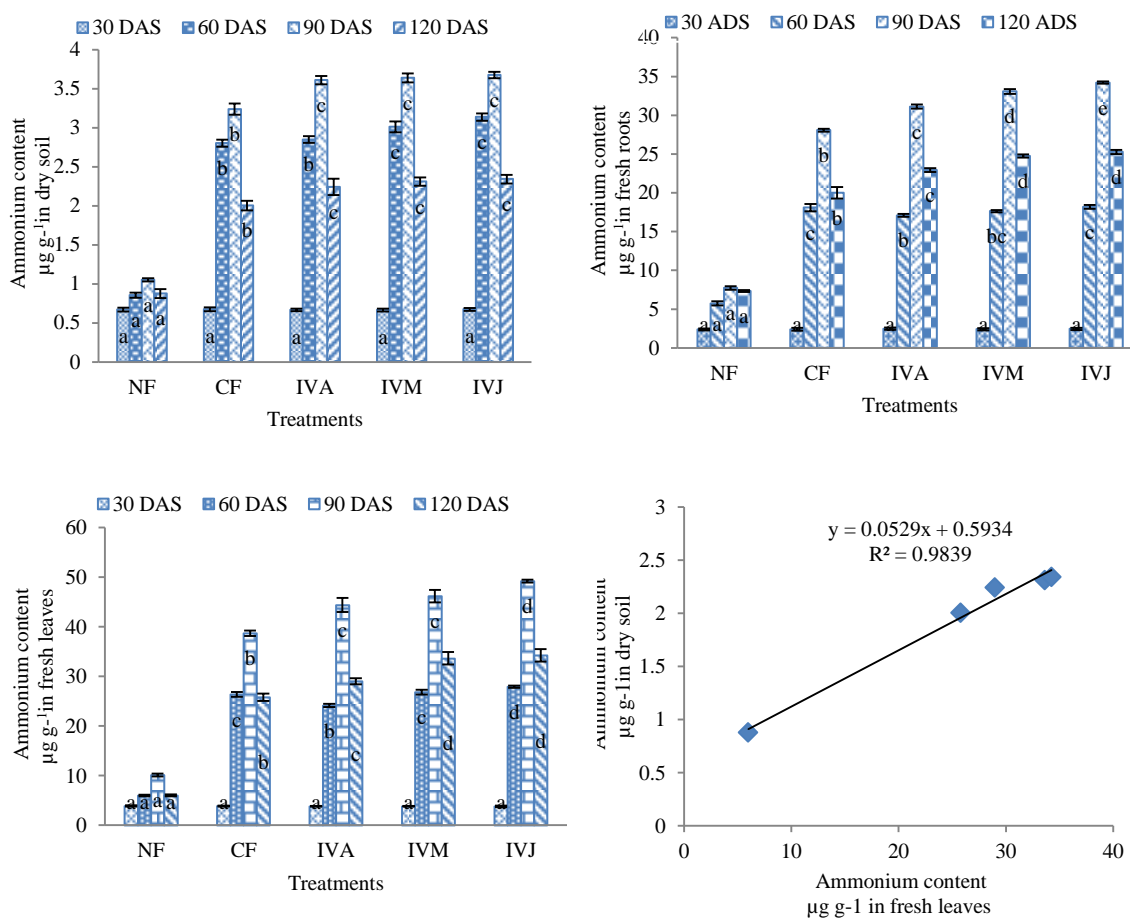


Figure 4.6.4 Ammonium content on soil and plant parts at 30, 60, 90 and 120 DAS. All the values are means of three replicates with two determinations ($n=6$) \pm SE (one way ANOVA). Values followed by different letters show significant differences between the treatments at $p < 0.05$. (Other details are described in Table. 4.6.1)

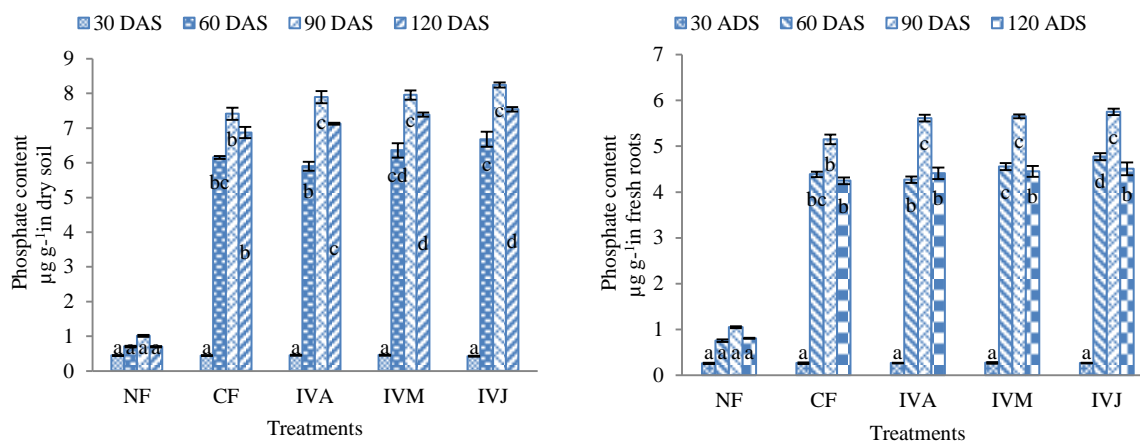
4.6.6 Level of Phosphate Content Assimilation in the Soil and Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

The phosphate content increased on application of IVJ was 90.71, 8.88, 1.98 and 5.43% at 120 DAS over the NF, CF, IVM and IVA respectively. By the application of IVM was increased by 90.52, 7.03 and 3.38% over the NF, CF and IVA at 120 DAS respectively. Application on IVA increased by 90.18, 3.64 over NF and CF at 120 DAS respectively (Figure 4.6.5).

The phosphate content assimilation in the plant roots increased when applied on IVJ was 84.66, 7.96%, 82.03, 5.76% at 60 and 120 days old plants, with the application of IVM 83.51, 3.51% and 81.79, 4.49% over NF and CF respectively. Application of IVA increases by 82.39 and 81.63% over NF at 60 and 120 DAS and 3.63% at 120 DAS over controls of CF.

By application of SGBs based application IVJ was increased phosphate content in leaves by 94.34, 5.86%, 92.80, 11.40%, 92.97 and 12.85% at 60, 90 and 120 DAS over NF and CF respectively. And also the leading application of IVM was recorded significantly by 94.11, 1.98%, 92.79, 10.30%, 92.67, and 9.12% over NF and CF at the 60, 90 and 120 DAS respectively (Figure 4.6.5).

By application of IVA was 93.73, 92.63, and 92.28% over NF at 60, 90 and 120 DAS and 8.39, 92.28, 4.34% over CF at 90 and 120 DAS respectively. The phosphate content of soil and plant leaves was also analysed linearly significant at 120 DAS.



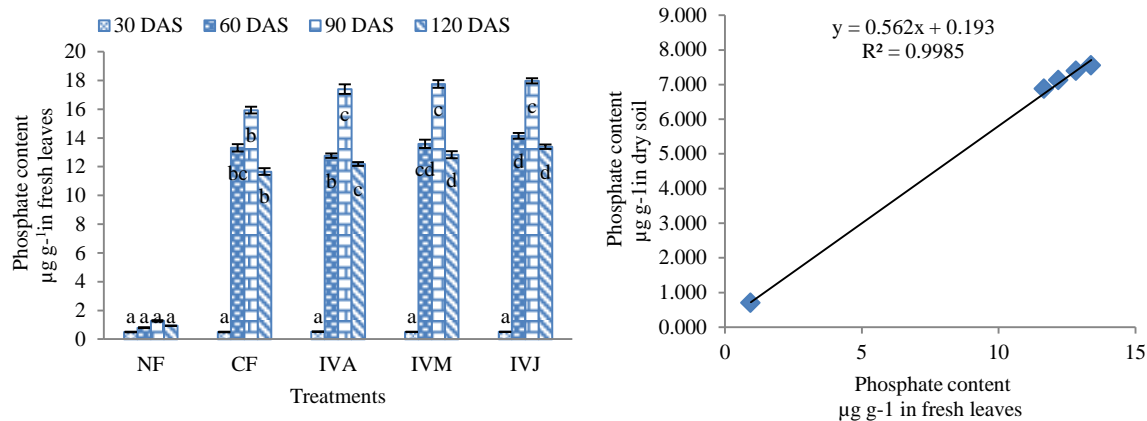


Figure 4.6.5 Levels of phosphate ($\mu\text{g g}^{-1}$) in dry soil and plant tissue of *T. aestivum* L. at 30, 60, 90 and 120 DAS in different treatments Values followed by different letters are significantly differences between the treatments at $P < 0.05$. (Other details are described in table 4.6.1).

4.6.7 Levels of NRA Content in Root and Leaves of Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

In root system the nitrate reductase (NR) activity was increased the level by 28.11 and 7.13% on amending of IVJ over the CF at 60 and 90 DAS. The application applied with IVM was also increased 19.37 and 2.84% in leaves of wheat plant at the 60 and 90 DAS over CF (Figure 4.6.6).

The nitrate reductase activity as same in roots, application with IVJ was significantly recorded by 24.28 and 31.15% at 60 and 90 DAS. On application on IVM was also increased by 16.39 and 27.68% over NF and CF respectively (Figure 4.6.6).

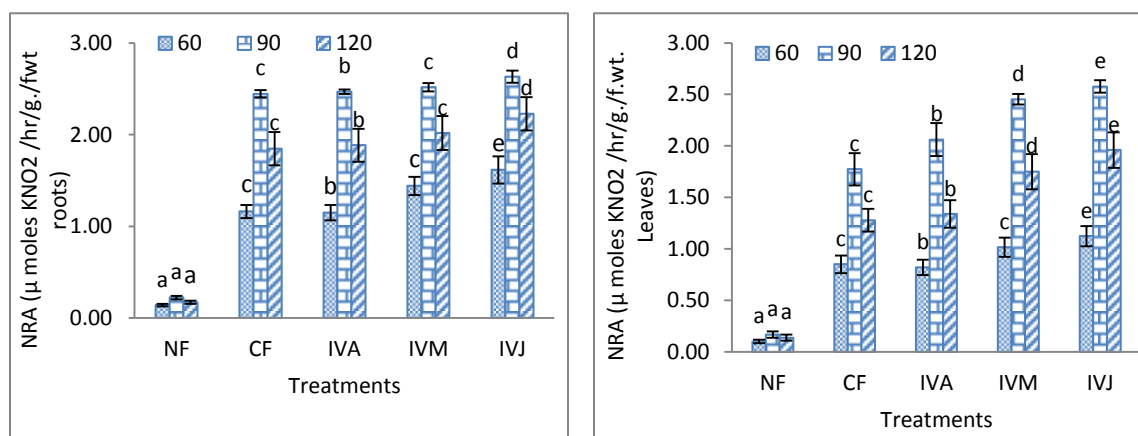


Figure 4.6.6 Levels of NRA ($\mu\text{g g}^{-1}$) in dry soil and plant tissue of *T. aestivum* L. at 30, 60, 90 and 120 DAS in different treatments Values followed by different letters are significantly differences between the treatments at $P < 0.05$. (Other details are described in table 4.6.1).

4.6.8 Level of Dehydrogenase Activity DHA in Rhizospheric Soil, of Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

The dehydrogenase activity (DHA) in soil was significantly increased on application of IVJ was 54.63, 25.64, 12.91 and 6.31% over NF, CF, IVA and IVM at 60 DAS. The application on IVM was also increased by 20.63 and 7.05% in soil of wheat plant at the 60 DAS over CF and IVA and on application of IVA by 14.60 over CF at 60 DAS (Figure 4.6.7).

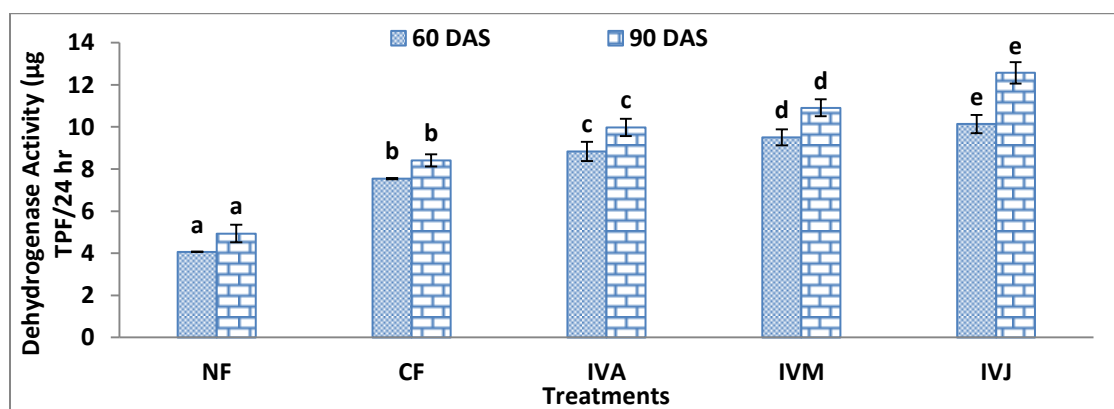


Figure 4.6.7 Dehydrogenase activity ($\mu\text{g TPF}/24 \text{ hr}$) in soil of experimental plot cultivated with *T. aestivum* L. at 60 and 90 DAS in different treatments. One way analysis of variance was used to compare the means. Values followed by different letters are significantly different at $P < 0.05$. (Other details are described in table 4.6.1)

4.6.9 Level of Alkaline Phosphate Activity (mg/g/hr) in Soil, of Wheat (*T. aestivum* L.) Plant Parts on Application of Immobilized Biofertilizers

Alkaline phosphate activity (APA) (mg/g/hr) in soil increased application on IVJ was 40.61, 19.87, 13.93 and 7.16% over NF, CF, IVA and IVM at 60 DAS. Application on IVM was also increased by 35.71, 13.69 and 7.29% at 60 DAS respectively. In soil APA significantly increased by amending organic matrix over NF and CF by 30.99 and 6.89% at 60 DAS respectively on application of IVA. Similarly APA also significantly increased at 90 DAS (Figure 4.6.8).

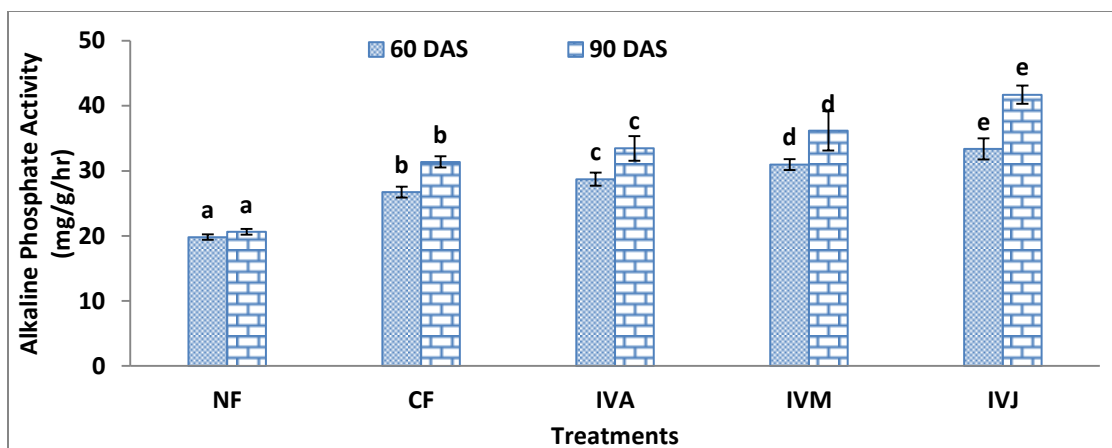


Figure 4.6.8 Alkaline phosphate activity (mg/g/hr) in soil of experimental plot cultivated with *T. aestivum* L. at 60 and 90 DAS in different treatments. One way analysis of variance was used to compare the means. Values followed by different letters are significantly different at $P < 0.05$. (Other details are described in table 4.6.1)

4.6.10 Grain, Straw Yield and Harvesting Index of Wheat Crop by the Application of Biofertilizers with Organic Matrix

Biofertilizers immobilized with organic matrix increases significant seed yield of wheat (*T. aestivum* L.) during cultivation. The percentage increased the seed yield (q/ha) on application of immobilized form of biofertilizers with organic matrix used jaggery as binder (IVJ) over the application of no fertilizer (NF) and free form of chemical fertilizers (CF) after the harvest of crop was 77.97 and 21.71% respectively. Application with Immobilized form of biofertilizers with organic matrix using molasses substrate as binder (IVM) has increases the percentage of yield was 76.36 and 15.99% over the controls of NF and CF respectively. Application with Immobilized form of biofertilizers with organic matrix using acacia gum substrate as binder (IVA) has increases by 75.77 and 13.89% over the controls of NF CF respectively at 120 DAS. Application of IVJ increases the yield over the IVA and IVM by 9.08 and 6.81% respectively at 120 DAS.

The straw production were also increased significantly by the application of Immobilized form of biofertilizers with organic matrix using jaggery substrate as binder (IVJ) at 120 DAS was 69.65 and 16.82% over the NF and CF. Immobilized form of biofertilizers with organic matrix using molasses substrate as binder (IVM) recorded by 68.92 and 14.80% respectively and IVA by 67.88 and 11.95% at 120 DAS old plants.

By the application of immobilized form of biofertilizers with organic matrix using various substrates as immobilizing agent in the formulation of SGBs is directly related to biomass of wheat crop produced and crop yield during cultivation of wheat. The harvest index was increased by the application of immobilized form of biofertilizers with organic matrix using jaggery substrate as a binder by 18.63, 3.77, 2.51 and 3.02% over NF, CF, IVA and IVM at 120 DAS respectively. Application applied with immobilized form of biofertilizers with organic matrix using molasses as immobilizing agent was recorded by 16.10 and .77% over NF and CF, by the application of IVA was 16.53 and 1.29% over NF and CF respectively at 120 DAS.

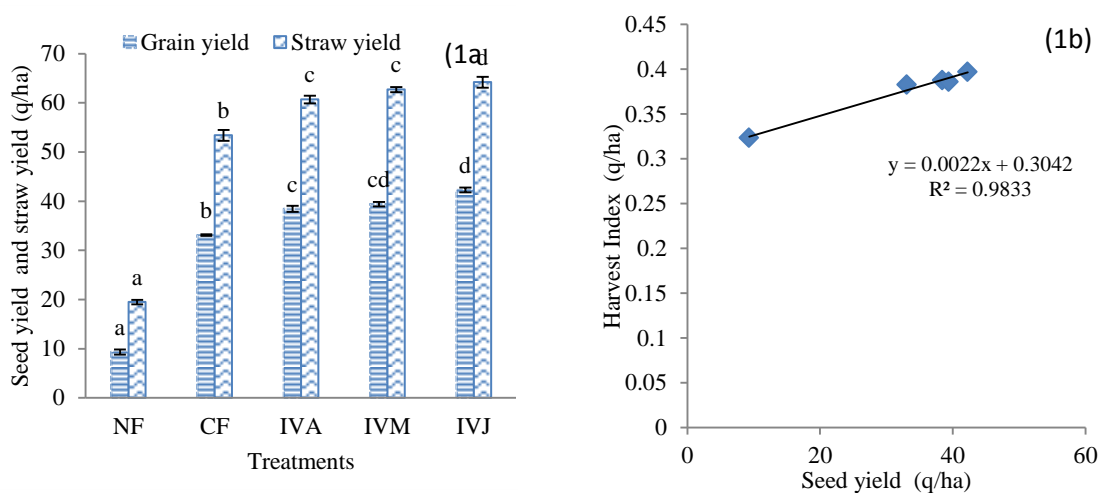


Figure 4.6.9 Effect of organic matrix based co-immobilized biofertilizers on seed yield, straw yield and harvesting index at 120 (DAS) old plants. Values are mean of data ($n=6$) \pm SE. differences are statistically significant and shown different alphabets using Duncan's multiple range test (DMRT). Data analysed by one way ANOVA at <0.05 . (Other details are described in table 4.6.1)



Plate: 4.6.1 Effect of immobilized biofertilizers on wheat plants at 60 DAS. (Other details are described in table 4.6.1)

4.7. Growth and productivity of wheat (*T. aestivum* L. cv. PBW-502) applied with charcoal based biofertilizers (*A. chroococcum* and *B. subtilis*) in triple dose under the stress condition (salinity and high cadmium) on 30, 60, 90 and 120 days after sowing (DAS)

4.7.1 Growth Parameters of wheat plant applied with Immobilized and Optimized Biofertilizer on 30, 60, 90 and 120 days after sowing (DAS)

The significant increase in growth parameters was recorded on application of charcoal based biofertilizers (*A. chroococcum* and *B. subtilis*; R/D = 0.6 kg h⁻¹) with enhanced dose (*A. chroococcum* and *B. subtilis*; R/D = 0.18 kg h⁻¹) immobilized in organic carriers under salinity and high cadmium (Cd) stresses. The application of immobilized biofertilizer significantly increased shoot height by IVJ(SL) was 50.58, 48.38, 43.00, 17.78, 38.04, 19.05, 30.05 and 43.21% at 60 DAS, 42.15, 39.51, 31.86, 6.65, 28.15, 20.15, 11.05 and 29.38% at 120 DAS respectively over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Table-4.7.1).

Application on IVJ(SL) significantly increased number of leaves over their respective doses of charcoal based biofertilizers immobilized form on 90 DAS was 71.13, 61.86, 53.61, 17.53, 60.82, 47.42, 31.96 and 53.61% over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Table-4.7.1).

The percentage of fresh weight of shoot was significantly recorded by 73.81, 54.61, 37.42, 15.24, 44.83, 19.06, 28.71 and 46.44% at 60 DAS and 65.66, 46.66, 27.07, 15.04, 35.43, 30.51, 25.34 and 43% at 120 DAS old plants on application of IVJ(SL) over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Table-4.7.1).

The dry weight of shoot was significantly increased on application of 72.26, 48.52, 47.92, 10.22, 41.57, 24.49, 14.27 and 33.71% at 60 DAS and 64.37, 47.99, 26.99, 23.05, 35.88, 23.14, 12.43 and 41.25% at 120 days after sowing respectively over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Table 4.7.1).

Table 4.7.1 Effect of Immobilized Biofertilizers under Stress of Salinity and Cadmium, on Plant Growth Parameters of Wheat (*T. aestivum* L. cv. PBW-502) Shoot on 30, 60, 90 and 120 DAS

	30 days	60 days	90 days	120 days
Plant Height				
NF	23.67±2.04 ^b	35.67±1.65 ^a	45.10±1.34 ^a	49.30±2.35 ^a
CF	23.80±3.05 ^b	58.45±1.33 ^e	75.20±2.48 ^e	82.47±4.48 ^{ef}
BFFOM	22.23±3.34 ^{ab}	37.25±1.88 ^a	48.08±1.10 ^a	51.55±0.83 ^a
IVS(SL+Cd)	20.07±1.46 ^a	41.13±1.78 ^b	53.57±1.73 ^b	58.07±3.61 ^b
IVJ(SL)	23.20±3.72 ^{ab}	72.17±4.55 ^f	80.05±5.09 ^e	85.22±2.89 ^f
IVJ(Cd)	23.53±1.67 ^{ab}	59.33±3.26 ^e	74.12±2.97 ^e	79.55±2.84 ^{de}
IVJ(SL+Cd)	22.70±3.87 ^{ab}	44.72±2.28 ^c	60.87±4.83 ^c	61.23±2.01 ^b
SCVJ(SL+Cd)	35.85±2.35 ^c	58.42±3.93 ^e	65.87±3.89 ^d	68.05±6.59 ^c
IVM(SL)	22.53±1.75 ^{ab}	50.48±3.31 ^d	69.70±2.77 ^d	75.80±2.94 ^d
IVM(SL+Cd)	22.45±1.63 ^{ab}	40.98±1.45 ^b	56.31±4.85 ^b	60.18±3.53 ^b
No. of leaves				
NF	2.50±0.55 ^a	3.33±0.52 ^a	4.67±0.52 ^a	5.17±0.98 ^a
CF	3.00±0.00 ^a	7.33±1.75 ^b	9.17±1.17 ^c	11.33±3.01 ^{cd}
BFFOM	3.00±0.00 ^a	4.83±0.75 ^a	6.17±0.75 ^{ab}	7.00±1.10 ^{ab}
IVS(SL+Cd)	3.17±0.41 ^a	5.17±0.75 ^a	7.50±1.64 ^{bc}	7.50±1.22 ^{ab}
IVJ(SL)	3.67±1.03 ^a	10.17±2.40 ^c	16.17±1.60 ^f	16.00±1.41 ^e
IVJ(Cd)	3.00±0.00 ^a	9.50±2.51 ^c	13.33±2.50 ^e	12.67±2.25 ^d
IVJ(SL+Cd)	3.17±0.41 ^a	4.67±0.52 ^a	6.33±0.52 ^{ab}	7.83±1.33 ^b
SCVJ(SL+Cd)	5.00±0.63 ^a	13.50±1.76 ^d	8.50±2.26 ^c	11.67±2.42 ^{cd}
IVM(SL)	3.83±0.75 ^a	9.83±1.72 ^c	11.00±1.55 ^d	12.00±2.53 ^d
IVM(SL+Cd)	3.00±0.00 ^a	4.67±0.52 ^a	7.50±1.22 ^{bc}	9.33±1.75 ^{bc}
Fresh wt. of Shoot				
NF	0.54±0.05 ^a	1.05±0.07 ^a	1.64±0.18 ^a	1.97±0.10 ^a
CF	0.57±0.05 ^a	2.74±0.23 ^{de}	4.04±0.29 ^{ef}	4.82±0.15 ^f
BFFOM	0.52±0.05 ^a	1.83±0.34 ^b	2.55±0.11 ^b	3.05±0.18 ^b
IVS(SL+Cd)	0.53±0.12 ^a	2.52±0.32 ^d	2.86±0.06 ^c	4.18±0.36 ^{de}
IVJ(SL)	0.57±0.06 ^a	4.02±0.15 ^g	5.08±0.24 ^g	5.73±0.28 ^g
IVJ(Cd)	0.52±0.10 ^a	3.41±0.12 ^f	4.26±0.19 ^f	4.87±0.22 ^f
IVJ(SL+Cd)	0.50±0.09 ^a	2.22±0.15 ^c	3.12±0.14 ^c	3.70±0.17 ^c
SCVJ(SL+Cd)	1.50±0.09 ^b	3.26±0.11 ^f	3.76±0.38 ^{de}	3.98±0.40 ^d
IVM(SL)	0.58±0.06 ^a	2.87±0.10 ^e	3.91±0.19 ^e	4.28±0.23 ^e
IVM(SL+Cd)	0.52±0.06 ^a	2.16±0.25 ^c	3.55±0.46 ^d	3.26±0.07 ^b
Dry wt. of Shoot				
NF	0.06±0.02 ^a	0.21±0.01 ^a	0.49±0.05 ^a	0.79±0.04 ^a
CF	0.06±0.01 ^a	0.65±0.05 ^f	1.21±0.09 ^{ef}	1.85±0.13 ^e
BFFOM	0.05±0.01 ^a	0.39±0.01 ^b	0.77±0.03 ^b	1.16±0.07 ^b

IVS(SL+Cd)	0.05±0.01 ^a	0.40±0.03 ^b	0.86±0.02 ^c	1.62±0.21 ^d
IVJ(SL)	0.06±0.01 ^a	0.76±0.03 ^g	1.49±0.10 ^g	2.22±0.10 ^f
IVJ(Cd)	0.05±0.01 ^a	0.68±0.02 ^f	1.17±0.06 ^e	1.71±0.09 ^d
IVJ(SL+Cd)	0.05±0.01 ^a	0.44±0.03 ^c	0.94±0.04 ^c	1.43±0.06 ^c
SCVJ(SL+Cd)	0.06±0.01 ^a	0.57±0.02 ^f	1.18±0.09 ^e	1.71±0.09 ^d
IVM(SL)	0.24±0.02 ^b	0.65±0.02 ^e	1.28±0.06 ^f	1.95±0.09 ^e
IVM(SL+Cd)	0.05±0.01 ^a	0.50±0.06 ^d	1.06±0.14 ^d	1.31±0.03 ^c

The optimized dose of biofertilizers at 30, 60, 90, and 120 days after shown (DAS) . One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values by different letters are significantly differences between the treatments at 5% level. [Where NF; No fertilizer, CF- Free form of recommended chemical fertilizers, BFFOM; Free form of biofertilizers and organic matrix, IVS(SL+Cd); Immobilized form of biofertilizers with salinity and Cd (Sareash as binder), IVJ(SL); Immobilized form of biofertilizers with salinity, IVJ(Cd); Immobilized form of biofertilizers with Cd (Jaggery as binder), IVJ(SL+Cd); Immobilized form of biofertilizers with salinity and Cd (Jaggery as binder), SCVJ(SL+Cd); Seed coated with biofertilizers and vermicompost+ salinity and Cd, IVM(SL); Immobilized form of biofertilizers with salinity (Molasses as binder), IVM(SL+Cd); Immobilized form of biofertilizers with salinity and Cd (Molasses as binder)]

Application of immobilized biofertilizers increased the root length, number of roots, fresh and dry weight of roots significantly over no fertilizers on 30, 60, 90 and 120 days after sowing (DAS) under stress conditions. An incremental dose of charcoal based as well as organic matrix immobilized biofertilizers threefold increases the plant growth very significantly. The prepared immobilized form of biofertilizers with organic matrix under this study enhanced the growth parameters. The percentage of root length increased by 60.84, 31.07, 20.05, 10, 15.58, 12.27, 10 and 14.40% at 90 DAS was recorded on application of IVJ(SL) over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Table-4.7.2).

Biofertilizers (1.8 kg ha^{-1}) in immobilized form IVJ(SL) caused a very significant growth in root biomass over the recommended dose (0.6 kg ha^{-1}) of biofertilizers and recommended dose of chemical fertilizers (urea; 150 kg ha^{-1} and DAP 75; kg ha^{-1}). The fresh weight of root increased by 66.31, 49.04, 42.71, 16.87, 41.60, 17.82, 16.87 and 44.57% at 90 day, 66.56, 50.15, 43.68, 16.97, 41.52, 23.77, 22.02 and 44.28% at 120 DAS respectively over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) on application of the IVJ(SL) (Table-4.7.2).

The percentage increase of 57.58, 54.61, 52.78, 21.34, 38.05, 12.93, 14.70 and 47.38% at 120 DAS in dry weight of roots on the application of IVJ(SL) over NF, CF, BFFOM, IVS(SL+Cd), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Table-4.7.2).

Table 4.7.2 Effect of Immobilized Biofertilizers under Stress of Salinity and Cadmium, on Plant Growth Parameters of Wheat (*T. aestivum* L. cv. PBW-502) Roots on 30, 60, 90 and 120 DAS

No. of Roots				
NF	3.33±1.03 ^a	5.17±1.83 ^a	6.50±0.55 ^a	7.00±0.63 ^a
CF	4.00±0.89 ^{a b}	10.33±2.25 ^b	15.17±4.31 ^{bc}	16.33±6.22 ^{bc}
BFFOM	4.00±0.89 ^{a b}	9.00±2.76 ^b	10.83±1.83 ^b	12.33±4.50 ^b
IVS(SL+Cd)	4.33±1.21 ^{abc}	10.50±2.51 ^b	13.00±2.19 ^{bc}	14.33±3.01 ^b
IVJ(SL)	4.33±1.03 ^{bc}	15.17±4.88 ^c	20.00±4.47 ^d	20.67±4.89 ^c
IVJ(Cd)	4.17±0.75 ^{bc}	12.17±2.40 ^{bc}	15.67±3.44 ^c	17.00±3.79 ^{bc}
IVJ(SL+Cd)	4.00±0.63 ^{ab}	8.67±1.97 ^{de}	12.83±4.31 ^{bc}	13.50±5.43 ^b
SCVJ(SL+Cd)	4.83±0.41 ^c	11.00±3.79 ^b	14.00±3.79 ^{bc}	14.00±4.38 ^b
IVM(SL)	5.67±0.52 ^c	11.00±2.37 ^b	16.17±3.92 ^{cd}	14.50±2.35 ^b
IVM(SL+Cd)	4.00±0.89 ^{a b}	10.50±2.51 ^b	12.83±3.54 ^{bc}	13.17±2.79 ^b
Root length				
NF	2.57±0.47 ^a	3.68±0.47 ^a	4.03±0.69 ^a	5.55±0.50 ^a
CF	2.67±0.40 ^a	7.40±0.66 ^{cde}	8.82±0.60 ^{cd}	10.03±0.68 ^{cd}
BFFOM	2.84±0.40 ^a	6.62±0.77 ^b	7.10±0.40 ^b	8.05±0.33 ^b
IVS(SL+Cd)	2.67±0.50 ^a	6.86±0.56 ^c	8.24±0.62 ^c	8.85±0.40 ^{bc}
IVJ(SL)	2.71±0.53 ^a	8.02±0.43 ^f	10.30±0.54 ^e	10.55±0.80 ^d
IVJ(Cd)	2.85±0.14 ^a	7.77±0.37 ^f	9.27±0.83 ^d	9.08±0.80 ^b
IVJ(SL+Cd)	2.55±0.33 ^a	7.10±0.30 ^{cd}	8.70±0.56 ^d	9.00±0.55 ^{bcd}
SCVJ(SL+Cd)	4.48±0.47 ^b	7.77±0.41 ^{d ef}	9.04±0.48 ^d	8.88±0.60 ^{bcd}
IVM(SL)	2.85±0.29 ^a	7.61±0.21 ^{def}	9.27±0.83 ^d	9.95±0.30 ^{cd}
IVM(SL+Cd)	2.61±0.44 ^a	7.35±0.18 ^{cde}	8.82±0.44 ^{cd}	8.35±0.36 ^{bc}
Fresh wt. of Root				
NF	0.03±0.01 ^a	0.07±0.01 ^a	0.10±0.01 ^a	0.12±0.01 ^a
CF	0.03±0.01 ^{a b}	0.17±0.02 ^c	0.28±0.03 ^d	0.30±0.02 ^e
BFFOM	0.03±0.00 ^a	0.09±0.01 ^{a b}	0.15±0.01 ^{ab}	0.18±0.02 ^b
IVS(SL+Cd)	0.02±0.01 ^a	0.12±0.04 ^{a b}	0.17±0.02 ^b	0.20±0.02 ^{bc}
IVJ(SL)	0.03±0.01 ^{ab}	0.19±0.02 ^c	0.30±0.03 ^b	0.35±0.03 ^f
IVJ(Cd)	0.03±0.01 ^a	0.17±0.02 ^c	0.25±0.02 ^d	0.29±0.02 ^{de}
IVJ(SL+Cd)	0.04±0.01 ^b	0.12±0.04 ^b	0.17±0.01 ^b	0.21±0.02 ^c
SCVJ(SL+Cd)	0.03±0.01 ^{ab}	0.23±0.02 ^d	0.25±0.02 ^b	0.27±0.03 ^d
IVM(SL)	0.18±0.01 ^c	0.12±0.04 ^b	0.25±0.02 ^b	0.28±0.01 ^{de}
IVM(SL+Cd)	0.03±0.00 ^a	0.11±0.01 ^b	0.17±0.01 ^b	0.20±0.02 ^{bc}

Dry wt. of Root				
NF	0.04±0.00 ^a	0.04±0.01 ^a	0.05±0.01 ^a	0.06±0.01 ^a
CF	0.04±0.02 ^a	0.08±0.00 ^d	0.12±0.01 ^e	0.13±0.01 ^f
BFFOM	0.05±0.00 ^a	0.05±0.01 ^{ab}	0.05±0.01 ^{ab}	0.06±0.01 ^a
IVS(SL+Cd)	0.05±0.01 ^a	0.06±0.02 ^c	0.05±0.01 ^a	0.06±0.01 ^{ab}
IVJ(SL)	0.04±0.01 ^a	0.10±0.01 ^e	0.13±0.01 ^f	0.14±0.01 ^f
IVJ(Cd)	0.05±0.00 ^a	0.09±0.01 ^{de}	0.10±0.00 ^d	0.11±0.00 ^d
IVJ(SL+Cd)	0.05±0.00 ^a	0.06±0.02 ^c	0.07±0.01 ^c	0.08±0.01 ^c
SCVJ(SL+Cd)	0.07±0.01 ^b	0.11±0.01 ^f	0.12±0.01 ^e	0.12±0.01 ^e
IVM(SL)	0.04±0.00 ^a	0.08±0.01 ^d	0.11±0.01 ^e	0.12±0.01 ^{de}
IVM(SL+Cd)	0.04±0.01 ^a	0.05±0.01 ^{bc}	0.06±0.00 ^b	0.07±0.01 ^b

Effect of optimized dose of biofertilizers on growth of roots on 30, 60, 90, and 120 days after shown (DAS), (Other details are described in Table. 4.7.1)

4.7.2 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Rhizospheric Soil, of Wheat (*T. aestivum* L.) Plant Applied with Immobilized Form of Biofertilizers under Stress of Salinity and Cadmium

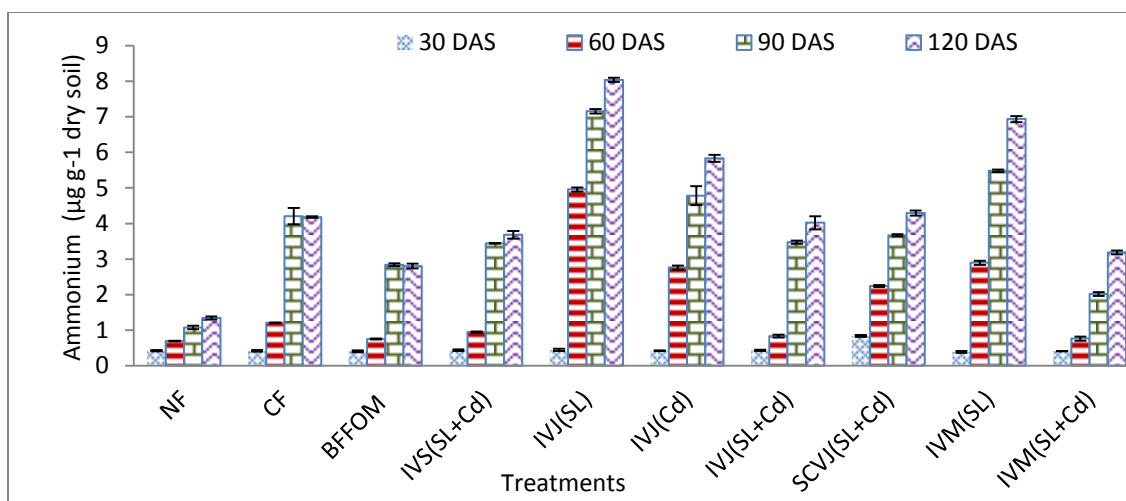
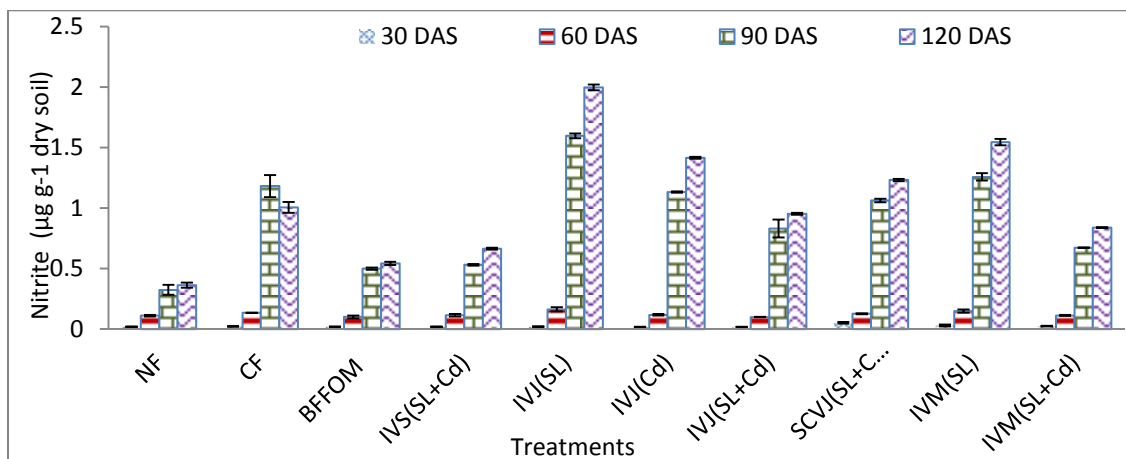
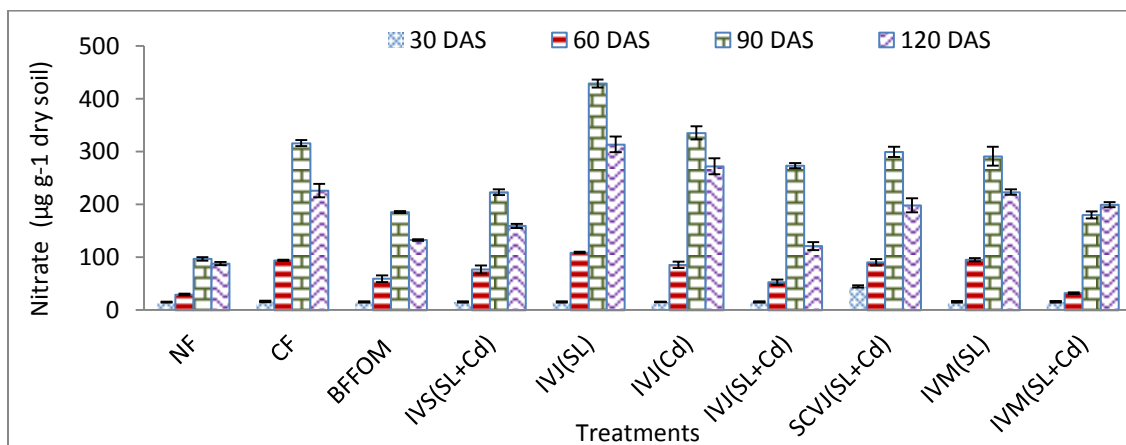
The soil nitrate content increased by the application on IVJ(SL) was 72.90, 45.33, 29.28, 21.37, 51.63, 17.09, 12.50 and 70.42% at 60 and 77.40, 56.68, 47.93, 21.77, 36.18, 30.16, 32.09 and 58.00 at 90 DAS over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Figure 4.7.1).

The nitrite content in the soil on application with IVJ(SL) recorded significantly and it increased at different level by 32.01, 38.10, 29.81, 27.78, 38.80, 22.02, 8.51 and 31.09% and 79.71, 68.68, 66.73, 29.14, 47.98, 33.47, 21.26 and 57.94% and 81.80, 72.81, 66.73, 29.14, 52.26, 38.30, 22.65 and 57.94% at 60, 90 and 120 DAS respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Figure 4.7.1).

Ammonium content was recorded different level by IVJ(SL) by ammonium in soil 86.02, 84.87, 80.86, 44.29, 83.22, 54.71, 41.54 and 84.60% at 60 DAS 83.28, 65.16, 54.24, 27.50, 49.96, 46.60, 13.72 and 60.35% at 120 DAS respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Figure 4.7.1).

The phosphate content in the root, the application with IVJ(SL) has recorded significantly and it increase at different level by 76.97, 45.96, 32.54, 17.17, 33.36,

18.05, 13.78 and 37.39% and 86.78, 50.53, 32.40, 19.46, 35.67, 23.75, 11.66 and 45.86% at 60 and 120 DAS respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.1).



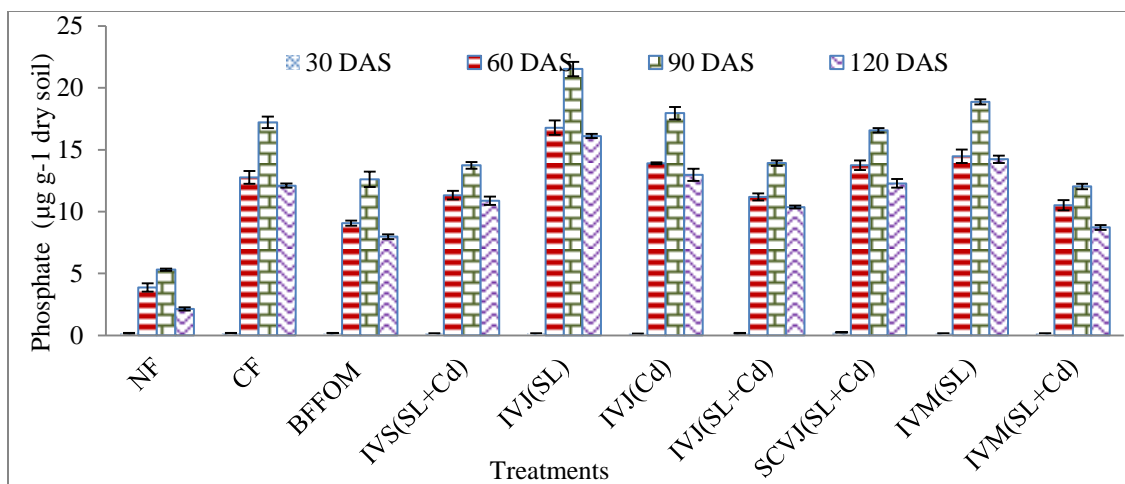


Figure 4.7.1 The effect of optimized dose of biofertilizers on nutrient availability in the soil on 30, 60, 90, and 120 days after sowing. All the values are means of three replicates with two determinations ($n=6$) \pm S.E). (DAS). (Other details are described in Table. 4.7.1)

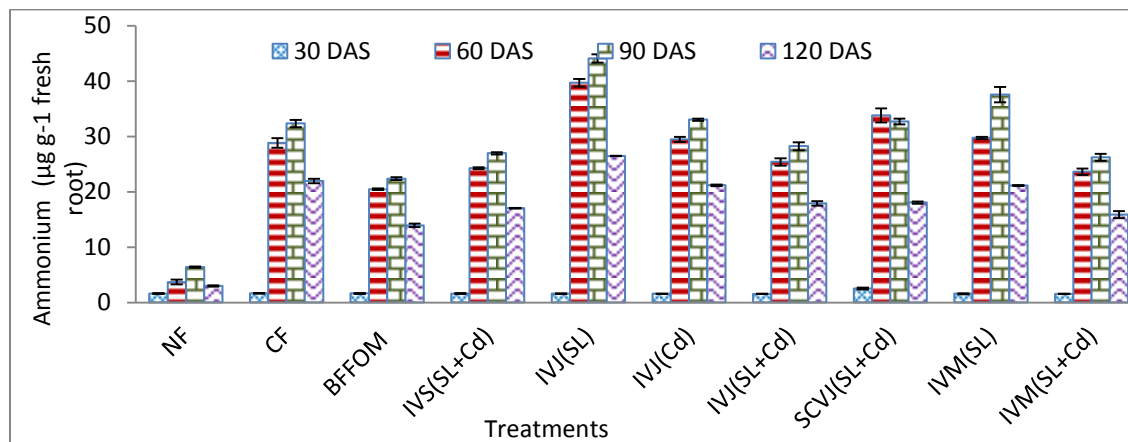
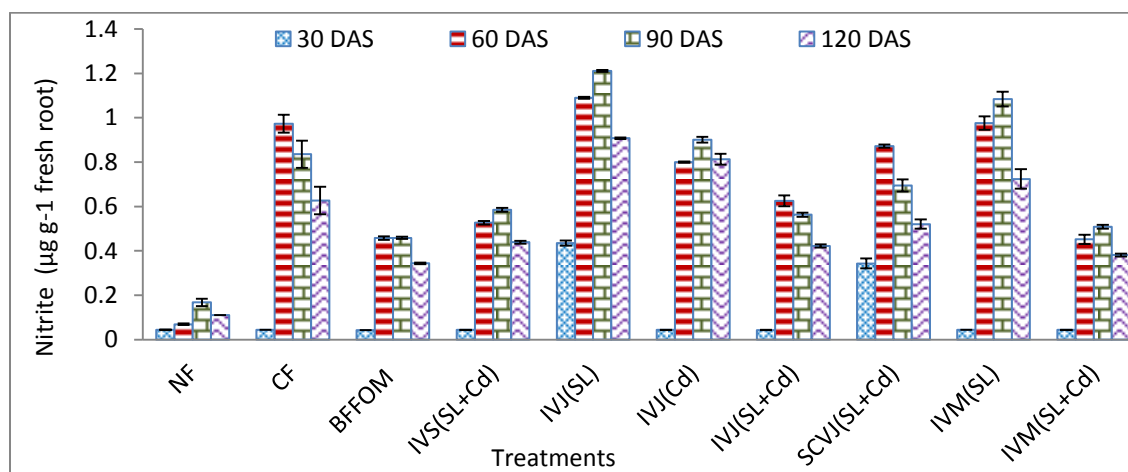
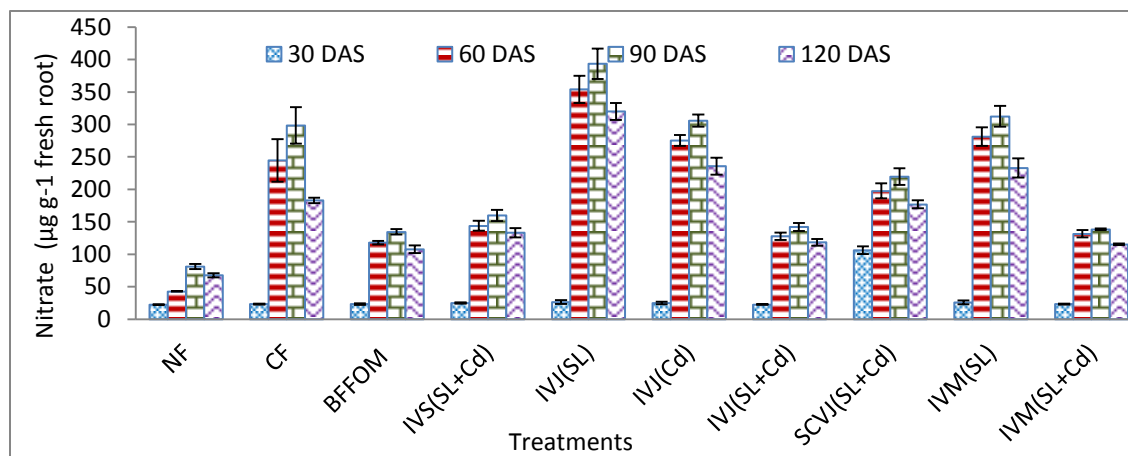
4.7.3 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Roots of Wheat (*T. aestivum* L.) Plant Applied with Immobilized Form of Biofertilizers under Stress of Salinity and Cadmium

The organic matrix immobilized biofertilizers also increased root nitrate content in under stress condition on application of IVJ(SL) increased by 87.90, 66.71, 59.36, 22.22, 63.88, 44.18, 20.57, 62.86% at 60 days after sowing respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.2).

The root nitrite increased by 93.67, 57.99, 51.65, 26.64, 42.64, 19.99, 10.43 and 58.53% at 60 DAS 87.86, 62.12, 51.65, 10.43, 53.51, 42.64, 20.26, 57.99% at 120 DAS, respectively, with application of IVJ(SL) over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.2).

The ammonium content in root of wheat plant was increased by ammonium in root 90.68, 48.43, 38.88, 25.82, 36.01, 14.82, 25.10 and 40.51% at 60 DAS, 88.73, 47.40, 35.65, 20.02, 32.41, 31.82, 20.15 and 40.12% at 120DAS with the application on IVJ(SL) over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.2).

The root phosphate increased on application of IVJ(SL) was , 95.17, 53.53, 28.50, 23.00, 33.61, 18.90, 10.53 end 37.96% at 60 DAS 93.33, 51.24, 24.97, 19.20, 30.33, 15.44, 1.79 end 34.90% at 90 DAS over applications on NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.2).



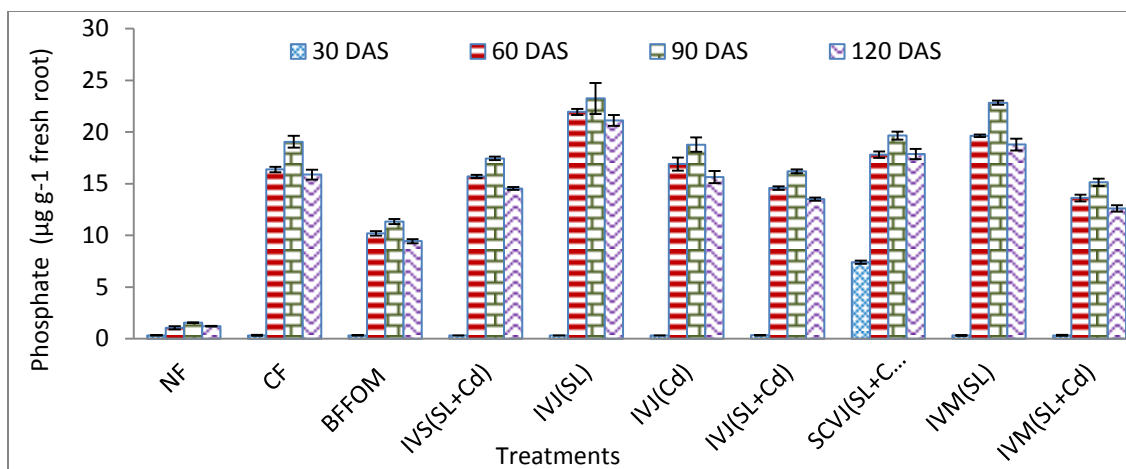


Figure 4.7.1 The effect of optimized dose of biofertilizers on nutrient availability in the soil on 30, 60, 90, and 120 days after sowing. All the values are means of three replicates with two determinations ($n=6 \pm S.E.$). (Other details are described in Table. 4.7.1)

4.7.4 Level of Nitrate, Nitrite, Ammonium and Phosphate Content in Leaves of Wheat (*T. aestivum* L.) Plant Applied with Immobilized Form of Biofertilizers under Stress of Salinity and Cadmium

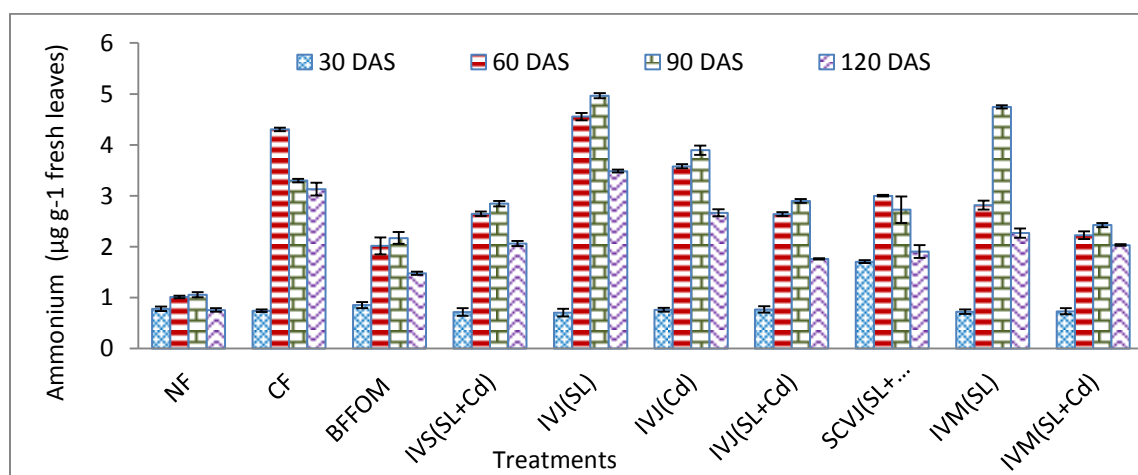
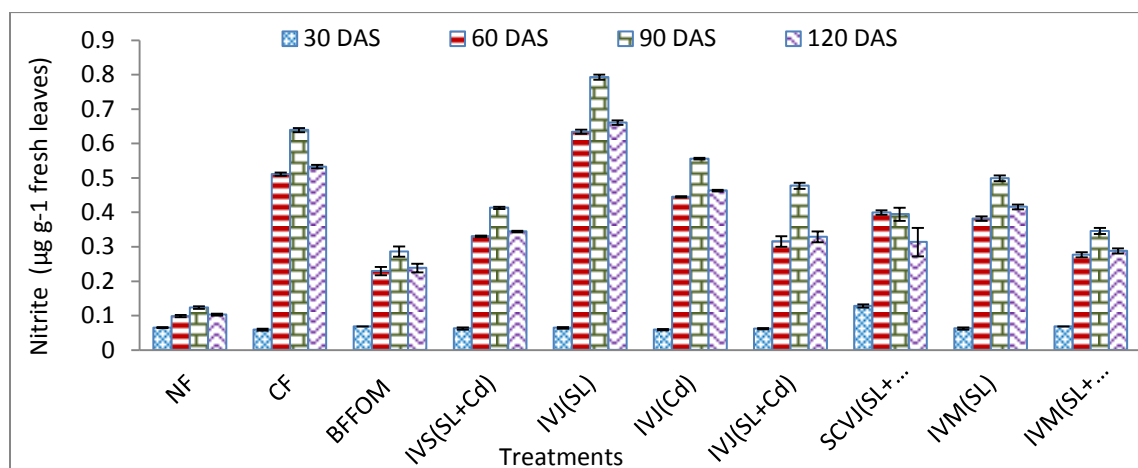
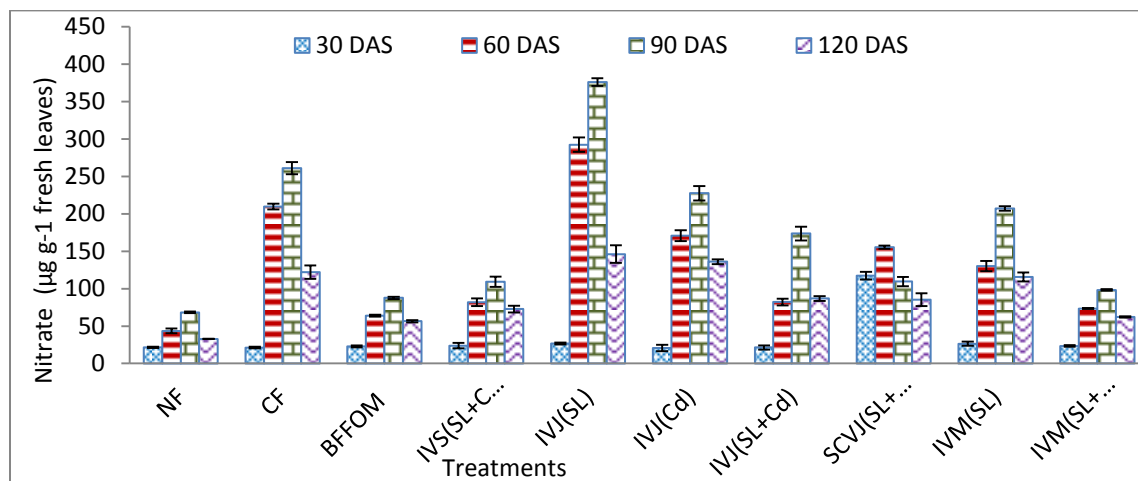
Nitrate content in leaves increased when applied with enhanced dose of biofertilizers (1.8 kg ha^{-1}) immobilized with organic matrix; IVJ(SL) used jaggery as a nutritive binder was recorded by 85.12, 78.13, 72.01, 41.63, 71.92, 46.87, 55.46 and 74.88% at 60 DAS and 77.54, 61.38, 50.25, 7.00, 40.52, 41.69, 20.82 and 57.36% at 120 days after sowing respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.3).

Nitrite content in wheat leaves increased by 84.45, 63.90, 47.90, 29.85, 39.80, 50.25, 37.07 and 56.37% 90 days after sowing applied with IVJ(SL) over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.3).

In leaves the ammonium content assimilates and was recorded different level IVJ(SL) by 7.80, 55.75, 41.89, 21.43, 42.05, 34.13, 38.18, 51.18% sixty 78.33, 57.66, 40.83, 23.54, 49.46, 45.33, 34.92 and 41.69% at 60 and 120 DAS respectively over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) (Figure 4.7.3).

The increased phosphate content assimilation in leaves by the application of IVJ(SL) was 86.89, 80.17, 65.74, 25.98, 56.24, 36.43, 38.78 and 72.81% at 60 DAS

respectively over on applications of NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) respectively (Figure 4.7.3).



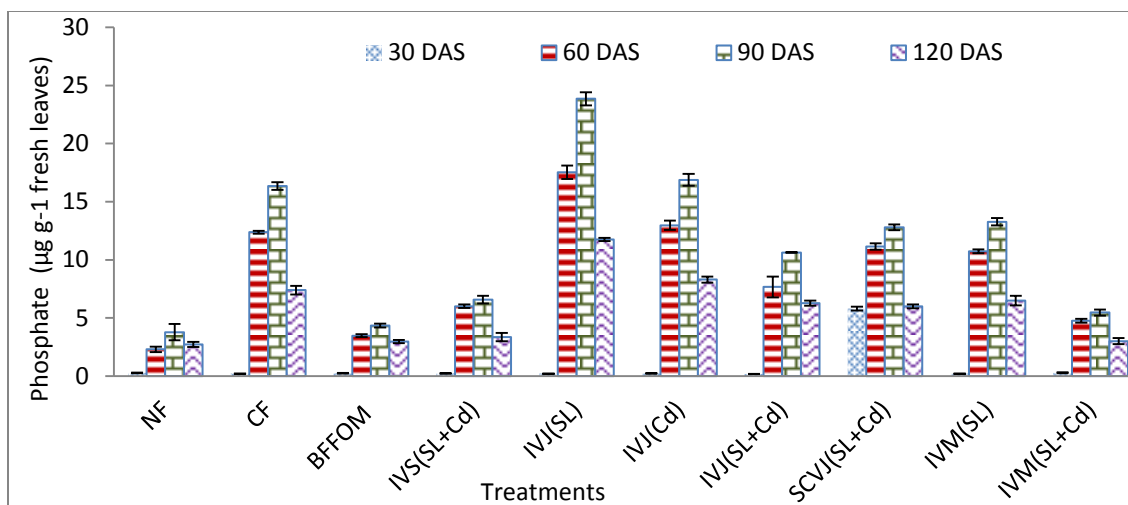


Figure 4.1.3 Nutrient concentrations in the leaves on 30, 60, 90, and 120 DAS the seeds. (Other details are described in Table. 4.7.1)

4.7.5 Yield and Productivity of Wheat (*T. aestivum* L.) Plant Applied with Immobilized Form of Biofertilizers under Stress of Salinity and Cadmium

Application of immobilized biofertilizers (*A. chroococcum* and *B. subtilis*) increased the yield (g plant^{-1}). Enhanced dose of biofertilizers increased grain yield by 63.07, 56.54, 49.67, 21.26, 56.54, 38.4, 18.19 and 68.46% respectively on application of IVJ(SL) over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) after harvesting at 120 DAS (Figure 4.7.4).

The straw yield was significantly increased by 65.66, 46.66, 27.07, 15.04, 35.43, 30.51, 25.34 and 43% on after harvesting with the application on of IVJ(SL) over NF, BFFOM, IVS(SL+Cd), IVJ(SL), IVJ(Cd), IVJ(SL+Cd), SCVJ(SL+Cd), IVM(SL) and IVM(SL+Cd) after harvesting at 120 DAS respectively (Figure 4.7.4).

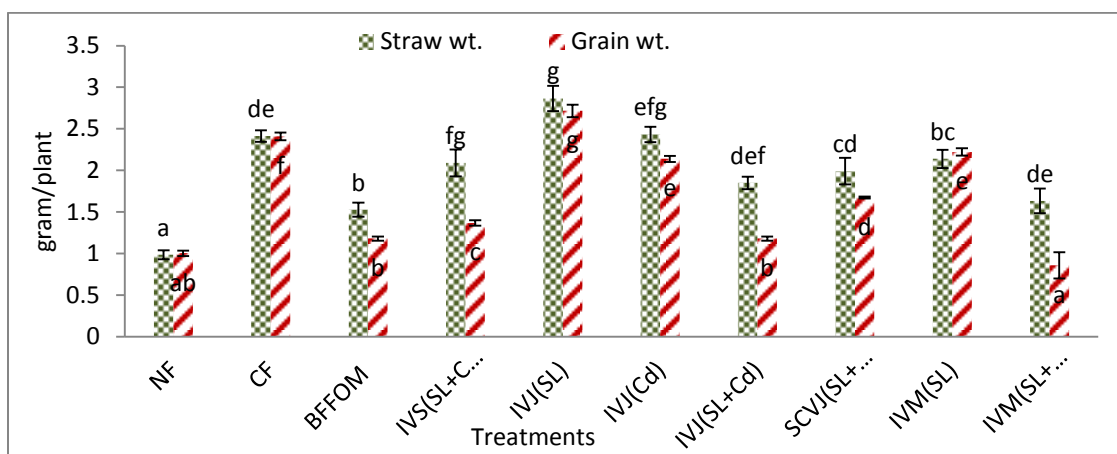


Figure 4.7.4 Application of optimized dose of biofertilizers in immobilized form on soil significantly increased grain yield and straw yield. (Other details are described in Table. 4.7.1)

4.8. Growth and Productivity of Wheat (*T. aestivum* L. cv. PBW-502) Applied with Charcoal Based Biofertilizers (*A. chroococcum* and *B. subtilis*) in Triple Dose under Drought Condition on 30, 60, 90 and 120 Days After Sowing (DAS)

4.8.1 Growth Parameters of Wheat Applied with Immobilized biofertilizer under Drought Stress, Recorded on 30, 60, 90 and 120 Days After Sowing (DAS)

There was a significant increases in growth parameters on application of immobilized form of charcoal based optimised biofertilizers (*A. chroococcum* and *B. subtilis*; R/D = 0.18 kg h⁻¹) with vermicompost as a carrier. Application on IVJ(NI), increased shoot height, number of leaves, fresh and dry weight of shoot significantly (p<0.05) over their respective dose on 30, 60, 90 and 120 days after sowing (Table-4.8.1).

The application of biofertilizers significantly increased shoot height on IVJ(NI) was 50.15, 9.51, 44.26, 41.61, 46.52, 9.48, 48.45, 26.56, 32.07 and 55.18% at 90 DAS and 47.44, 9.74, 41.17, 37.79, 42.38, 9.13, 44.57, 22.83, 26.99 and 52.91% at 120 DAS over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) respectively (Table-4.8.1).

The number of leaves was increased by 65.38, 13.46, 46.15, 42.31, 52.88, 7.69, 53.85, 42.31, 45.19 and 66.35% at 90 DAS and 72.27, 4.20, 50.42, 46.22, 53.78, 16.81, 56.30, 38.66, 49.58 and 73.11% at 120 DAS in IVJ(NI) over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) respectively. Similarly the number of tillers was increased by 68.18, 22.73, 31.82, 27.27, 40.91, 13.64, 54.55, 31.82, 31.82 and 68.18% at 90 DAS in IVJ(NI) over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) respectively (Table-4.8.1).

The percentage fresh weight of shoot was significantly increased on application of IVJ(NI) was 67.94, 10.76, 51.33, 49.47, 62.99, 8.34, 60.34, 41.41, 48.03 and 71.67% at 90 DAS and 76.12, 16.44, 68.13, 55.43, 71.25, 15.51, 64.43, 32.38, 39.71 and 80.22%, on 120 days old plant over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) respectively (Table-4.8.1).

The dry weight of shoot was also significantly increased by 69.72, 10.61, 53.36, 49.84, 63.69, 9.37, 60.71, 41.57, 49.22 and 71.59% on 90 DAS and 74.23, 17.35, 58.38, 55.40, 71.96, 15.19, 65.72, 33.95, 40.88 and 80.33% on 120 days after sowing respectively by the application of IVJ(NI) over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) (Table 8.1.1). Application of immobilized biofertilizers increased shoot length, number of leaves, fresh and dry weight of shoots significantly over no fertilizers on 30, 60, 90 and 120 days after sowing (DAS) under drought condition (Table-4.8.1).

Table 8.1.1 Effect of Free form, Seed Coated and Immobilized Forms of Charcoal Based Biofertilizers on Plant Growth Parameters of Wheat (*T. aestivum* L. cv. PBW-502) Shoot on 30, 60, 90 and 120 DAS under Drought Stress

	30 days	60 days	90 days	120 days
	Plant Height			
NF	26.15±1.51 ^b	38.37±1.09 ^{ab}	44.45±2.20 ^b	49.00±1.56 ^b
FCF	26.67±1.09 ^b	59.78±1.78 ^g	80.68±2.85 ^h	84.15±1.92 ^h
FBF	26.53±1.32 ^b	42.38±2.08 ^c	49.70±2.51 ^{de}	54.85±1.76 ^d
BFOM (NI)	24.98±0.94 ^{ab}	45.40±1.90 ^d	52.07±1.22 ^e	58.00±1.29 ^e
FOM (NI)	25.53±1.50 ^{ab}	40.28±1.83 ^{bc}	47.68±1.39 ^{cd}	53.72±1.90 ^d
SCVJ(NI)FOM	56.53±1.92 ^d	76.50±2.52 ⁱ	80.72±3.16 ^h	84.72±1.37 ^h
IVJ(NI)	25.52±1.38 ^{ab}	67.13±4.76 ^h	89.17±0.65 ⁱ	93.23±1.90 ⁱ
BFOM(D)	23.68±1.32 ^a	41.00±1.18 ^{bc}	45.97±2.08 ^{bc}	51.68±0.63 ^c
IVJ(D)	23.75±0.99 ^a	51.75±3.56 ^e	65.48±2.97 ^g	71.95±1.22 ^g
SCVJ(D)FOM	40.70±3.88 ^c	55.27±1.54 ^f	60.57±1.46 ^f	68.07±1.42 ^f
FOM(D)	23.52±0.95 ^a	35.67±1.27 ^a	39.97±1.22 ^a	43.90±2.06 ^a
	No. of leaves			
NF	3.00±0.00 ^a	3.67±0.82 ^a	6.00±0.00 ^a	5.50±0.55 ^a
FCF	3.00±0.00 ^a	9.00±0.89 ^e	15.00±1.26 ^d	19.00±1.41 ^f
FBF	3.00±0.00 ^a	5.33±0.52 ^{bc}	9.33±0.82 ^{bc}	9.83±0.98 ^{bc}
BFOM (NI)	3.00±0.00 ^a	5.83±0.41 ^c	10.00±1.41 ^c	10.67±1.21 ^{cd}
FOM (NI)	3.00±0.00 ^a	4.50±0.55 ^{ab}	8.17±1.17 ^b	9.17±1.60 ^{bc}
SCVJ(NI)FOM	5.33±0.82 ^b	14.00±1.67 ^g	16.00±1.41 ^{de}	16.50±1.87 ^f
IVJ(NI)	3.00±0.00 ^a	10.50±1.22 ^f	17.33±2.16 ^e	19.83±1.47 ^f
BFOM(D)	2.50±0.55 ^a	5.33±1.03 ^{bc}	8.00±1.55 ^b	8.67±1.63 ^b
IVJ(D)	2.50±0.55 ^a	7.83±0.98 ^d	10.00±1.90 ^c	12.17±1.60 ^d
SCVJ(D)FOM	5.00±0.63 ^b	9.17±0.98 ^e	9.50±1.52 ^{bc}	10.00±1.41 ^{bc}
FOM(D)	2.50±0.55 ^a	3.67±0.52 ^a	5.83±0.41 ^a	5.33±0.52 ^a
	No. of tillers			
NF	1.00±0.00 ^a	1.17±0.41 ^a	1.17±0.41 ^a	1.50±0.84 ^a
FCF	1.00±0.00 ^a	2.67±0.52 ^{de}	2.83±0.75 ^{cd}	3.33±0.52 ^{cd}
FBF	1.00±0.00 ^a	2.33±0.52 ^{cd}	2.50±0.55 ^{cd}	2.50±0.55 ^b
BFOM (NI)	1.00±0.00 ^a	2.50±0.55 ^{de}	2.67±0.52 ^{cd}	2.67±0.52 ^{bc}
FOM (NI)	1.00±0.00 ^a	1.83±0.41 ^{bc}	2.17±0.41 ^{bc}	2.50±0.55 ^b

SCVJ(NI)FOM	2.50±0.55 ^c	3.00±0.00 ^e	3.17±0.41 ^{de}	3.33±0.52 ^{cd}
IVJ(NI)	1.00±0.00 ^a	3.00±0.00 ^e	3.67±0.52 ^e	4.00±0.63 ^d
BFOM(D)	1.00±0.00 ^a	1.33±0.52 ^{ab}	1.67±0.52 ^{ab}	2.67±0.52 ^{bc}
IVJ(D)	1.00±0.00 ^a	1.83±0.75 ^{bc}	2.50±0.55 ^{cd}	2.67±0.52 ^{bc}
SCVJ(D)FOM	1.67±0.52 ^b	2.33±0.52 ^{cd}	2.50±0.55 ^{cd}	2.67±0.52 ^{bc}
FOM(D)	1.00±0.00 ^a	1.00±0.00 ^a	1.17±0.41 ^a	1.33±0.52 ^a
Fresh wt. of Shoot				
NF	1.21±0.02 ^b	1.79±0.09 ^b	2.41±0.10 ^b	3.55±0.11 ^b
FCF	1.22±0.02 ^b	3.84±0.12 ⁱ	6.71±0.13 ^h	12.43±0.17 ⁱ
FBF	1.22±0.01 ^b	2.13±0.02 ^e	3.66±0.07 ^e	4.74±0.05 ^d
BFOM (NI)	1.22±0.01 ^b	2.15±0.03 ^e	3.80±0.16 ^{ef}	6.63±0.18 ^{de}
FOM (NI)	1.21±0.01 ^b	1.92±0.06 ^c	2.78±0.14 ^c	4.28±0.23 ^c
SCVJ(NI)FOM	2.85±0.08 ^d	4.03±0.13 ^j	6.89±0.07 ^h	12.57±0.35 ⁱ
IVJ(NI)	1.23±0.02 ^b	3.36±0.07 ^h	7.52±0.08 ⁱ	14.87±0.22 ^j
BFOM(D)	1.15±0.01 ^a	2.03±0.02 ^d	2.98±0.39 ^d	5.29±0.16 ^e
IVJ(D)	1.16±0.02 ^a	2.29±0.13 ^f	4.41±0.16 ^g	10.06±0.38 ^g
SCVJ(D)FOM	2.17±0.01 ^c	3.06±0.05 ^g	3.91±0.19 ^f	8.97±0.12 ^f
FOM(D)	1.15±0.02 ^a	1.58±0.10 ^a	2.13±0.06 ^a	2.94±0.14 ^a
Dry wt. of Shoot				
NF	0.07±0.00 ^b	0.52±0.03 ^b	0.68±0.05 ^a	1.95±0.06 ^b
FCF	0.15±0.00 ^g	1.11±0.04 ^h	2.01±0.04 ^g	6.25±0.05 ⁱ
FBF	0.15±0.01 ^{gh}	0.61±0.01 ^{cd}	1.05±0.07 ^d	3.15±0.03 ^e
BFOM (NI)	0.08±0.00 ^{cd}	0.63±0.02 ^d	1.13±0.04 ^e	3.37±0.01 ^f
FOM (NI)	0.08±0.00 ^b	0.57±0.06 ^c	0.82±0.04 ^b	2.12±0.03 ^c
SCVJ(NI)FOM	0.16±0.01 ^f	1.18±0.05 ⁱ	2.04±0.02 ^f	6.41±0.05 ⁱ
IVJ(NI)	0.13±0.00 ^h	1.01±0.06 ^g	2.25±0.02 ^h	7.56±0.03 ^j
BFOM(D)	0.08±0.00 ^c	0.62±0.02 ^d	0.88±0.07 ^c	2.59±0.06 ^d
IVJ(D)	0.09±0.01 ^d	0.68±0.04 ^e	1.31±0.05 ^f	5.00±0.12 ^h
SCVJ(D)FOM	0.13±0.00 ^e	0.94±0.01 ^f	1.14±0.03 ^e	4.47±0.06 ^g
FOM(D)	0.06±0.01 ^a	0.47±0.04 ^a	0.64±0.02 ^a	1.49±0.04 ^a

All the values are means of three replicates with two determinations (n=6) ±S.D.), One way ANOVA is performed to compare the means of different treatments at p < 0.05. Values followed by different letters are significant differences between the treatments. [Where, NF-No fertilizer, FCF-Free form recommended dose of urea and DAP, FBF- free form of biofertilizers, BFOM (NI)-free form of biofertilizers with organic matrix under normal irrigation, FOM (NI)-free form of organic matrix under normal irrigation, SCVJ(NI)FOM- Seed coat with biofertilizers and vermicompost + free form organic matrix, IVJ(NI)- Immobilized form of biofertilizers with normal irrigation, BFOM(D)- Biofertilizers and free form of organic matrix under drought, IVJ(D)- Immobilized form of biofertilizers under drought condition SCVJ(D)FOM- Seed coat with biofertilizers and vermicompost + free form organic matrix under drought condition, FOM(D)- free form of organic matrix under drought condition]

Application of biofertilizers immobilized with organic carriers increased root length, number of roots, fresh and dry weight of roots significantly over no fertilizers on 30, 60, 90 and 120 days after sowing (DAS), the increase in the plant growth was however, more pronounced when measured on 30 days after sowing (Table 4.8.2). Root length increased due to application on IVJ(NI) was 59.85, 7.26, 29.78, 25.33, 37.48, 6.96, 33.04, 20.59, 25.93 and 60.15% at 90 DAS and 53.69,

8.07, 31.57, 26.27, 33.80, 7.79, 33.24, 21.84, 27.57 end 57.44% at 120 DAS respectively over applications on NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) (Table 4.8.2).

Number of roots was increased on application of IVJ(NI) by 73.23, 45.67, 60.63, 57.48, 62.99, 25.98, 64.57, 48.03, 48.82 and 74.80% at 60 DAS and 78.79, 50.51, 58.59, 55.05, 60.10, 37.37, 61.11, 54.04, 56.57 and 76.77% at 120 DAS respectively over applications on NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) (Table 4.8.2).

Addition of 1.8 kg ha⁻¹ biofertilizers in immobilized form IVJ(NI) significantly increased the root biomass over the recommended dose of biofertilizer (0.6 kg ha⁻¹) and recommended dose of chemical fertilizers. The increased in root biomass was 59.58, 11.86, 50.93, 44.03, 55.85, 7.72, 55.30, 24.43, 29.48 and 65.09% on 90 DAS and 55.10, 13.66, 47.94, 38.51, 51.41, 11.04, 52.83, 19.40, 29.18 and 62.75% on 120 days after sowing respectively, by the application of organic matrix immobilized biofertilizers in triple dose (IVJ(NI)) over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) (Table 4.8.2).

The percentage dry weight of root was increased by 63.84, 11.68, 58.27, 48.91, 58.70, 6.90, 53.57, 30.53, 31.10 and 69.72% on 90 DAS and 68.61, 11.38, 55.86, 50.11, 63.55, 9.37, 60.76, 41.99, 45.54 and 71.77% recorded in 120 days old plants by the application of IVJ(NI) over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D) (Table 4.8.2).

Table 4.8.2 Effect of charcoal based *A. chroococcum* and *B. subtilis* biofertilizers on plant growth parameters of wheat (*T. aestivum* L. cv. PBW-502) Roots on 30, 60, 90 and 120 DAS under drought stress

	No. of Roots			
NF	4.50±1.64 ^a	5.67±1.63 ^{ab}	8.33±1.21 ^{ab}	7.00±0.63 ^a
FCF	4.00±0.89 ^a	11.50±1.76 ^e	20.17±1.60 ^f	16.33±6.22 ^b
FBF	4.00±0.89 ^a	8.33±1.97 ^c	11.67±2.50 ^{cd}	13.67±3.01 ^b
BFOM (NI)	4.33±1.21 ^a	9.00±2.76 ^{cd}	12.83±3.49 ^{cd}	14.83±1.72 ^b
FOM (NI)	4.00±0.63 ^a	7.83±1.17 ^{bc}	10.50±1.22 ^{bc}	13.17±2.79 ^b
SCVJ(NI)FOM	7.67±0.82 ^c	15.67±3.50 ^f	19.17±3.76 ^f	20.67±4.89 ^c
IVJ(NI)	4.17±0.75 ^a	21.17±2.04 ^g	27.83±1.72 ^g	33.00±2.28 ^d
BFOM(D)	3.83±0.75 ^a	7.50±0.55 ^{abc}	10.67±1.21 ^{bc}	12.83±1.33 ^b
IVJ(D)	3.50±0.55 ^a	11.00±2.00 ^{de}	16.00±1.55 ^e	15.17±2.04 ^b

SCVJ(D)FOM	5.67±0.52 ^b	10.83±0.98 ^{de}	13.33±1.21 ^d	14.33±3.14 ^b
FOM(D)	3.67±0.52 ^a	5.33±0.82 ^a	7.50±0.84 ^a	7.67±0.82 ^a
	Root length			
NF	2.57±0.47 ^a	3.67±0.35 ^a	4.52±0.42 ^a	5.55±0.50 ^a
FCF	2.67±0.40 ^a	9.13±0.51 ^f	10.43±0.68 ^f	11.02±0.77 ^d
FBF	2.58±0.51 ^a	6.45±0.37 ^c	7.90±0.47 ^{cd}	8.20±0.84 ^{bc}
BFOM (NI)	2.83±1.20 ^a	6.75±0.46 ^{cd}	8.40±0.49 ^d	8.84±0.39 ^c
FOM (NI)	2.55±0.33 ^a	5.90±0.51 ^b	7.03±0.33 ^b	7.93±0.42 ^{bc}
SCVJ(NI)FOM	7.67±0.98 ^a	9.93±0.41 ^g	10.47±0.51 ^f	11.05±0.72 ^d
IVJ(NI)	2.71±0.35 ^a	9.37±0.42 ^f	11.25±0.57 ^g	11.98±0.51 ^d
BFOM(D)	2.43±0.45 ^a	5.62±0.39 ^b	7.53±0.35 ^{bc}	8.00±0.26 ^{bc}
IVJ(D)	2.41±0.24 ^a	7.17±0.23 ^{de}	8.93±0.42 ^e	9.37±0.32 ^c
SCVJ(D)FOM	5.15±0.44 ^b	7.53±0.26 ^e	8.33±0.31 ^d	8.68±0.57 ^b
FOM(D)	2.38±0.26 ^a	3.43±0.34 ^a	4.48±0.33 ^a	5.10±0.17 ^a
	Fresh wt. of Root			
NF	0.20±0.01 ^b	0.23±0.01 ^b	0.29±0.02 ^b	0.35±0.03 ^b
FCF	0.19±0.03 ^b	0.57±0.04 ^g	0.63±0.03 ^g	0.68±0.02 ^h
FBF	0.19±0.03 ^{ab}	0.29±0.01 ^{cd}	0.35±0.02 ^d	0.41±0.01 ^d
BFOM (NI)	0.19±0.02 ^b	0.30±0.01 ^d	0.40±0.01 ^e	0.48±0.03 ^e
FOM (NI)	0.20±0.01 ^b	0.25±0.03 ^{bc}	0.32±0.01 ^c	0.38±0.01 ^c
SCVJ(NI)FOM	0.35±0.04 ^d	0.64±0.05 ^h	0.66±0.03 ⁱ	0.70±0.02 ^h
IVJ(NI)	0.19±0.01 ^b	0.59±0.02 ^g	0.72±0.03 ^j	0.79±0.01 ⁱ
BFOM(D)	0.17±0.01 ^{ab}	0.26±0.03 ^{bc}	0.32±0.01 ^c	0.37±0.02 ^{bc}
IVJ(D)	0.17±0.02 ^a	0.37±0.03 ^e	0.54±0.03 ^g	0.63±0.01 ^g
SCVJ(D)FOM	0.26±0.03 ^c	0.43±0.03 ^f	0.50±0.02 ^f	0.56±0.03 ^f
FOM(D)	0.16±0.01 ^a	0.19±0.01 ^a	0.25±0.01 ^a	0.29±0.02 ^a
	Dry wt. of Root			
NF	0.04±0.00 ^b	0.06±0.00 ^b	0.07±0.00 ^b	0.08±0.00 ^a
FCF	0.04±0.00 ^b	0.14±0.00 ^h	0.16±0.01 ^g	0.22±0.00 ^g
FBF	0.04±0.00 ^b	0.07±0.00 ^{cd}	0.08±0.01 ^c	0.11±0.01 ^c
BFOM (NI)	0.04±0.00 ^b	0.08±0.00 ^d	0.09±0.00 ^e	0.13±0.01 ^d
FOM (NI)	0.04±0.00 ^b	0.07±0.00 ^c	0.08±0.00 ^c	0.09±0.01 ^b
SCVJ(NI)FOM	0.09±0.01 ^d	0.15±0.01 ⁱ	0.17±0.00 ^h	0.23±0.00 ^g
IVJ(NI)	0.04±0.00 ^b	0.13±0.00 ^g	0.19±0.00 ⁱ	0.25±0.00 ^h
BFOM(D)	0.04±0.00 ^a	0.08±0.00 ^d	0.09±0.00 ^d	0.10±0.01 ^b
IVJ(D)	0.04±0.00 ^a	0.08±0.01 ^e	0.13±0.01 ^f	0.15±0.00 ^f
SCVJ(D)FOM	0.07±0.00 ^c	0.12±0.00 ^f	0.13±0.01 ^f	0.14±0.00 ^e
FOM(D)	0.04±0.00 ^a	0.05±0.01 ^a	0.06±0.00 ^a	0.07±0.00 ^a

All the values are means of three replicates with two determinations (n=6) ±S.D.) (Other details are described in Table. 4.8.1)

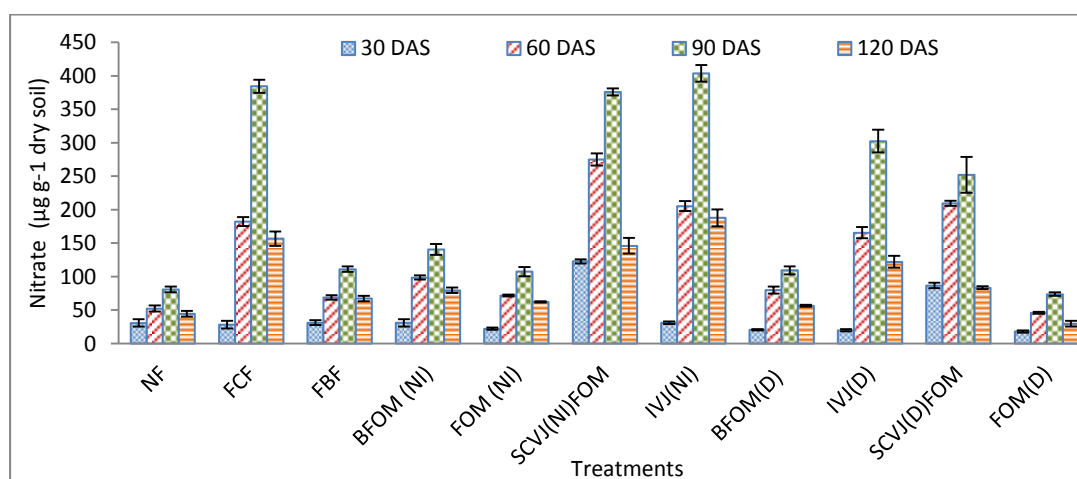
4.8.2 Availability of Nitrate, Nitrite, Ammonium and Phosphate Content in the Soil on Application of Immobilized Biofertilizers Super Granules under Drought Stress Condition

Nitrate content was highest when applying the applications of immobilized biofertilizers with vermicompost as a carrier; IVJ(NI) during cultivation of wheat crop. Increased percentage by IVJ(NI) was 74.59, 11.25, 66.48, 51.91, 65.08, 61.03, 19.28 and 77.64% at 60 DAS, 79.98, 4.79, 72.48, 65.17, 73.36, 72.90, 25.11 and 81.71% at 90 DAS and 76.26, 16.64, 64.23, 57.65, 66.85, 69.97, 35.01 and 84.17% at 120 DAS over applications on NF, FCF, FBF, BFOM (NI), FOM (NI), BFOM(D), IVJ(D) and FOM(D) respectively (Figure 4.8.1).

The increased nitrite content according to the application of IVJ(NI) in soil was Nitrite in soil 68.53, 7.97, 43.13, 25.72, 56.22, 55.77, 30.75, 13.01 and 73.35% at 60 DAS and 75.78, 12.58, 53.67, 43.71, 59.73, 19.69, 52.34, 16.80, 34.23 and 79.90% at 120 DAS significantly over NF, FCF, FBF, BFOM (NI), FOM (NI), BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) respectively (Figure 4.8.1).

On application of IVJ(NI) was increased ammonium content in soil at different concentration level by 74.16, 4.37, 59.04, 38.59, 50.55, 16.37, 49.97, 28.36, 36.19 and 75.26% at 90 DAS 77.41, 17.15, 70.36, 41.84, 51.04, 24.84, 58.42, 26.47, 38.41 and 41.70% at 120 DAS respectively over NF, FCF, FBF, BFOM (NI), FOM (NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) (Figure 4.8.1).

Phosphate content in soil was also increased on application of IVJ(NI) by 183.39, 10.86, 74.46, 58.89, 71.61, 76.02, 44.56, 11.49 and 86.05% at 60 DAS over NF, FCF, FBF, BFOM (NI), FOM (NI), BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) respectively (Figure 4.8.1).



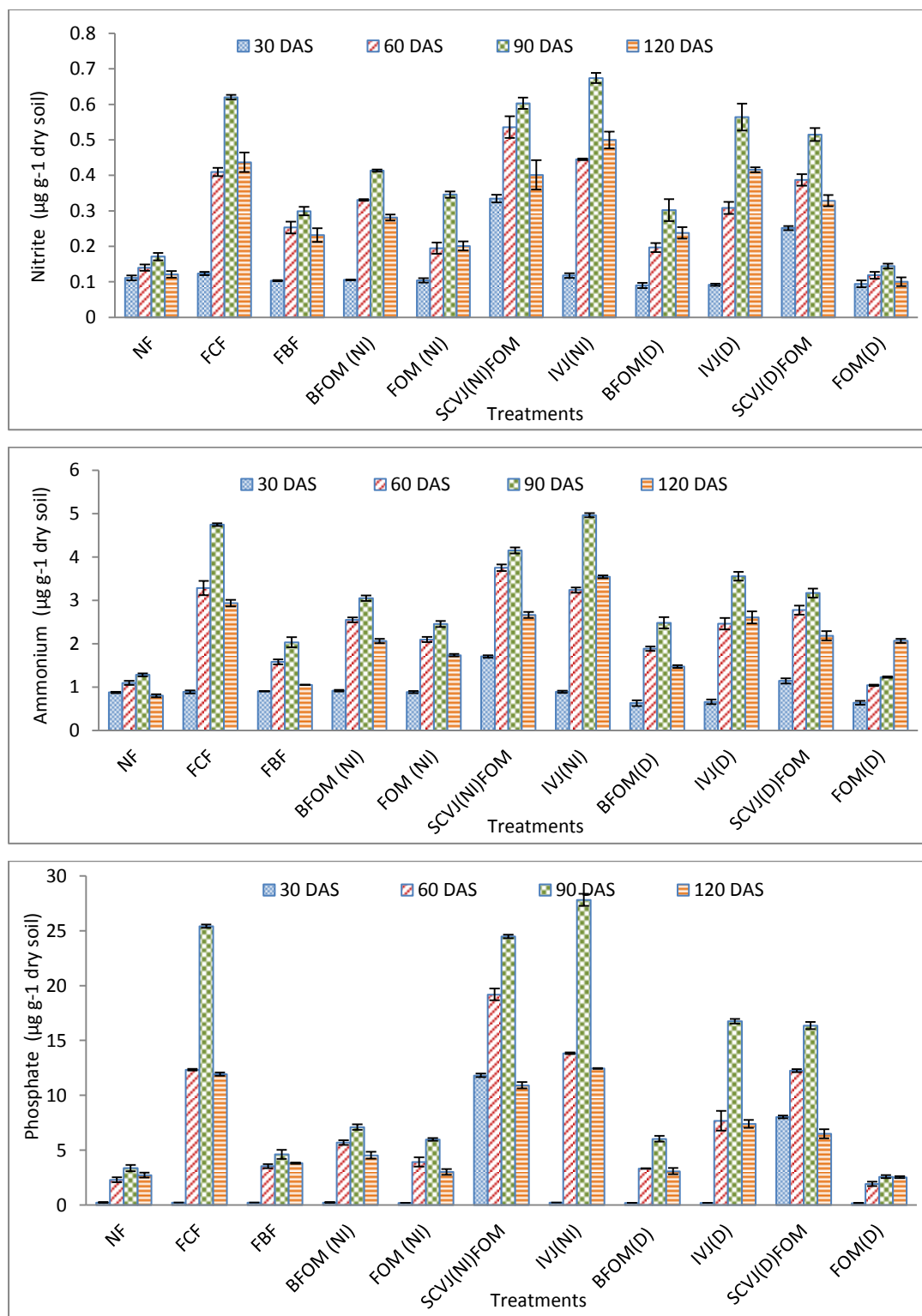


Figure 4.8.1 Effect of immobilized biofertilizers on the nutrient availability in the soil on 30, 60, 90, and 120 days after shown (DAS). All the values are means of three replicates with two determinations ($n=6$) \pm S.E), (Other details are described in Table. 4.8.1)

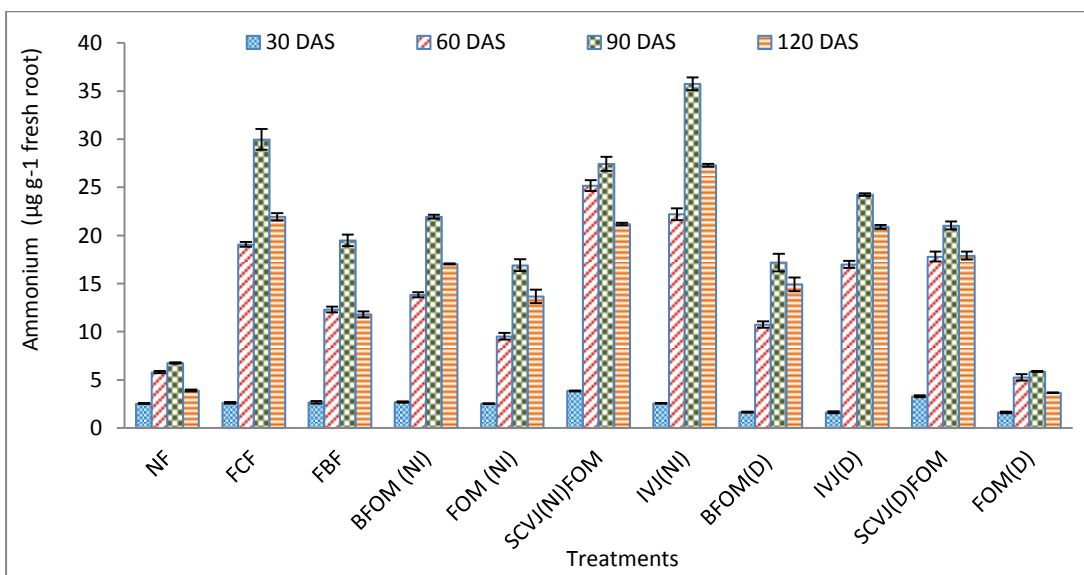
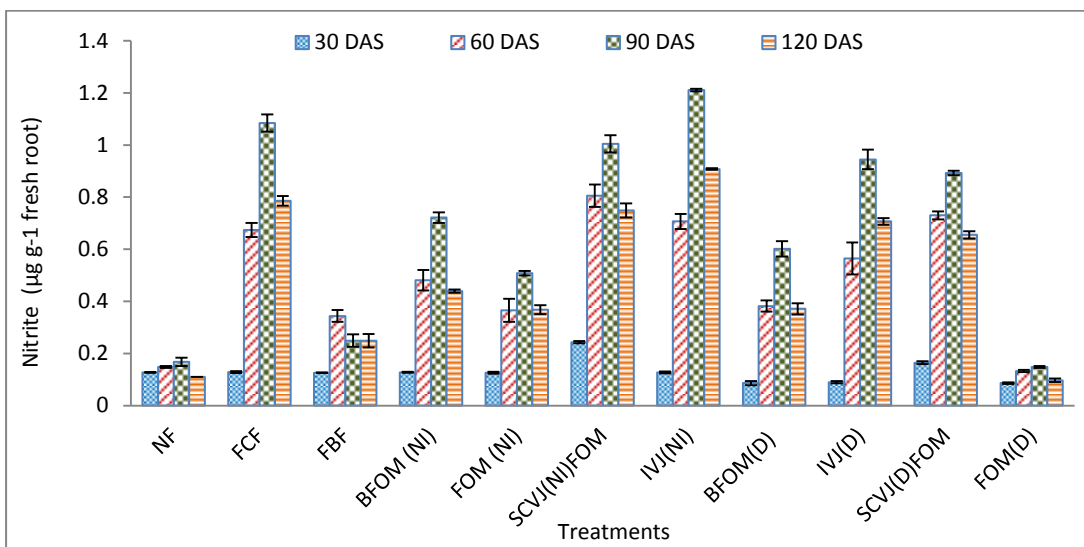
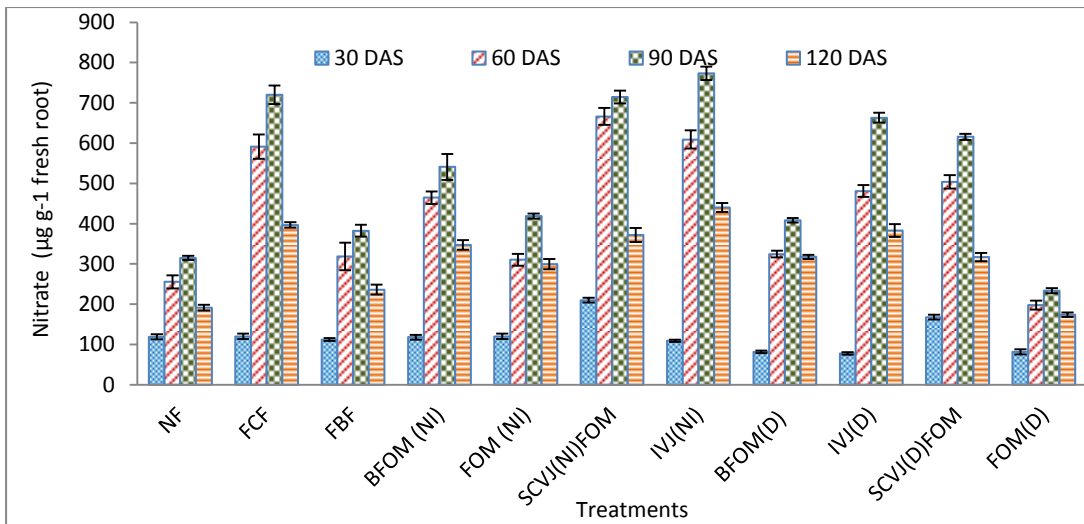
4.8.3 Availability of Nitrate, Nitrite, Ammonium and Phosphate Content in the Roots on Application of Immobilized Biofertilizer Based Super Granules under Drought Stress Condition

Immobilized form of biofertilizers with vermicompost based super granules increased the nitrate content in roots was recorded significantly on application of IVJ(NI) by Nitrate in root 58.05, 2.93, 47.69, 23.72, 49.03, 46.72, 21.01, 17.25 and 67.54% and 56.50, 9.84, 46.31, 21.14, 31.89, 27.84, 13.02, 27.96 and 60.57% at 60, and 120 DAS respectively over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D), SCVJ(D)FOM, FOM(D). Similarly application of Immobilized biofertilizers with IVJ(D) and SCVJ(D)FOM also increased nitrate content in roots of wheat plant at different days of intervals over NF, FBF, BFOM(NI) and FOM(NI) (Figure 4.8.2).

The increased nitrite content in roots by application of IVJ(NI) at 60 DAS and 120 DAS was 79.02, 4.65, 51.32, 31.95, 48.25, 45.96, 20.11 and 81.25% and 87.86, 13.46, 72.62, 51.67, 59.50, 59.10, 22.17 and 89.32% over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D) and FOM(D) (Figure 4.8.2).

The increased percentage of ammonium content was 73.88, 14.13, 44.53, 37.64, 57.15, 51.66, 23.47, 19.84 and 76.36% at 60 DAS 85.76, 19.62, 56.80, 37.56, 49.93, 45.33, 23.48, 34.41 and 86.56% at 120 DAS in roots of wheat plant by application on super granule of IVJ(NI) over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D) and SCVJ(D)FOM, FOM(D) (Figure 4.8.2).

On application of IVJ(NI) which enhanced the nutrient availability of phosphate content in plant roots was recorded highest by Phosphate in root 94.19, 8.03, 29.57, 20.40, 32.85, 42.24, 9.71, 0.40 and 95.93% and 94.21, 14.40, 43.09, 36.11, 43.77, 55.29, 29.78, 35.07 and 95.94% at 60 and 120 DAS respectively over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) (Figure 4.8.2).



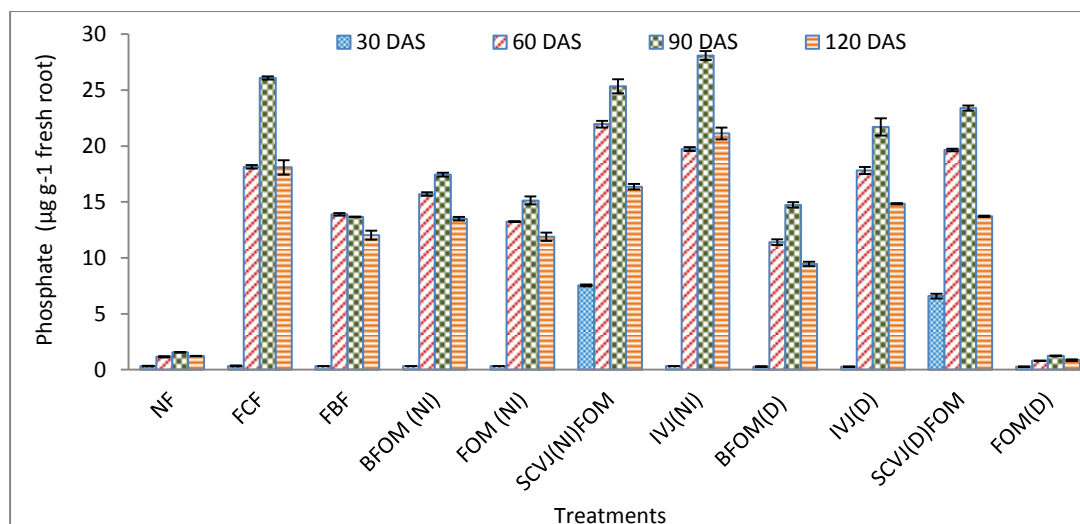


Figure 4.8.2 The immobilized biofertilizers increased nutrient availability in the roots on 30, 60, 90, and 120 days after shown (DAS). All the values are means of three replicates with two determinations ($n=6$) \pm S.E). (Other details are described in Table. 4.8.1)

4.8.4 Availability of Nitrate, Nitrite, Ammonium and Phosphate Content in the Leaves by the Application of Immobilized Biofertilizers Based Super Granules under Drought Stress Condition

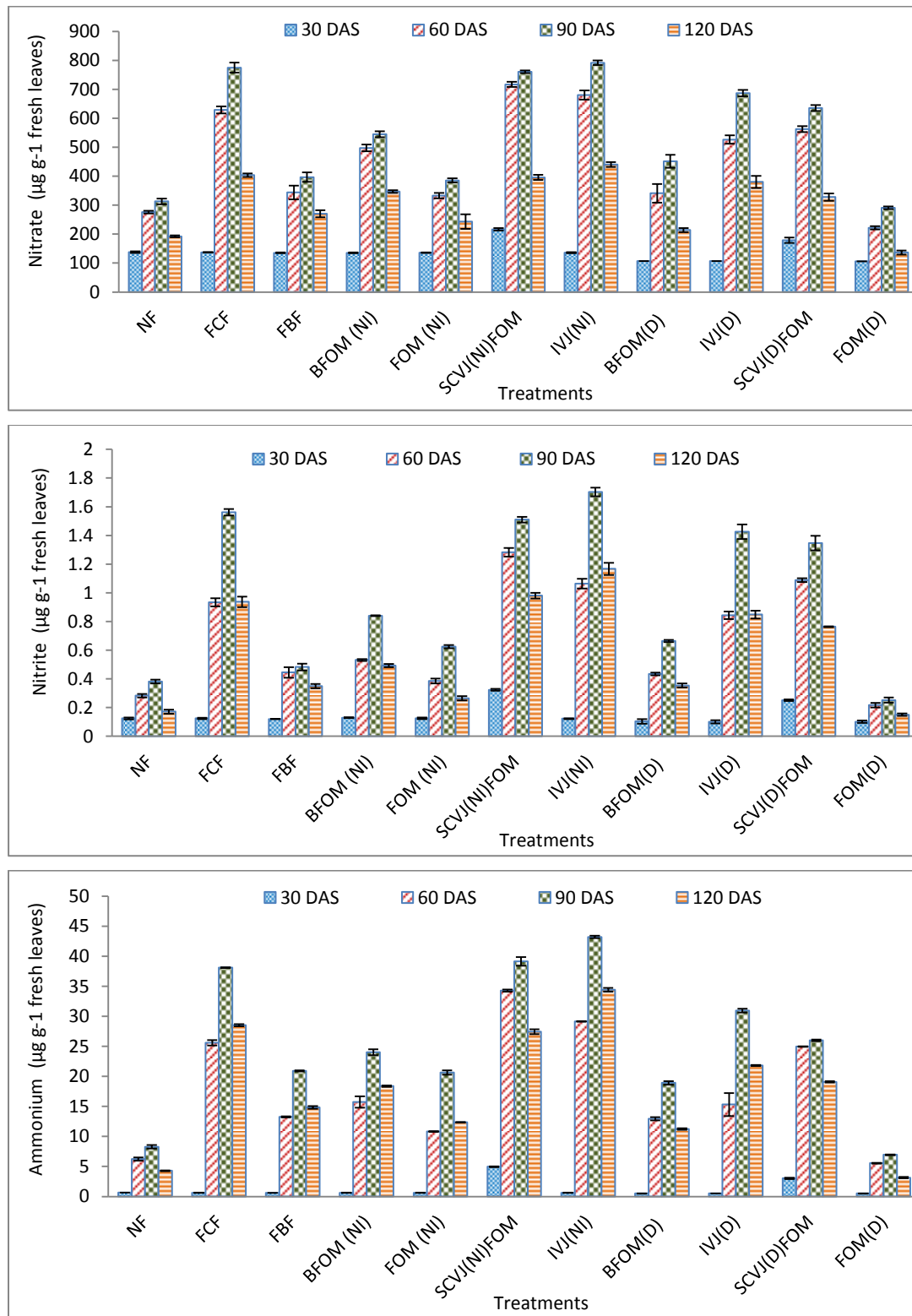
Nitrate content was recorded highest with the application of IVJ(NI) in plant leaves by 60.46, 2.13, 49.89, 31.20, 51.32, 3.94, 42.96, 13.24, 19.75 and 63.23% at 90 DAS and 56.40, 8.42, 38.63, 21.25, 44.77, 10.19, 51.44, 13.70, 25.56 and 68.97% at 120 DAS over NF, FCF, FBF, BFOM(NI), FOM(NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) respectively (Figure 4.8.3).

Similarly by the application of IVJ(NI) also increased the nitrite content in plant leaves over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D) and FOM(D) was 73.40, 12.18, 58.13, 50, 63.76, 59.15, 20.67 and 79.60% at 60 DAS and 85.29, 19.72, 70.05, 57.73, 77.30, 69.68, 27.30 and 87.11% at 120 DAS respectively (Figure 4.8.3).

On application of IVJ(NI) the nutrient availability of ammonium content in plant leaves was recorded highest by 78.60, 12.16, 54.55, 46.01, 62.87, 55.64, 47.47, 14.31, 81% and 87.52, 17.15, 56.85, 46.59, 64.13, 67.34, 36.59, 44.53 and 90.88% at 60 and 120 DAS respectively over NF, FCF, FBF, BFOM(NI), FOM(NI), BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) (Figure 4.8.3).

The phosphate content in leaves was highest recorded on the application of IVJ(NI) by 77.85, 12.07, 42.13, 36.70, 52.67, 15.08, 53.43, 16.83, 28.22 and 79.88% at 90 DAS 86.78, 19.22, 46.41, 32.40, 45.86, 20.76, 50.53, 23.75, 35.68 and 88.15%

at 120DAS respectively NF, FCF, FBF, BFOM(NI), FOM(NI), SCVJ(NI)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) respectively (Figure 4.8.3)



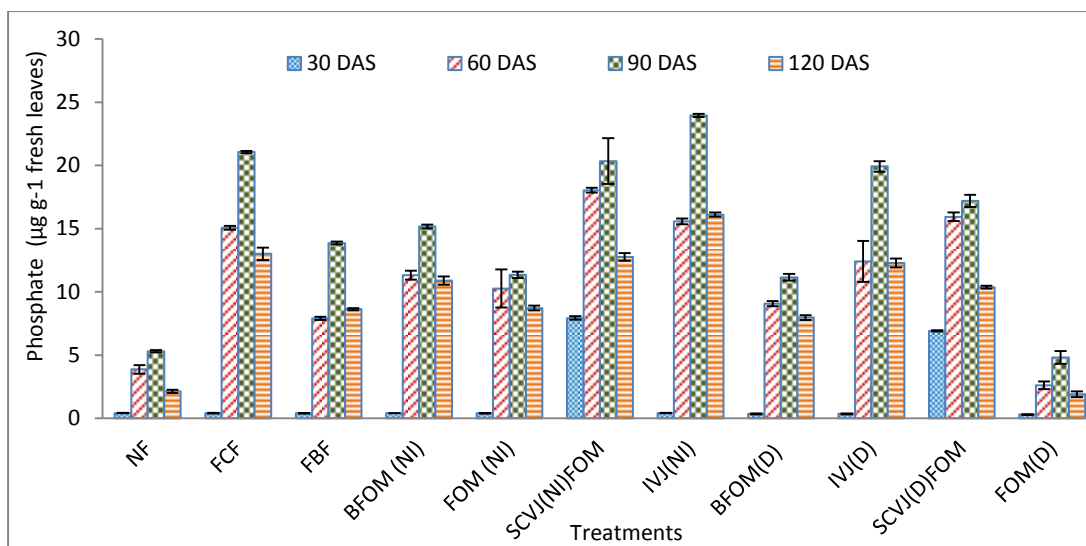


Figure 4.8.3 Nutrient concentrations in the leaves increased on 30, 60, 90, and 120 DAS. All the values are means of three replicates with two determinations ($n=6 \pm S.E.$). (Other details are described in Table. 4.8.1)

4.8.5 Grain and Straw Yield of Wheat (*T. aestivum* L.) Plant Applied with Immobilized Biofertilizers Based Super Granules under Drought Stress Condition

Application on immobilized biofertilizers in form of the super granules increased the yield percentage by 72.09, 14.76, 60.95, 54.22, 67.47, 26.92, 67.12, 30.86, 40.97 and 77.06% on application of IVJ(Ni) over NF, FCF, FBF, BFOM(Ni), FOM(Ni), SCVJ(Ni)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) after harvesting at 120 DAS (Figure 4.8.4).

The percentage of straw yield significantly increased on application of IVJ(Ni) was 71.2, 8.75, 62.39, 47.19, 66.02, 12.9, 57.91, 21.2, 29.44 and 78.32% on after harvesting over NF, FCF, FBF, BFOM(Ni), FOM(Ni), SCVJ(Ni)FOM, BFOM(D), IVJ(D), SCVJ(D)FOM and FOM(D) respectively (Figure 4.8.4).

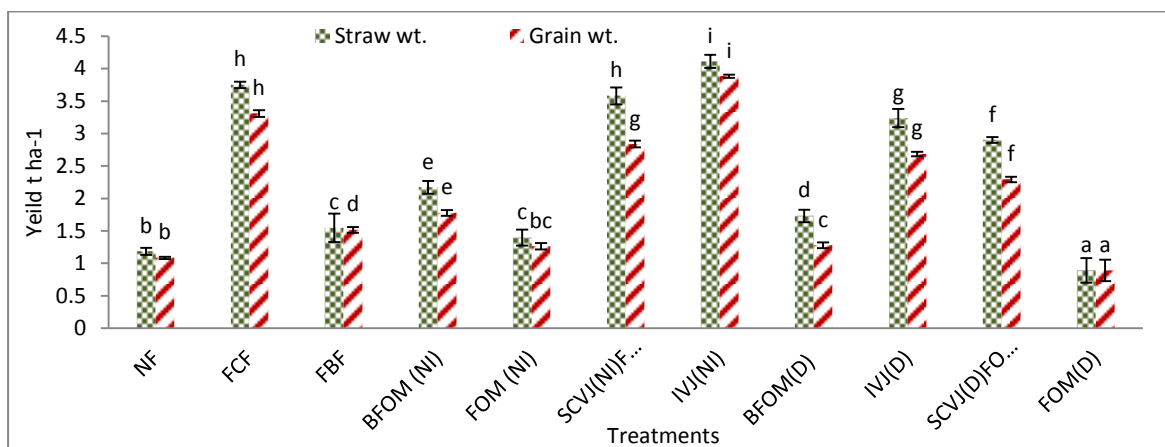


Figure 4.8.4 Grain and straw yield significantly increased on application of immobilized biofertilizers on soil. Values are mean of data ($n=6 \pm SE$), differences are statistically significant and shown different alphabets using Duncan's multiple range test (DMRT). Data analysed by one way ANOVA at <0.05 . (Other details are described in Table. 4.8.1)

4.8.6 Changes in Physico-Chemical Properties of Soil by the Application of Biofertilizers in free form, Seed Coated form and Immobilized within Organic Matrix

The immobilized biofertilizers decreased soil pH more significantly than no fertilizer and free form of biofertilizers applied in field. Water holding capacity of the soil increased during the crop cultivation, which was higher in the plots applied with the chemical fertilizers (CF) (Table 8.4.3). The organic matrix immobilized biofertilizers increased organic carbon (%) and organic matter (%) in the soil very significantly in comparison to the no fertilizers or conventional chemical fertilizers. In addition total N, available N, available P and soluble K were significantly increased on harvest in the presence of immobilized biofertilizers over the other treatments (Table 8.4.3).

Table 4.8.3 Changes in Physico-Chemical Properties of Soil on Application of Free Form of Biofertilizer, Seed Coated Form and Immobilized Form with Organic Matrix

		NF	FBF	CF	FBF+OM	BFS	BF-3(SV)	IVJ
pH	SS	8.27 ± .04	8.64 ± .06	8.04 ± .03	8.15 ± .05	8.35 ± .05	8.76 ± .05	7.91 ± .05
	H	7.56 ± .03	7.78 ± .05	7.65 ± .02	7.47 ± .02	8.02 ± .04	8.12 ± .03	7.47 ± .10
EC ds/m ²	SS	0.88 ± .02	0.41 ± .02	0.60 ± .05	0.84 ± .05	0.87 ± .05	0.90 ± .03	0.48 ± .03
	H	0.47 ± .02	0.35 ± .05	0.28 ± .01	0.22 ± .05	0.65 ± .02	0.21 ± .04	0.19 ± .04
WHC %	SS	49 ± 3.05	46 ± .05	47 ± .10	50 ± .02	47 ± 3.02	45 ± .08	46 ± .02
	H	30 ± .05	58 ± .03	58 ± .05	62 ± .04	59 ± .04	66 ± .06	69 ± .03
OC %	SS	0.38 ± .03	0.40 ± .02	0.38 ± .09	0.41 ± .04	0.42 ± .05	0.40 ± .03	0.32 ± .02
	H	0.42 ± .06	0.57 ± .05	0.61 ± .03	0.63 ± .02	0.57 ± .02	0.57 ± .05	0.57 ± .05
OM	SS	0.65 ± .05	0.68 ± .02	0.65 ± .05	0.70 ± .02	0.72 ± .09	0.68 ± .03	0.55 ± .05
	H	.72 ± .02	.98 ± .01	1.05 ± .03	1.08 ± .05	.98 ± .04	.98 ± .05	.98 ± .01
N (kg/ha)	SS	124.21 ± 4.08	128.01 ± 2.09	125.41 ± 4.03	118.12 ± 5.02	121 ± 4.21	126.16 ± 5.2	119 ± 6.86
	H	169.25 ± 5.26	198.34 ± 4.32	225.64 ± 9.05	220.75 ± 8.02	205.32 ± 9.25	248.52 ± 8.93	285.66 ± 10.05
P ₂ O ₅ (kg/ha)	SS	7 ± 0.4	8 ± 0.05	8 ± 0.05	6 ± 0.04	9 ± 0.05	10 ± 0.08	9 ± 0.03
	H	10 ± 0.06	18 ± 0.04	25 ± 0.03	29 ± 0.08	26 ± 0.08	30 ± 0.05	48 ± 0.04
K (kg/ha)	SS	165.24 ± 8.5	168.21 ± 9.02	165.65 ± 10.55	150.43 ± 5.12	172.22 ± 8.92	166.56 ± 10.30	165.24 ± 8.54
	H	195 ± 10.25	215 ± 12.06	265 ± 16.4	220 ± 12.12	252 ± 20.29	288 ± 18.03	297 ± 12.5

Analysis of physico-chemical parameters before seed sowing and at harvest stage. All the values are means of three replicates with two determinations (n=6)±SD. [Where, NF; No Fertilizer, FBF; Free Form of Biofertilizers (Optimized dose; *A. chroococcum*- 1.80 kg ha⁻¹ and *B. subtilis* -1.80 kg ha⁻¹), CF; Recommended dose of Urea-150 kg ha⁻¹ and DAP- 75 kg ha⁻¹, FBF+OM; free form of biofertilizers with organic matrix free form, BFS; Seed coated with biofertilizers, IVJ; immobilized form of biofertilizers with vermicompost, BF-3(SV); Seed coated with biofertilizers and organic matrix]

4.9 Cost Benefits Analysis

The total cost of cultivation of wheat was calculated by detailed economic analysis of input cost and income obtained and it was the sum of common and valuable costs of different treatments. The cost calculation was carried out on the basis of prevailing market rates during the crop years (Table 4.9.1).

Table 4.9.1 Net loss/gain in terms of economic return for wheat (*Triticum aestivum* L.) cultivated using biofertilizers in free form, seed coated form and immobilized organic matrix based super granular forms

	Input cost ha ⁻¹					Income ha ⁻¹			
	Approx. Labour+ Field Prep. (ha ⁻¹)	Seed ha ¹ (ha ⁻¹)	Fertilizer (ha ⁻¹)	Other/ Irrigation /Organic Matrix (ha ⁻¹)	Total (Rs ha ⁻¹)	Net seed yield (t ha ⁻¹)	Approx. rate /ton	Cost of product (ha ⁻¹)	Net (ha ⁻¹) Loss (-) / gain(+)
NF	13,000.00	3,600.00	0.00	2,500.00	19,100.00	0.79	16,000.00	2,640	(-) 16,460.00
CF	13,000.00	3,600.00	3000.0	2,500.00	22,100.00	3.31	16,000.00	52,960	(+) 30,860.00
FBF-III	13,000.00	3,600.00	0.00	2620.00	19,220.00	1.20	16,000.00	19,200	(-) 20.00
FBF+OM	13,000.00	3,600.00	0.00	3120.00	19,720.00	1.77	16,000.00	28,320	(+) 8,600.00
BF-3(SV)	13,000.00	3,600.00	0.00	3360.00	19,960.00	1.9	16,000.00	30,400	(+) 10,440.00
SCVJ+OM	13,000.00	3,600.00	0.00	3120.00	19,720.00	2.8	16,000.00	44,800	(+) 25,080.00
BFS-III	13,000.00	3,600.00	0.00	2,870.00	19,470.00	1.73	16,000.00	27,680	(+) 8,210.00
IVJ(SL)	13,000.00	3,600.00	0.00	3,420.00	20,020.00	2.71	16,000.00	43,360	(+) 23,340.00
IVA	13,000.00	3,600.00	0.00	6,000.00	22,600.00	3.84	16,000.00	61,440	(+) 38,840.00
IVM	13,000.00	3,600.00	0.00	3,800.00	20,400.00	3.86	16,000.00	61,760	(+) 41,360.00
IVJ	13,000.00	3,600.00	0.00	3420.00	20,020.00	3.88	16,000.00	62,080	(+) 42,060.00

Labour day⁻¹= 300.00/-; Organic Matrix (100 Kg) = 500.00/-; Urea (100 Kg) = 1000.00/-; Biofertilizers/Kg= 120.00/-; DAP (100 Kg) = 2000.00/-; Binder(saresh)/ kg= 300.00/- ; Binder(molasses)/ It. = 80.00/- ; Binder (jaggery)/ kg= 35.00/- [Where, NF= No fertilizers; CF= conventional chemical fertilizers (urea and DAP); FBF-III= free form of Biofertilizers; FBF+OM = Free form of biofertilizer and free form of organic matrix; BF-3(SV)= Biofertilizers in triple dose with vermicompost in seed coated form; SCV+OM = biofertilizers coated with biofertilizer and vermicompost + free form of organic matrix; BFS-III =Biofertilizers in triple dose in seed coated form; IVJ(SL) = Immobilized biofertilizers in vermicompost used binder as jaggery in applied in saline condition; IVA= Immobilized biofertilizers in vermicompost used binder as jaggery; IVM = Immobilized biofertilizers in vermicompost used binder as molasses; IVJ= Immobilized biofertilizers in vermicompost used binder as jaggery.



CHAPTER-5

DISCUSSION

Chapter 5

DISCUSSION

Excessive loading of chemical fertilizers is a common feature in green revolution belts, which causes many economic, environmental and health related problems (Gupta et al., 2008; Rawat et al., 2011; Kumar et al., 2014a, b). Low efficiency of uptake of nutrients in many crops is another factor that aggravates the process of leaching, volatilization, and emissions of nutrients of the added soluble chemical fertilizers, which are readily released in the soil and atmosphere (Akiyama, 2000; Tilman et al., 2002; Jagadeeswaran et al., 2005; Menendez et al., 2008; Monem et al., 2010; Myrbeck et al., 2012; Kumar et al., 2014a). Such losses in various forms pollute the ecosystems, degrade air and water quality and enhance the process of global warming (Velmurugan et al., 2008; Rawat et al., 2010; Weligama et al., 2010; Rawat et al., 2012).

It has been reported that application of biofertilizer is a common practices for crop cultivation throughout the world as it increases plant productivity and grain yield (Gudadhe et al., 2005; Rajpar et al., 2006; Zaman et al., 2010; Guizazu et al., 2013; Xin-Kai et al., 2012; Sapkota et al., 2014; Kumar et al., 2014a, b; 2015a, b). Biofertilizers can modify soil conditions and enrich soil fertility. Which play a critical role in soil nutrient cycling, and agricultural fertilization that significantly affects the communities and activities of microbes involved in the soil nitrogen cycle (Sun et al., 2015). The mode of application (seed coating, and soil inoculation) of biofertilizers may also affects efficacy of microbes (Ramus et al., 2000; Al-Moudi and Moujahed 2006; Arshad et al., 2008; Kizilkaya, 2008; Malusa et al., 2012; Herrmann and Lesueur, 2013; Zang et al., 2014).

The present data indicates that the application of enhanced dose of biofertilizers i.e. three fold of its recommended dose increased wheat productivity significantly. The fivefold (BF-V) and sixfold (BF-VI) doses of biofertilizer maintained the plant growth productivity but failed to increase grain yield, which was higher than the threefold dose of biofertilizer. The enhanced doses of

biofertilizer increased the plant growth parameters i.e., biomass of the plant, nitrate, nitrite, ammonium and phosphate content in rhizosphere soil and plant parts as measured on 30, 60, 90 and 120 days after sowing the seeds (Ashok et al., 2014; Kumar et al., 2014a, b; 2015a, b). The triple dose dose of biofertilizer increased plant growth parameters over recommended dose of biofertilizer, single dose of biofertilizer, double dose of fertilizer and enhanced fivefold dose of biofertilizer in terms of fresh weight of plant and biomass, nutrient availability and yield productivity (Table - 4.1.1; 4.1.2 and Figure 4.1.1-4.1.4).

Today, interest in *Azotobacter* as inoculants for agriculture has only recently been revived in granular form, free form, seed inoculation and as root colonization forms etc. (Cocking, E.C., 2003; Al- Amoudi, O.A., Moujahed, H.M., 2006; Kundu et al., 2009; Sharma et al., 2011, 12; Nayak et al., 2012; Otinga et al., 2013; Kumar et al., 2013a,b,c; Kizilkaya et al., 2012; 2014; Ashok et al., 2014; Kumar et al., 2015a,b). Seed coating with charcoal based biofertilizer have been shown that, the enhanced doses of biofertilizer can produce more yield with certain technical interventions and improve nutritional qualities (Packowski and Berryhill 1979; Yang, 2003; Ogut et al., 2005; Cheng and Yang et al., 2006;). The application of enhanced dose of biofertilizer as seed coated form increased the root and shoots length and mainly increased the fresh weight of shoot and root ratio. Seed coated with biofertilizer in threefold (BFS-III) increased the plant growth parameters over without adding fertilizers, single dose of biofertilizer (BFS-I), twofold (BFS-II) and sevenfold (BFS-VII) by obtained significantly total plant biomass was 16.44, 4.23, 3.66 and 4.31% at 120 days after shown (DAS) respectively (Table -4.2.1; 4.2.2 and Figure 4.2.1-4.2.4).

Another mode on application of seed coated with enhanced doses of biofertilizer and organic matrix significantly increased the shoots height, root length, fresh and dry weight of shoot and root. Seed coated with threefold of biofertilizer and vermicompost; BFS-3(SV) increased the plant growth parameters over without fertilizer adding, single dose of biofertilizer and vermicompost coated seed; BFS-1(SV), similarly twofold of BFS-2(SV), fivefold BFS-5(SV), sixfold BFS-6(SV), and sevenfold BFS-7(SV) by obtained significantly total plant biomass was 75, 3.23, 1.61, 1.61, 4.03 and 6.45% at 90 days after shown (DAS) respectively. The nutrient

content in wheat cultivated soil was increased in all the treatments as compared to control. It may be stated that the increased soil fertility and improvement in the nitrogen fixing and phosphate solubilisation in rhizospheric soil. The nitrogenous and phosphate content in soil and plant tissue part significantly recorded in increased level (Table - 4.3.1, 4.3.2 and Figure 4.3.1- 4.3.4).

The present data of this study indicates that the application of enhanced dose of biofertilizer increased the wheat productivity. The application of biofertilizer in free form, seed coat form and in immobilized form separately in pot as well as plot condition indicate that the enhanced doses of biofertilizers increased the plant growth parameters in terms of shoot height, root length, number of leaves, number of roots, number of tillers and fresh and dry weight of shoot and root as measured on 30, 60, 90 and 120 days after sowing the seed. But when pot plant compare with plot plant it showed the significance different between them in terms of their growth productivity. The plant biomass of pot was less than the plot, the significant different was there is no tillers or short height in pot condition. The yield productivity was also less as compared to the plot plant (Figure- 5.1.1).

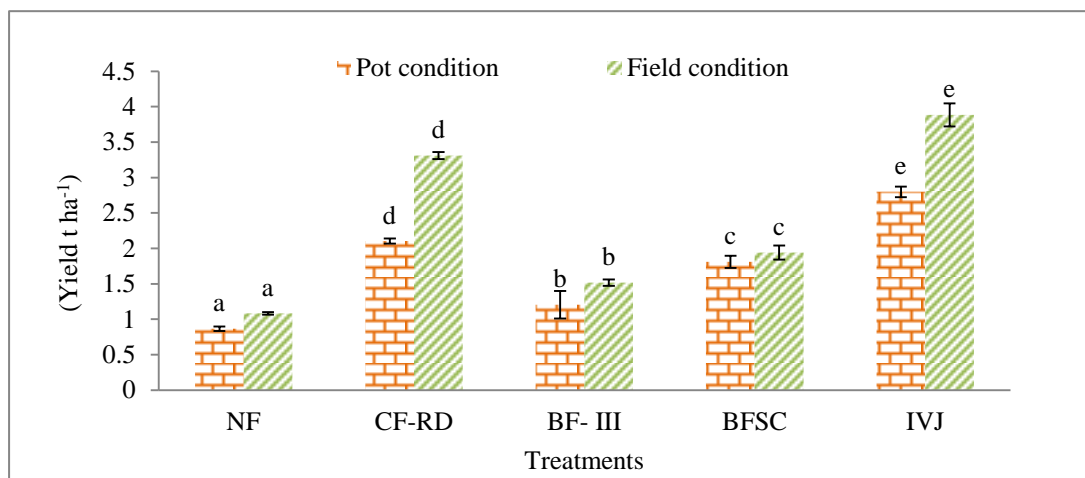


Figure 5.1.1 Yield of wheat crop affected by enhanced dose of biofertilizer with different mode of application at pot and field conditions. One way ANOVA is performed to compare the means of different treatments at $p < 0.05$. Values followed by different letters are significantly differences between the treatments [Where NF- No fertilizers, CF-RD- Recommended dose of urea and DAP, BF-III- Triple dose of biofertilizers, BFSC- Seed coated with biofertilizers and IVJ- Immobilized form of biofertilizers with organic binder jaggery]

The enhanced dose of biofertilizer in different agro-climatic conditions reported significantly (Ashok et al., 2014; Kumar et al., 2013a, Kumar et al., 2014a, b; Sharma et al., 2011; 2012). In this experiment it was found that the threefold (optimal dose) of biofertilizer (recommended dose) doses increased the plant growth

and yield over the no fertilizer, free form of biofertilizer and free form of conventional chemical fertilizers. It appears that a dose optimization for use of biofertilizer is needed in different crops and different agro climatic conditions to obtain better efficacy of these fertilizers. To achieve that, the improvement of the microbial biofertilizers quality and availability is crucially needed near future for the sustainable development.

Organic matrix are good for sustainable agriculture in present scenario, it enhanced the crop productivity, soil fertility and enhance the efficacy of biofertilizers (Yang,2003; Dahiya et al., 2004; Kumar et al., 2012; 2015a Ashok et al., 2014; Sharma and Singh, 2011; Kumar et al., 2013a, b; 2014a, b; 2015b)) inoculants have to be designed in organic matrix to provide a dependable/suitable source of beneficial bacteria that survive in the soil and become available to the plant) too (Gopinath et al., 2008; Shaharoon et al., 2008; Hasanuzzaman et al., 2010; Ardakani, et al., 2011; Sharma et al., 2012; Minaxi et al., 2013; Singh et al., 2013; Kumar et al., 2014 a, b; 2015a, b). Organic matrix based super granules contained lower chemical fertilizers but shows higher nitrate, nitrite, ammonium and phosphate in rhizosphere and its wheat roots and leaves as measured on 30, 60, 90 and 120 days after sowing the seeds during cultivation. As well as it improves soil properties and soil nutritional level at harvest stage of the crop. This is possibly because of slow release and slow leaching of the nutrients in soil leaching (Kondo et al., 2005; Tang et al., 2006; Grant et al., 2012; Yushi et al., 2013; Ibrahim et al., 2014; Steng et al., 2014). The cow-dung, neem leaves and acacia gum binder available in the immobilized matrix may also be adding to the organic matter and nutritional and microbial status of soil same with farm yard manure and cow-dung based super granules. The increase in organic matter, organic carbon and soil microbial activities on application of organic manures have been reported for some cropping system (Sharma et al., 2011, 2012; Wild et al., 2014; Tamilselvi et al., 2015). The neem leaves are known to contain urease inhibitors which subsequently may decrease the release of free ammonia from the granules (Mohanty et al., 2008; Abbasi et al., 2011; Abalos et al., 2012).

The elemental properties of organic matrix and immobilized super granules were detected by the SEM-EDX analyser. SEM-EDX of organic matrix and immobilized super granules and free form of chemical fertilizers (urea and DAP)

indicate that the C, O, Mg, Al, Si, P, S, K and Zr present in these fertilizers. The ratio of CHN of the different organic matrix; clay soil, neem leaf, cow-dung, commercial chemical fertilizers and various organic matrix immobilized granular slow release fertilizers is shown in Figure 4.1- 4.12. The results indicate that organic matrix based fertilizers possess higher carbon percentage and slightly lower or same percentage of nitrogen as compared to urea and DAP, clay soil and neem leaf powder.

The analytical analysis by Fourier transmission infrared radiation (FTIR) of various organic matrix based immobilized super granules showed that the highest absorption bands ranges from 2850-3000 cm^{-1} to 3200-3500 cm^{-1} (Figure 4.13-4.20 and Table 4.1). Generally, the bands around 3300 cm^{-1} represent O-H and N-H stretching vibrations that are mainly represent the proteins and carbohydrates (D' Souza et al., 2008). The absorption peak band at 3375 cm^{-1} depicts the N-H bonded secondary amines in the organic matrix. The absorption peak at 3300-3400 are related to stretching vibration of structural OH and NH groups of granules consisting of organic matrix based urea granules, potassium dihydrogen and ammonium dihydrogen phosphate (Olad and Rashidzadeh, 2012).

A absorption bands peak at 1630-1695 cm^{-1} and 1720-1740 cm^{-1} is due to C=O stretching mode related to urea (Emrani et al., 2011). The absorption broad band at 3200- 3500 cm^{-1} may also be due to OH groups in the polysaccharides of granules and carboxylic acid functional groups in the substrate (Pourjavadi et al., 2012). The appearance of bands between 2850-3000 cm^{-1} is due to the combined stretching of CH_3 and CH_2 groups in all granule formulations. The protein absorption bands between 1500-1560 cm^{-1} contains amide I and amide II bands. The bands between 1500 and 1000 cm^{-1} belong to the fingerprint region (Rashidzadeh and Olad, 2014), amide III and the functional groups of nucleic acids and carbohydrates contributed to these absorption bands in samples. A very sharp band of wave number 1733.8 which represents acid, aldehyde and ketone groups appeared in the neem leaves, showing the presence of saturated aldehydes. The absorption band in the ranges from band region of 500-540 is due to S-S (disulphide) weak group in certain granule mixtures. The studied IR spectrum of urea and DAP based granules showing bands in the range of 1400-1450 and 1630-1680 are the characteristic absorption bands of urea (Liu et al., 2008). The absorption bands of spectra on various granule formations showed the presence of important functional

groups of various biomolecules and other inorganic nutritional compounds present in them.

The co-immobilized super granule fertilizers possess higher carbon percentage due to it contains cow dung, vermicompost and FYM organic matrix as carrier (Ramos and Martinez 2006; Sahni et al., Prabha et al., 2007; 2008; Vivas et al., 2009; Wang et al., 2010; Warman and Anglopez, 2010; Lazcano and Dominguez 2011; Singh et al., 2013). In which vermicompost co-immobilized biofertilizer showed the significant elemental properties which are required by the plant for better growth and productivity.

Data of this study indicate that the organic matrix based immobilized biofertilizers in form of super granules increase the microbial population (Kumar et al., 2005; Sharma et al., 2011a, b; Dong et al., 2014; Kumar et al 2015b), it contains 15 to 18 cfu g⁻¹ bacteria per granule. The biofertilizer (threefold) co-immobilized in organic matrix with different combinations in the form of super granules. The vermicompost based immobilized super granules contains significant microbial growth and population followed by farm yard manure and cow dung (Table 4.4.14). The data of this study also indicates that the application of immobilized form of biofertilizers i.e. *Azotobacter chroococum* and *Bacillus subtilis* increased the total biomass of the crop plant, which is directly correlated to the growth and productivity of the plants. This study also indicates that the dose optimization and the use of organic carrier to formulate super granules provide the available nutrients to the plant for better yield productivity and enrich the soil fertility.

Whereas co-immobilized *Azotobacter* and *Bacillus* with organic matrix and used different binders and stored it in different temperature showed the significant results. Microbial growth in super granules at different storage temperature shows the various aspects. The organic matrix (vermicompost) immobilized with biofertilizers used jaggery as binder shows the significant growth at storage condition followed by organic matrix (vermicompost) immobilized with biofertilizers used binder as molasses and organic matrix (vermicompost) immobilized with biofertilizers used *acacia* (plant gum) as binder at different temperature (Table 4.4.15). **(There is no previous work/references with molasses and jaggery used as binders to formulate super granular fertilizer in agricultural field).**

Kumar et al., 2015a, b; Sharma et al. 2011a, b; 2012 demonstrated that the application of organic matrix in agricultural field, increase nutritional and microbial status of soil. The significant increase of soil microbial population and activities in the presence of organic manures was reported (Sharma et al. 2011a, b; Kumar et al., 2014a, 2015b). It has been reported that the organic manure such as cow-dung, vermicompost, farm yard manure, poultry manure and other organic waste materials combined used with biofertilizers during the crop cultivation increased the microbial population and its activity in soil and also increased the productivity of crop in the agricultural field (Ramos and Martinez 2006; Sahni et al., Prabha et al., 2007; 2008; Gong et al., 2009; Warman, and Anglopez, 2010; Singh et al., 2013; Dong et al., 2014; Radha and Rao, 2014; Wild et al., 2014; Tamilselvi et al., 2015).

The application of fertilizers have been found directly co-related to the productivity of cereals in general and wheat in particular (Abedi et al., 2010; Cerny et al., 2010; Laik et al., 2014). Nitrogen losses in gaseous forms pollute the ecosystems, degrade water quality and enhance the process of global warming (Adesemoye et al., 2009; Jiang et al., 2010; Rawat et al., 2010; Weligama et al., 2010; Liu et al., 2014; Plaza-Bonilla et al., 2014). Organic manures e.g. cow-dung, compost, vermicompost and farm yard manure (FYM) etc. have been found as an alternative to the chemical fertilizers in organic farming systems (Ardakani et al., 2011; Moharana et al., 2012; Nayak et al., 2012; Otinga et al., 2013; Yadav et al., 2013). Biofertilizers have been identified as another alternative to chemical fertilizers to increase soil fertility and crop production in sustainable manner (Kundu et al., 2009; Wu et al., 2005; Perez- Montano et al., 2014; Ramesh et al., 2014). In recent years, biofertilizers have emerged as an important component of the integrated nutrient management programs and hold a great promise to improve crop yield with minimum environmental degradation. Strains of *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Azospirillum*, *Pseudomonas*, *Bacillus* and *Acetobacter* etc. have been developed as biofertilizers for cereals, pulses, vegetables, oil seeds, cotton, sugarcane, wheat etc (Mahajan et al., 2003; Ogut et al., 2005; Shaukat et al., 2006; Broschat and Moore, 2007; Adesemoye et al., 2009; Swarnalakshmi et al., 2013; Lavakush et al., 2014).

This study has performed to investigate the effect of the biofertilizers and organic matrix on growth and yield of wheat at different days after sowing in field conditions. Application of organic matrix immobilized with biofertilizers enhanced

the content of higher nitrate, nitrite, ammonium and phosphate in rhizospheric soil and plant parts as measured on 30, 60, 90 and 120 days after sowing (Figure 4.3.1-4.3.4). These super granules improved soil physiochemical and biological properties and nutrients availability which was assessed during crop cultivation. Higher nitrate levels in plant leaves indicate that application of these fertilizers enhanced the nitrogen uptake by roots (Ashok et al., 2014; Kumar et al., 2013a, 2014a, b). Sharma et al., 2011 and 2012 have also showed that application of farm yard manure, green manure and biofertilizers increase soil microbial properties in optimal irrigation and no tillage conditions. The experimental study revealed that the threefold dose of biofertilizers over recommended dose was found to be optimal dose. This data depicts that the formulations of super granules can help to bring out the loading of chemical fertilizers urea and DAP and can produce more wheat yield with better nutritional qualities. In addition, immobilized of these biofertilizers to a biodegradable, low cost organic matrix contained local and cheap agro-waste materials like cow-dung, FYM, vermicompost, neem leaves powder, clay soil (Ashok et al., 2014; Kumar et al., 2013a, 2014a, b; 2015a, b Minj and Singh 2015).

The recommended dose of a nutrient is generally used by field trials in the given agro-climatic conditions, however many factors e.g. soil type, environmental stress, availability of organic matter and nutrients in soil and other agro-climatic and genetic factors may affect the nutrient use efficiency for specific crops in agricultural fields (Kumar et al., 2013a, Kumar et al., 2014a,b). This study indicates that the new formulation of super granules prepared in different organic carrier and binders they release the available nutrient to the rhizospheric soil, provide essential nutrient to the plant. The organic carrier based super granules protect the microbes from direct contact to the soil and various environmental stresses, organic matrix and nutritive organic binders provide nutritional and healthy environment to the microbes also (Heijnen et al. 1992; Adil et al., 2005; Moujahed, H.M., 2006; Kundu et al., 2009; Densilin et al., 2011; Sharma et al., 2011a,b, 12; Nayak et al., 2012; Otinga et al., 2013; Kumar et al., 2013a,b,c; Kizilkaya et al., 2012; 2014; Ashok et al., 2014; Kumar et al., 2015b). Cow-dung available in the matrix beading to the organic matter and nutritional and microbial status of soil. Sharma et al. 2011a, b, have also demonstrated a significant increase in soil microbial activities in the presence of organic manures. Organic matrix such as vermicompost is the better

sources to the microbes provide sufficient nutrients and shelter. Similarly with the FYM support the growth and enhanced the efficacy of biofertilizers.

The low nutrient levels in soil leads to low crop productivity due to less availability of essential nutrients needed for plant growth, metabolism and reproductive yield. Therefore, additional fertilizers (especially N fertilizers) are loaded to increase crop yield. Since application of fertilizers is directly related to plant yield in cereals like CF- R/D, The N cycle is an essential and complex biogeochemical cycle that has a great impact on soil fertility (Jetten, 2008; Mc-Allister et al., 2012; Godinot et al., 2014). In wheat, excessive loading is a common feature in green revolution belts, which causes many environmental, economic and health related problems (Singh et al., 2010; Abedi et al., 2010; Cerny et al., 2010; Ryan et al., 2012; Kong et al., 2013; Singh et al., 2014; Laik et al., 2014). Low efficiency for the uptake of fertilizers in many crops is another factor that aggravates the leaching, volatilization and emissions related losses of the loaded soluble/chemical fertilizers which are readily released in the soil and atmosphere (Akiyama, 2000; Guo et al., 2014). Over 50% of the applied N can be lost from agricultural systems as N trace gases and reactive nitrogen species (Adesemoye et al., 2009, Weligama et al., 2010; Cui et al., 2012; Liu et al., 2014; Plaza-Bonilla et al., 2014). Similarly when P, another growth limiting nutrient, is applied in high percentage, sometimes up to 90%, is precipitated by metal complexes in the soil and can later lead to P pollution (Adesemoye et al., 2009).

Bacteria inoculation significantly increased number of spike/m², plant height, spike length, 1000 kernel weight, and grain yield dry matter increased fresh and dry weight, P and K availability, uptake nutrient and the plant growth (Sundara et al., 2002; Gizaway, 2009; Ahmed et al., 2011; Abou et al., 2012; Zafar et al., 2012; Gupta et al., 2013). In soil stress condition, bacteria possess stress tolerance along with the plant growth- promoting traits (Tiwari et al., 2011) also *B. subtilis* and *B. cereus* gave the most frequent positive growth responses (wheat seedling, root weight, shoot weight and shoot length) (Ryder et al., 1999). Use of biofertilizers (*B. amyloliquefaciens* and *A. brasilense*) improve drought stress tolerance of wheat which tolerant to osmotic stress, fixates nitrogen were the best inoculation for yield of wheat grains (Omar et al., 2000, 2009). Wheat growth under drought stress conditions through priming with beneficial bacteria considered biofertilizer

(*Azospirillum*) has ability to attenuate several stress consequences in plants which strongly supports the potential of such an approach to control drought stress in wheat (Kloepper, 2003; Cohen et al., 2008; Kasim et al., 2013). They are able to produce plant hormones such as auxin, and proteins like polyamines, fix N, increase root growth and enhance growth of plant under the stress condition (Ramos et al., 2002; Bhaskara Rao and Charyulu, 2005; de-Bashan et al., 2005; Russo et al., 2008; Cassan et al., 2009). The beneficial effects of microorganisms are important in agricultural and environmental significance it can resist the unfavourable effects of stress. (Bashan et al., 1991; Castellanos et al., 2000; El-Komy et al., 2003; Pereyra et al., 2006, 2009 Fischer et al., 2007; Spaepen et al., 2008; Arzanesh et al., 2009; Pereyra et al., 2009). They have ability to alleviate the stress (Arzanesh et al., 2011). The *A. chroococcum* inoculations influenced plant growth and the nitrogen content of various plant parts, the plant growth promotion due to stimulation of native microbial communities of the rhizosphere/rhizoplane region (Pandey et al., 1999).

Immobilizing of biofertilizer with organic matrix used nutritive organic binders such as *acacia* gum (sareh) (Sharma et al., 2011; 2012; Kumar et al., 2012; 2013a, b; 2014 a, b; 2015 a, b), molasses and jaggery (Kirk and Othmer 1967; Bhosale and Gadre, 2001; Kalogiannis et al., 2003; Aksu and Tugba-Eren 2005; Li et al., 2007; Oliveira et al., 2007; Ren et al., 2010; Kucukasik et al., 2011; Cappelletti et al., 2012; Abou-Taleb et al., 2014; Trivedi and Shah 2014). The data indicated that the use of organic matrix along with nutritive organic binders plays an important role to enhance the efficacy of charcoal based biofertilizers (Kundu et al., 2009; Ardakani et al., 2011; Piromyou et al., 2011; Yadav et al., 2011; Panhwar et al., 2012; Singh et al., 2013; Kumar et al., 2014a, b; Yadav et al., 2014). The data also indicate that the organic matrix provide additional nutrition and micro environment to these microbes in the granules which increase its population and activity significantly leading to enhanced nutrient availability for the crop for a longer duration (Sharma et al., 2011; 2012). The nutrient content was significantly increased in soil and plants parts. The nutrient levels was observed in organic matrix immobilized biofertilizers with vermicompost used jaggery as a binder (IVJ) treated plants showed increased nitrate assimilation at vegetative and reproductive stages that enhanced plant growth and seed yield. Increase the nitrate, nitrite and ammonium assimilation in soil and in many crops have been reported with the

application of slow release fertilizers, which include wheat (Ingle et al., 2010; Kumar et al., 2014 a, b; 2015 b), rice (Saigusa, 1999; Dahiya et al., 2004, Singh et al., 2006; Kumar et al., 2012; Ashok et al., 2014), Indian mustard (Sharma and Singh, 2011). Organic matrix immobilized triple dose of charcoal based biofertilizers used jaggery as binders have reported higher growth of plant especially fresh weight of plant by 53.20 and 9.49% on application on IVJ over no added fertilizer and recommended dose of chemical fertilizers respectively. IVJ the best formulation over recommended dose of soluble chemical fertilizers. For this there is a need to formulate super granules for better plant growth, development and productivity. The nutrient content in soil as well as plant parts recorded significantly by application on immobilized biofertilizer with vermicompost used jaggery as binder (IVJ) (Table 4.6.1, 4.6.2 and Figure 4.6.1-4.6.5).

Anthropogenic influence and global change of climate there is an actual problem of plant adaptation to the odd environments affects the production of crops (Gupta et al., 2002; IPCC, 2007; Ovrutskaya, 2012). Under stress conditions the plant faces a dilemma that lowers agricultural production due to reduction in grain yield by drought and heat stresses, it considered that environmental constraints is an important agricultural problems, affecting from optimal for their plant growth, development and productivity (Vinocur and Altman 2005; Sekmen et al., 2012; Kreczmer et al., 2012; Zhang et al., 2013).

Growth and productivity of crop and soil inhabiting microbial population is negatively affected by soil salinity (Nautiyal et al., 2013). But there is some salt resistant, rhizosphere competent bacteria like *Bacillus amyloliquefaciens* and other inoculation with selected PGPR could improve plant health in saline stress condition and increased plant growth (Nautiyal et al., 2013; Patel and Saraf, 2013). But organic matrix carrier provided the nutrition and a good shelter to the microbes where they obtain their food and protection from external stresses (Van-Elsas and Heijnen, 1990; Rodriguez-Navarro et al. 1991; Heijnen et al. 1992).

Biofertilizer in immobilized with organic carrier showed significant results under over abiotic and biotic stresses (Kloepper, 2003; Lucy et al., 2004; Wu et al., 2005; Conrath et al., 2006; Fischer et al., 2007; Spaepen et al., 2008; Dimkpa et al., 2009; Omar et al., 2009; Tiwari et al., 2011; Tapias et al., 2012; Jha and Subramanian, 2014). This experimental study depicts that the immobilized

biofertilizers with vermicompost used jaggery as binder (IVJ) has significantly recorded the growth parameters, nutrient availability and yield under stress condition. Application with IVJ was increased yield by 72.09, and 14.76%, under drought condition and 63.07 and 12% under saline condition over without fertilizer and recommended dose of chemical fertilizer (Figure 4.7.5 and 4.8.5).

A direct correlation between the application of NPK fertilizers and crop productivity have been reported for wheat cultivation (Yadav, 2003; Kumar and Nanwal, 2006; Osborne, 2007; Brar et al., 2013; Duan et al., 2014), biofertilizers, e.g., *A. chroococcum*, *B. subtilis*, *Azospirillum*, *Acetobacter* (Kumar et al., 2001, Ogut et al., 2005; Lavakush et al., 2014; Perez-Montano et al., 2014; Ramesh et al., 2014) and organic fertilizers (Sharma and Prasad, 1999; Yadav et al., 2013; Laik et al., 2014; Singh et al., 2014) organic binders (Kumar et al., 2013a,b,c; Kizilkaya et al., 2012; 2014; Ashok et al., 2014; Kumar et al., 2015a, b) have been applied to the wheat fields as alternative and eco-friendly. It has been demonstrated that the immobilized super granules recorded the significant increase in soil nutrient content and obtained better plant growth and yield in the presence of organic matrix and organic binders. Organic matrix such as vermicompost is the better sources to provide sufficient nutrients support the growth and enhanced the efficacy of biofertilizers.

Cost Benefits Analysis

From cost analysis study, it was found that our new formulated organic matrix immobilized bio-fertilizers based “Super Granules” were eco-friendly and cost effective in terms of total gain per hectare to the farmers. These formulations are very effective in increasing crop productivity and yield over the commercial chemical fertilizers, free form of charcoal based biofertilizers and no fertilizers.

The total cost cultivation was calculated by detailed economic analysis of input cost and obtained income and it was the sum of common and variable costs of different treatments. The cost calculation was carried out on the basis of prevailing market rates during the crop years. This data concerned with the cost calculation of grain yield showed that super granular biofertilizer i.e. IVJ-III gave maximum net returns of Rs.(+) 42,060.00 followed by (IVM-III) gave maximum net returns of Rs. (+) 41,360.00, (IAM-III) gave maximum net returns of Rs.(+) 38,840.00, SCVJ+OM

gave maximum net returns of Rs. (+) 25,080.00 followed by recommended dose of soluble chemical fertilizers CF-R/D;Rs. (+) 30,860.00, FBF+OM gave maximum net returns of Rs. (+) 8,600.00 and BF-3(SV) gave maximum net returns of Rs.(+) 10,440.00 in wheat crop cultivation. The nutrient release from super granules having threefold dose of biofertilizers, immobilized in organic carriers (clay soil, vermicompost, and neem leaves) with organic binders as jaggery, molasses and saresh (gum of *Acacia* spp.) resulted in maximum net returns as compared to free form application of biofertilizers and recommended dose of chemical fertilizers.



CHAPTER-6

SUMMARY

&

CONCLUSIONS

Chapter 6

SUMMARY AND CONCLUSIONS

Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world, which is cultivated over a wide range of climatic conditions. Due to climatic constraints and shrinkage of per capita arable land wheat productivity has suffered losses worldwide. In India also wheat production has declined due to climate change and anthropogenic activities such as industrialization, urbanization, construction of roads, railway tracks, etc. It is estimated that in India the arable land will be reduced to 0.10 ha⁻¹ by 2050. On the other hand India's population is growing at the rate of 1.9% per annum which has increased the demand for food production. To fulfil the needs of growing population, dependency on conventional fertilizers as a source of nutrients to enhance yield has increased. The excessive use of chemical fertilizers has caused drastic effects on environment, economy and public health, which has resulted in environmental pollution, ecological damage, degraded soil fertility and increased cost of cultivation. The conventional chemical fertilizers are more efficient for crop productivity in a given unit area, however, a significant amount of applied fertilizers i.e. 30-50% approximately get lost by leaching, runoff, volatilization and emission losses. These losses account economic losses and indeed, environmental degradation and health hazards. To reduce the impact and load of conventional chemical fertilizers there is a need for some alternative measures that can provide food security for growing population in ecologically sustainable and cost effective manner.

Sustainability in agriculture sector can be attained through developing an efficient nutrient management system with the use of organic manures and biofertilizers that holds potential to maintain soil fertility and crop productivity at lower cost. Among various organic management approaches, application of biofertilizers in agricultural field considered most efficient and cost effective practice. Biofertilizers are products containing living cells of different types of microorganisms, which have ability to convert nutritionally important elements nitrogen, phosphorus and potassium (N, P and K) from unavailable to available form through biological process such as atmospheric N fixation; and solubilisation and mineralization of rock phosphate, etc. Thus biofertilizers can decrease the dependency on use of chemical N and P fertilizers making them available to plants and

significantly improving nutrient status of soil. They not only play major role in reducing use of chemical fertilizers but also reduce the rate of nutrient loss due to leaching, volatilization and fixation after fertilizer application to the soil and helps in improving yield in sustainable manner.

Biofertilizers when applied directly in soil or on seeds lose their efficacy as they fail to compete with existing microbial population and acclimatize themselves under prevailing environmental conditions. Their efficacy can be improved by immobilizing them with non-toxic, biodegradable materials which provide shelter and nutrients to survive and flourish in soil even under unfavourable conditions. Organic manures such as cow-dung, compost, vermicompost, poultry manure, farm yard manure (FYM), crop residue and green manure etc. which are recommended as an alternative to the chemical fertilizers in farming system are non-toxic, biodegradable materials that have capacity to provide a range of nutrients and improve physical and biological properties of soil. Immobilization of biofertilizers also delays their release in soil thereby extending time period for their availability. Thus immobilization of biofertilizers with organic manures as carrier is a sustainable strategy to enhance the efficiency of biofertilizer to increase crop productivity as well as soil fertility.

The present investigation entitled “Enhancing the Efficacy of *Azotobacter chroococcum* and *Bacillus subtilis* by Dose Optimization and Immobilization within Organic Carrier for High Wheat (*T. aestivum* L.) Productivity” was carried out to develop and study the optimal conditions for enhanced productivity of wheat (*T. aestivum* L.) using an efficient organic nutrient system based on basic principles of the plant nutrient and simple technological intervention.

From the present study following conclusions were drawn out:

- ❖ **The experiment on assessment of effect of consortium of biofertilizers on wheat productivity and dose optimization when applied as seed coat and in free form in pot:** study revealed that the threefold dose of biofertilizers (recommended dose) increased the growth and yield compared to crops grown in soil without fertilizer, with recommended dose and twofold of recommended dose on application in form of seed coat as well as in free form. However, when biofertilizer was applied as seed coat showed better results in comparison to free form application. Further increase in dose concentration does not produced

significant effect on growth and productivity of wheat. At threefold dose the yield was recorded to be increased by 43.34, 19.93 and 17.57% when applied as seed coat and 17.46, 5.41 and 4.84% when applied in free form over no fertilizer, single dose, and twofold dose, respectively.

- ❖ **The experiment on assessment of effect of consortium of biofertilizers on wheat productivity and dose optimization, applied as seed coat and free form in plots:** study shown that the threefold dose of biofertilizers (recommended dose) was optimum to enhance the growth and yield of wheat crop compared to control (without fertilizer), recommended dose and twofold of recommended dose on both, seed coat and free form of application and increase in dose concentration beyond threefold does not produced any significant effect on growth and productivity of wheat.

Similar to pot experiment in plot experiment when biofertilizers was applied as seed coat showed better results compared to free form of application. The yield productivity was recorded to be increased by 48.71, 17.71 and 8.45% on seed coat application and 20.18, 8.47 and 6.09% in case of free form of biofertilizers over no fertilizer, single dose and twofold dose, respectively.

- ❖ **Formulation and Characterization of Super granules:** The super granules were formulated by immobilizing biofertilizers with different organic matrix i.e. vermicompost, farm yard manure and cow dung with binders Acacia, molasses and Jaggery; clay soil as inert carrier and neem leaves. The super granules prepared in different combinations were analyzed through SEM-EDX and FTIR to determine elemental properties and functional groups of granules and the result obtained revealed that the super granules prepared with vermicompost with all three binders contains higher percentage of CHN ratio.

The microbial population in super granules was maintained in storage even at different temperature. The immobilization of biofertilizers in organic matrix and organic binder significantly supported and enhanced the microbial population. The highest microbial population was recorded in super granules prepared with vermicompost and jaggery at different storage time and varying temperature.

- ❖ **Application of super granules for wheat cultivation in pots:** All combination of organic matrix and binders immobilizing biofertilizers were applied in pots to enhance wheat productivity. The study showed super granules of biofertilizers

immobilized with vermicompost and jaggery produced best results in terms of plant growth and yield in comparison to no fertilizer, chemical fertilizer, and other combinations of immobilized biofertilizers.

❖ **Application of best performing super granules of biofertilizers immobilized with vermicompost and jaggery under environmental stress conditions:**

Under drought condition super granules of vermicompost and jaggery (IVJ-D) increased percentage of grain yield by 59.63, 43.51, 33.78, and 52.92% over no fertilizer (NF), free form biofertilizers (FBF), free form biofertilizers + free form of organic matrix (BFFOM) and free form of organic matrix (FOM). Under salinity super granules of vermicompost and jaggery (IVJ-SL) increased yield by 77.08, 11.27, 69.10 and 21.26% over no fertilizer (NF), free form chemical fertilizers (CF), free form biofertilizer + free form of organic matrix (BFFOM) and immobilized biofertilizers in vermicompost used jaggery as binder under stress condition in cadmium (IVJ-Cd). Under cadmium stress condition, super granules of vermicompost and jaggery (IVJ-Cd) increased percentage of grain yield by 51.09, 43.11 and 34.72% over no fertilizer; (NF), free form biofertilizers+free form of organic matrix; (BFFOM) and immobilized biofertilizers in vermicompost used *acacia* gum as binder under stress condition in cadmium and salinity; (IVJ-Cd+SL).

- ❖ **Cost benefit analysis:** From cost benefit analysis study it was concluded that the use of vermicompost as organic matrix and jaggery as binder for formulation of super granules of biofertilizer are cost effective in terms of their easy availability, low cost and potential to improve wheat productivity per hectare thereby reducing economic pressure over farmers and replacing dependency on costly chemical fertilizers.

Conclusions

A consortium of biofertilizers (*A. chroococcum* and *B. subtilis*) at threefold of recommended dose was formulated in form of super granules by immobilizing with organic matrix (sole nutrient source) and binder to increase wheat (*T. aestivum* L. cv. PBW- 502) productivity. Super granules of immobilized biofertilizers were observed to increase the growth of wheat plants on 30, 60 and 90 DAS in terms of root and shoot length, number of roots and leaves as well as fresh and dry wt. of roots and

leaves, tiller number and grain and straw yield over the recommended dose (2.47 kg ha⁻¹) of the same biofertilizers. Biofertilizers immobilized in an organic matrix increased the efficacy of these biofertilizers over the non-immobilized conventional forms. An increase in plant growth of wheat by application of higher doses of biofertilizers and immobilized biofertilizers were correlated with the availability of NO₃⁻, NO₂⁻, NH₄⁺ and PO₄⁺ in the plant rhizosphere (0-15 cm) and their mobilization from soil to the plant parts was observed during wheat cultivation in experimental fields.

The results indicate that the dose of biofertilizers usually used for wheat cultivation is not a true reflection of the actual requirements of biofertilizers for different crops in different agro-climatic regions and it requires a revisit. In addition, immobilize of these biofertilizers to a biodegradable, low cost organic matrix contained local and cheap agro-waste materials like cow dung, vermicompost, FYM, neem leaves powder, clay soil and different organic binders i.e. *acacia* gum, molasses and jaggery enhanced its efficacy over the free form of biofertilizers and free form of chemical fertilizers. This opens a new dimension to develop; commercial organic fertilizers which can maintain the crop productivity parallel to the conventional chemical fertilizers and simultaneously can be eco-friendly, cost effective and soil enriching.

Recommendations

- ❖ We can improve the efficacy of biofertilizer by amending the other organic carrier in organic farming systems.
- ❖ We can prepare super granules for enhancing its efficacy by combination of bacteria with fungi.
- ❖ This experimental study was done in small field. The applications of these type of super granules apply in essential field conditions in sustainable agriculture.
- ❖ These low cost super granules can use in integrated crop management system to reduce the load of chemical fertilizers and reuse of organic waste.



REFERENCES

References

- Abalos, D., Sanz-Cobena, A., Misselbrook, T., Vallejo, A. **2012**. Effectiveness of urease inhibition on the abatement of ammonia, nitrous oxide and nitric oxides emissions in a non-irrigated Mediterranean barley field. *Chemosphere*. 89(3), 310-318.
- Abao, E.B., Bronson, K.F., Wassmann, R., Singh, U. **2000**. Simultaneous records of methane and nitrous oxide emissions in rice-based cropping systems under rainfed conditions. *Nut. Cycl. Agroecosyst.* 58, 131-139.
- Abbasi, M. K., Hina, M., Tahir, M.M. **2011**. Effect of *Azadirachta indica* (neem), sodium thiosulphate and calcium chloride on changes in nitrogen transformations and inhibition of nitrification in soil incubated under laboratory conditions. *Chemosphere* 82(11), 1629-1635
- Abdin, M.Z., Dwivedi, R.K., Abrol, Y.P. **2006**. Nitrogen in agriculture. In: Nitrogen Nutrition in Plant Productivity (Eds Singh RP, Shankar N and Jaiwal PK). *Stadium Press, LLC, Houston, Texas, USA*. 1-41
- Abedi, T., Alemzadeh, A., Kazemeini, S.A. **2010**. Effect of organic and inorganic fertilizers on grain yield and protein banding pattern of wheat. *Aust. J. Crop Sci.* 4(6), 384-389.
- Abou-el-Seoud, Abdel-Megeed, A. **2012**. Impact of rock materials and biofertilizations on P and K availability for maize (*Zea Maize*) under calcareous soil conditions. *Saudi Journal of Biological Sciences* 19, 55–63.
- Abou-Taleb, K.A., Abdel-Monem, M.O., Yassin, M.H and Draz, A.A., 2014. Nutritional factors affecting levan production by *Bacillus* sp.V8 strain isolated from rhizosphere bean (*Vicia faba*) plant. *Journal of Agricultural Technology* Vol. 10(4): 899-914.
- Adesemoye, A.O., Torbert, H.A., Kloepper, J.W. **2009**. Plant Growth-Promoting rhizobacteria allow reduced application rates of chemical Fertilizers. *Microbial Ecol.* 58, 921-929.
- Adesemoye, A.O., Torbert, H.A., Kloepper, J.W. **2010**. Increased plant uptake of nitrogen from ¹⁵N-depleted fertilizer using plant growth-promoting rhizobacteria. *Applied Soil Ecology* 46 54–58.

- Adil, A., Ramazan, C., Akmac, I., Figen, A., Fikretin, S., Ahin, B.C. **2005**. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology & Biochemistry* 38, 1482–1487.
- Adviento-Borbe, M.A.A., Haddix, M.L., Binder, D.L., Walters, D.T., Dobermann, A. **2007**. Soil greenhouse gas fluxes and global warming potential in four high yielding maize systems. *Glob. Chan. Biol.* 13, 1972-1988.
- Adviento-Borbe, M.A.A., Kaye, J.P., Bruns, M.A., Mcdaniel, M.D., Mccoy, M., Harkcom, S. **2010**. Soil greenhouse gas and ammonia emissions in longterm maize-based cropping systems. *Soil Sci. Soc. America J.* 74, 1623-1634.
- Ahmad, F., Ahmad, I., Khan, M.S. **2005**. Indole acetic acid production by the indigenous isolates of Azotobacter and fluorescent Pseudomonas in the presence and absence of tryptophan. *Turk J Biol* 29:29–34
- Ahmad, F., Ahmad, I., Khan, M.S. **2008**. Screening of free-living rhizospheric bacteria for the multiple plant growth promoting activities. *Microbiol Res*;163:173–81.
- Ahmad, R., Lim C.J., Kwon, S.Y. **2013**. Glycine betaine: a versatile compound with great potential for gene pyramiding to improve crop plant performance against environmental stresses. *Plant Biotechnol Rep* 7:49–57. doi:10.1007/s 11816-012 - 0266-8
- Ahmed, A.k., El Hindi, M.H., Badawi, M.A., El Morarsy, S.A., Kalboush, F.A.H.M. **2011**. Response of wheat to rates of nitrogen, biofertilizers and land levelling. *Crop and Environment* 2(1): 46-51.
- Ahmedna, M., Marshall, W.E and Rao, R.M., **2000**. Granular Activated Carbons From Agricultural By-Products: Preparation, Properties, and Application in Cane Sugar Refining. January 2000 Bulletin Number 869.
- Akhtar, M.J., Asghar, H.N., Shahzad, K., Arshad, M. **2009**. Role of plant growth promoting rhizobacteria applied in combination with compost and mineral fertilizers to improve growth and yield of wheat (*Triticum aestivum* L.). *Pak. J. Bot.* 41(1), 381-390.
- Akiyama, H., Tsuruta, H., Watanabe, T. **2000**. N₂O and NO emissions from soils after the application of different chemical fertilizers. *Chemosphere- Global Chan. Sci.* 2, 313-320.

- Al-Amoudi, O.A., Moujahed, H.M. **2006**. Some aspects of nitrogen assimilation in wheat plant (*Triticum aestivum* L.) as affected by different nitrate levels and Bacteria Inoculation. *Saudi J. Biol. Sci.* 13(2), 141-151.
- Almaraz, J.J., Mabood, F., Zhou, X., Madramootoo, C., Rochette, P., Ma, B.L., Smith, D.L. **2009**. Carbon dioxide and nitrous oxide fluxes in corn grown under two tillage systems in Southwestern Quebec. *Soil Sci. Soc. America J.* 73, 113-119.
- Amal, A., Zaki, G., Nabila, M., Hassanein, M.S. **2007**. Response of grain Sorghum to different nitrogen sources. *Res. Agric. Biol. Sci.* 3, 1002-1008.
- Amirjani, M. **2012**. Effect of cadmium on wheat growth and some physiological factors. *International journal of forest, soil and erosion*. Vol-2, No-1.
- Aneja, K.R. **2008**. A Text book of Basic and Applied Microbiology. New Age International Publishers, New Delhi.
- APHA-AWWA-WPCF. **2005**. Standard method for the examination water, waste water, 19th ed., Washington, DC., USA.
- Ardakani, M.R., Mazaheri, D., Mafakheri, S., Moghadhham, A., **2011a**. Absorption efficiency of NPK through triple inoculation of wheat (*Triticum aestivum* L.) by *Azospirillum brasilense*, *Streptomyces* sp. *Glomus intraradices* and manure application. *Physiology of molecular biology of plants* 17(2): 181-192.
- Ardakani, M.R., Mazaheri, D., Rad, A.H.S., Mafakheri, S. **2011b**. Uptake of micronutrients by wheat (*Triticum aestivum* L.) in a sustainable agroecosystem. *Middle-east J. Sci. Res.* 7(4), 444-451.
- Arpana, J., Bagyaraj, D.J. **2007**. Response of kalmegh to an arbuscular mycorrhizae fungus and a growth promoting rhizomicroorganism at two levels of phosphorus fertilizer. *Am. Eurasian J. Agric. Environ. Sci.* 2, 33-38.
- Arregui, L.M., Quemada, M. **2006**. Drainage and nitrate leaching in a crop rotation under different N-fertilizer strategies: application of capacitance probes. *Plant soil.* 288:57-69.
- Arrobas, M., Parada, M.J., Magalhaes, P., Rodrigues, M.A. **2011**. Nitrogen use efficiency and economic efficiency of slow release N fertilizers applied to irrigated turfs in a Mediterranean environment. *Nutr. Cycl. Agroecosyst.* 89, 329-339.

- Arshad, M., Shaharoon, B., Mahmood, T. **2008**. Inoculation with *Pseudomonas* spp. Containing ACC-Deaminase Partially Eliminates the Effects of Drought Stress on Growth, Yield, and Ripening of Pea (*Pisum sativum* L.). *Pedosphere* 18(5): 611–620.
- Arya, R.L., Varshney, J.G., Kumar, L. **2007**. Effect of integrated nutrient application in chickpea and mustard intercropping system in the semi-arid tropics of North India. *Comm. Soil. Sci. Plant Anal.* 38, 229-240.
- Arzanesh, M., Alikhani, H., Khavazi, K., Rahimian, H., Miransari, M. **2011**. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World Journal of Microbiology and Biotechnology*, Vol.27, pp.197-205
- Aseri, G.K., Jain, N., Panwar, J., Rao, A.V., Meghwal, P.R. **2008**. Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) in Indian Thar Desert. *Scientia Horticulturae* 117, 130–135
- Ashok, V., Kumar, S., Singh, R.P. **2014**. Response of Organic Matrix Entrapped biofertilizers on Growth, Yield and soil properties of Rice (*Oryza sativa* L.). *Asian J. Agric. Food Sci.* 2(3), 211- 220.
- Ashraf, M., Foolad, M.R. **2007**. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216.
- Ashraf, M., **2004**. Some important physiological selection criteria for salt tolerance in plants. *Flora* 199, 361-376.
- Atlason R.S., Lehtinen, T., Daviod-ottir, B., Gislad-ottir G., Brocza, F., Unnþorsson, R., Ragnarsd-ottir, K.V. **2015**. Energy return on investment of Austrian sugar beet: A small-scale comparison between organic and conventional production. *Biomaass and bioenergy* 75, 267-271
- Audrey, V and Philippe, F., 2015. Development of organic farming for the protection of water quality: Local projects in France and their policy implications. *Land Use Policy* 43 197–206
- Azeem, B., KuShaari, K., Man, Z. B., Basit, A., Thanh, T.H. **2014**. Review on materials & methods to produce controlled release coated urea fertilizer. *J. Control Release.* 181(10), 11-21.

- Babu, S., Marimuthu, R. Manivanna, V. Ramesh, S.K. **2001**. Effect of organic and inorganic manures on growth and yield of rice. *Agricultural Science Digest* 21:232–234.
- Bakht, J., Shafi, M., Jan, M. T., Shah, Z. **2009**. Influence of crop residue management, cropping system and N fertilizers on soil N and C dynamics and sustainable wheat (*Triticum aestivum* L.) production. *Soil Till. Res.* 104, 233-240.
- Bao-guo M.A., Wenhua L., Huiyong, W. **2012**. Effect of different fertilization on the N, P loss in rice-wheat rotation soil in south Hebei, China. *Proc. Engg.* 28, 640- 643.
- Bashan, Y., Harrison, S.K., Whitmoyer, R.E. **1990**. Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. *Appl. Environment. Microbiol.* 56, 769-775.
- Bashan, Y., Hernandez, J.-P., Leyva, L.A., Bacilio, M. **2002**. Alginate microbeads as inoculant carrier for plant growth-promoting bacteria. *Biol. Fertil. Soils* 35, 359–368.
- Bashan, Y., Holguin, G. **1994**. Root-to-root travel of the beneficial bacterium *Azospirillum brasilense*. *Appl. Environ. Microbiol.* 60, 2120-2131
- Bashan, Y., Holguin, G. **1995**. Inter-root movement of *Azospirillum brasilense* and subsequent root colonization of crop and weed seedlings growing in soil. *Microb. Ecol* 29, 269-281.
- Bashan, Y., Holguin, G. **1997a**. *Azospirillum*-plant relationships: environmental and physiological advances (1990-1996). *Can. J. Microbiol* 43, 103- 12 1.
- Bashan, Y., Holguin, G. **1997b**. Short- and medium- term avenues for *Azospirillum* inoculation. In Plant Growth-Promoting Rhizobacteria-present status and future prospects, A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo and S. Akino (eds.), pp. 130-149, Faculty of Agriculture, Hokkaido University, Sapporo, Japan.
- Bashan, Y., Levanony, H. **1987a**. Horizontal and vertical movement of *Azospirillum brasilense* Cd in the soil and along the rhizosphere of wheat and weeds in controlled and field environments. *J. Gen. Microbiol* 133, 3473-3480.

- Bashan, Y., Levanony, H. **1987b**. The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season. *Can. J. Microbiol* 33, 1074-1079.
- Bashan, Y., Levanony, H. **1988**. Adsorption of the rhizosphere bacterium *Azospirillum brasilense* Cd to soil, sand and peat particles. *J. Gen. Microbiol* 134, 1811-1820.
- Beck, E.H., Fetitig, S., Knake, C., Hartig, K., Bhattarai, T. **2007**. Specific and unspecific responses of plants to cold and drought stress. *J Biosci* 32:501–510.
- Becking, J.H. **1981**. The Family Azotobacteraceae, 2nd ED, Springer, Verlag, Berlin. 795-817.
- Bhaskara, R. K.V. and Charyulu, P.B.B.N. **2005**. Evaluation of effect of inoculation of *Azospirillum* on the yield of *Setaria italic* (L.). *Afr J Biotech* 4(9):989–995
- Bhatia, A., Pathak, H., Jain, N., Singh, P.K., Singh, A.K. **2005**. Global warming potential of manure amended soils under rice-wheat system in the Indo-Gangetic plains. *Atmos. Environ.* 39, 6976-6984.
- Bhatia, A., Sasmal, S., Jain, N., Pathak, H., Kumar, R., Singh, A. **2010**. Mitigating nitrous oxide emission from soil under conventional and no-tillage in wheat using nitrification inhibitors. *Agric. Ecosyst. Environ.* 136, 247-253.
- Bhosale, P and Gadre, R.V., 2001. Beta-Carotene production in sugarcane molasses by a *Rhodotorula glutinis* mutant. *J Ind Microbiol Biotechnol*;26:327–32
- BIS. **2006**. Bureau of Indian standards. www.bis.org.in.
- Biswas, D.R., Narayanasamy, G. **2006**. Rock phosphate enriched compost: an approach to improve low-grade Indian rock phosphate. *Bioresour. Technol.* 97,2243–2251.
- Black, I.A. **1934**. An examination of the Degtjareff method for determining soil, organic matter and proposed modification of the chromic acid titration method. *Soil Sci.* 34, 29-38.
- Blaylock, A.D., Kaufmann, J., Dowbenko, R.D. **2005**. Nitrogen fertilizers technologies. *West. Nut. Manag.* 6, 8-13. Bojnec, S., Latruffe, L. **2013**. Farm size, agricultural subsidies and farm performance in Slovenia. *Land Use Pol* 32, 207–217.

- Blum, A., Sinmena, B. **1994**. Wheat seed endosperm utilization under heat stress and its relation to thermo tolerance in the autotrophic plant. *Field Crops Res.* 37, 185–191.
- Boonsiri, K., Suangsang, D., Pirommi, S., Sea-ang, A., Tongying, W., Kontha, J., Weejitjan, A. **2009**. Effect of granular organic fertilizers on the growth and yield of pak choi and rice cv. Phitssanulok 60-2. *Asian Journal of Food and Agro-Industry*, S1 60-S1 63.
- Borken, W., Matzner, E. **2009**. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob Change Biol* 15:808–824.
- Borlaug, N.E. **2003**. Feeding a world of 10 Billion People. The TVA/IFDC Legacy: Travis P. Hignett Memorial Lecture, Muscle Shoals, AL, USA.
- Bosch-Serra, A.D., Yagiue, M.R., Teira-Esmatges, M.R. **2014**. Ammonia emissions from different fertilizing strategies in Mediterranean rainfed winter cereals. *Atmos. Environ.* 84, 204-212.
- Boyer, K.W., Horwitz, W., Albert, R. **1985**. “Interlaboratory Variability in Trace Element Analysis,” *Anal. Chem.* 57, 454459.
- Brar, B.S., Singh, K., Dheri, G.S., Kumar, B. **2013**. Carbon sequestration and soil carbon pools in a rice- wheat cropping system: Effect of long-term use of inorganic fertilizers and organic manure. *Soil Till. Res.* 128, 30-36.
- Briggle, I.J.W. 1967. Morphology of the wheat plant. pages 89-116 in K.S. Quisenberry, ed. *wheat and wheat Improvement* amerj.cai society of Agronomy, Inc., Madison, Wisconsin, USA.
- Broschat, T.K., Moore, K.K. **2007**. Release of ammonium-nitrogen, nitrate-nitrogen phosphorus, potassium, magnesium, iron and manganese from seven controlled release fertilizer. *Comm. Soil Sci. Plant Anal.* 38, 843-850.
- Cai, Z., Xing, G., Yan, X., Xu, H., Tsuruta, H., Yagi, K., Minami, K. **1997**. Methane and nitrous oxide emissions from rice paddy fields as affected by nitrogen fertilisers and water management. *Plant Soil* 196, 7-14.
- Cakmakic, R. **2006**. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology & Biochemistry* 38, 1482–1487
- Cakmakic, R., Donmez, M.F., Erdogan, U. **2007**. The effect of plant growth promoting rhizobacteria 865 on barley seedling growth, nutrient uptake,

- some soil properties, and bacterial counts. *Turk J 866 Agric For* 31:189–199.
- Cakmakic, R., Kantar, F., Sahin, F. **2001**. Effect of N₂-fixing bacterial inoculations on yield of sugar beet and barley. *J. Plant Nutr. Soil Sci.* 164, 527–531.
- Cameron, K.C., Di, H.J., Moir, J.L. **2013**. Nitrogen losses from the soil/plant system: A Review. *Ann Applied Biol.* 145-173.
- Cao, Y., Tian, Y., Yin, B., Zhu, Z. **2014**. Improving agronomic practices to reduce nitrate leaching from the rice-wheat rotation system. *Agric. Ecosyst. Environ.* 195, 61-67.
- Cappelletti, M., Bucchi, G., Mendes, J.D.S., Alberini, A., Fedi, S., Bertin, L., Frascari, D., **2012**. Biohydrogen production from glucose, molasses and cheese whey by suspended and attached cells of four hyperthermophilic *Thermotoga* strains. *J. Chem. Technol. Biotechnol.* 87, 1291–1301.
- Cappuccino, J.G., Sherman, N. **1987**. Microbiology: A laboratory Manual, 1st ED, The Benjamin/ Cummings publishing Co. Inc, New York. 105-300.
- Casida, L.E., Klein, D.A., Santoro, T. **1964**. Soil Dehydrogenase Activity. *Soil Sci*, 98, 371-376.
- Cassan, F., Perrig, D., Sgroy, V., Masciarelli, O., Pennab, C., Luna, V. **2009**. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). *European journal of soil biology* 45 : 28-35.
- Cataldu, D.A., Haroon, M., Schvander, L.E., Young, L. **1975**. Rapid colorimetric determination of nitrate in plant tissue by nitrification of salicylic acid. *Comm. Soil Sci. Plant Anal.* 6, 71-80.
- Ceccarelli, S. and Grando, S. **1996**. Drought as a challenge for the plant breeder. *Plant Growth Regulation* 20:149–155.
- Ceccarelli, S. S., and Grando, M. B. **2007**. Participatory plant breeding in water limited environments. *Experimental Agriculture* 43, 411–435.
- Ceccarelli, S., Acevedo, E. and Grando, S. **1991**. Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. *Euphytica* 56:169–185.

- Cerny, J., Balik, J., Kulhanek, M., Casova, K., Nedved, V. **2010**. Mineral and organic fertilization efficiency in long-term stationary experiments. *Plant Soil Environ.* 56(1), 28–36.
- Chandna, P., Khurana, M.L., Iadha, J.K., Punia, M., Mehla, R.S., Gupta, R. **2011**. Spatial and seasonal distribution of nitrate- N in groundwater beneath the rice-wheat cropping system of India: a geospatial analysis. *Environ. Monit. Assess.* 178, 545-562.
- Chang, C.H., Yang, S.S. **2006**. Addition of polyelectrolyte oxygen detoxifier in poultry and livestock waste compost accelerating its maturity and maintaining population of thermo-tolerant microbes during composting. *In: Proc. 6th Cross Strait Conf. Soil Sci. Fertil.* vol. 2, pp. 677–692.
- Channabasanagowda, N.K., Patil, B.N., Awaknavar, J. S., Ninganur, B. T., Hunje, R. **2008**. Effect of Organic Manures on Growth, Seed Yield and Quality of Wheat. *Karnataka J. Agric. Sci* 21(3) (366-368).
- Chen, D., Li, Y., Grace, P., Mosier, A.R. **2008**. N₂O emissions from agricultural lands: a synthesis of simulation approaches. *Plant Soil.* 309, 169-189.
- Chen, S., Qic, C., Huang, T., Zhou, W., Qi, Y., Gao, Y., Shen, J., Qiu, L. **2013**. Effect of 1 amino cyclopropane-1-carboxylic acid deaminase producing bacteria on the hyphal growth and primordium initiation of *Agaricus bisporus*. *Fungal ecology* 6, 110-118.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C. **2006**. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl Soil* 879-880
- Cheng, H., Yang, S.S. **2009**. Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresource Technology* 100, 1648–1658.
- Chiarini, L., Bevivino, A., Tabacchioni, S., Dalmastri, C. **1998**. Inoculation of *Burkholderia Cepacia*, *Pseudomonas Fluorescens* and *Enterobacter* sp. on *Sorghum Bicolor*: root colonization and plant growth promotion of dual strain inocula. *Soil Biology Biochemical.* Vol. 30, No. I, pp. 81-87.
- Chien, S.H., Prochnow, L.I., Cantarella, H. **2009**. Recent developments of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. *Adv. Agron.* 102, 267-306.

- Chirinda, N., Carter, M.S., Albert, K.R., Ambus, P., Olesen, J.E., Porter, J.R., Petersen, S.O. **2010**. Emissions of nitrous oxide from arable organic and conventional cropping systems on two soil types. *Agric. Ecosyst. Environ.* 136, 199-208.
- Cohen, A., Bottini, R., Piccoli, P. **2008**. *Azospirillum brasilense* sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. *Plant Growth Regul* 54:97–103. doi:10.1007/s10725-007-9232-9
- Conrath, U., Beckers, G., Flors, V., Garcia-Agustin P, Jakab G, Mauch F, Newman M, Pieterse C, Poinssot B, Pozo M, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-Mani, B. **2006**. Priming: getting ready for battle. *Mol Plant Microbe Interact* 19:1062–1071.
- Creus, C.M., Sueldo, R.J., Barassi, C.A. **2004**. Water relations and yield in *Azospirillum* inoculated wheat exposed to drought in the field. *Can J Bot* 82:228–273
- Crutzen, P.J. **1970**. The influence of nitrogen oxides on the atmospheric ozone content. *Quart. J. Royal Meteorol. Soc.* 96, 320-325.
- Cui, F., Yan, G., Zhou, Z., Zheng, X., Deng, J. **2012**. Annual emission of nitrous oxide and nitric oxide from a wheat-maize cropping system on a silt loam calcareous soil in the north china plain. *Soil Biol. Biochem.* 48, 10-19.
- Dahiya, S., Jaiwal, P.K., Singh, R.P. **2004**. Efficient nitrogen assimilation and high productivity in rice (*Oryza sativa* L.) applied with organic matrix based slow release nitrogen fertilizers. *Physiol. Mol. Biol. Plant.* 10, 83-92.
- Das, B., Chakraborty, D., Singh, V.K., Aggarwal, P., Singh, R., Dwivedi, B.S., Mishra, R.P. **2014**. Effect of integrated nutrient management practice on soil aggregate properties, its stability and aggregate-associated carbon content in an intensive rice-wheat system. *Soil Till. Res.* 136, 9-18.
- Datta, A., Adhya, T.K. **2014**. Effects of organic nitrification inhibitors on methane and nitrous oxide emission from tropical rice paddy. *Atmos. Environ.* 92, 533- 545.
- Datta, A., Nayak, D.R., Sinhababu, D.P., Adhya, T.K. **2009**. Methane and nitrous oxide emissions from an integrated rainfed rice–fish farming system of Eastern India. *Agric. Ecosyst. Environ.* 129, 228-237.

- Davison, E.A. **1991**. Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers JE, Whitman WB (eds.) Microbial production and consumption of greenhouse gases: methane, nitrogen oxides and halomethanes. *American Society for Microbiology, Washington, DC*, pp 219-352.
- Da-wei, H., Zhou-ping, S., Tian-lai, L., Hong-zhi, Y., Hua, Z. **2014**. Nitrogen Nutrition Index and Its Relationship with N Use Efficiency, Tuber Yield, Radiation Use Efficiency, and Leaf Parameters in Potatoe. *J. Integrat. Agric.* 13(5), 1008-1016.
- De Freitas, J.R. **2000**. Yield and N assimilation of winter wheat (*Triticum aestivum* L., var. Norstar) inoculated with rhizobacteria. *Pedobiologia* 44, 97–104.
- Dechorgnat, J., Nguyen, C.T., Armengaud, P., Jossier, M., Diatloff, E., Filleur, S., Daniel-Vedele, F. **2011**. From the soil to the seeds: the long journey of nitrate in plants. *J. Exp. Bot.* 62, 1349-1359.
- Defago, G., Berling, C. H., Burger, U., Haas, D., Kahr, G., Keel, C., Voisard, C., Wirthner, P. and Wüthrich, B. **1992**. Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas jluorescens*: potential applications and mechanisms. In *Biological control of soil-borne plant pathogens*, D. Homby (ed.), pp. 93-108, C.A.B. International, Wallingford, Oxon, UK.
- Delin, S., Stenberg, M. **2014**. Effect of nitrogen fertilization on nitrate leaching in relation to grain yield response on loamy sand in Sweden. *Europ. J. Agro.* 52, 291-296.
- Delvasto, P., Valverde, A., Ballester, A., Igual, J.M., Muñoz, J.A., González, F., Blázquez, M.L., Garcia, C. **2006**. Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. *Soil Biol. Biochem.* 38, 2645 2654.
- Densilin, D., Sivagami, S.M., Manju, P., Sudha, S., 2011. Effect of Individual and Combined Application of Biofertilizers, Inorganic Fertilizer and Vermicompost on the Biochemical Constituents of Chilli (Ns - 1701). *Biofertilizers and Biopesticides volume 2*.
- Desbrosses, G., Varoquaux, F., Touraine, B. **2012**. Arabidopsis as a model system to decipher the diversity and complexity of plant responses to plant growthpromoting Rhizobacteria. In: Maheshwari DK, ed. Bacteria in

- agrobiolology: plant probiotics. Berlin, Heidelberg, Germany: Springer-Verlag, 227–250.
- Diez, J. A., Roman, R., Caballero, R., Caballero, A. **1997**. Nitrate leaching from soils under a maize- wheat-maize sequence, two irrigation schedules and three types of fertilizers. *Agri. Eco. Environ.* 65: 189-199.
- Diez-Lopez, J.A., Hernaiz-Algarra, P., Arauzo-Sanchez, M., Carrasco-Martin, I. **2008**. Effect of a nitrification inhibitor (DMPP) on nitrate leaching and maize yield during two growing seasons. *Span. J. Agric. Res.* 6, 294-303.
- Dimkpa, O.C., Merten, D., Svatos, A., Buchel, G., Kothe, E. **2009**. Metal-induced oxidative stress impacting plant growth in contaminated soil is alleviated by microbial siderophores. *Soil Biology & Biochemistry* 41, 154–162.
- Dinis, I., Ortolani, L., Bocci, R., Brites, C., 2015. Organic agriculture values and practices in Portugal and Italy. *Agricultural Systems* 136 39- 45.
- Dobereiner, J. and Day, J.M. **1976**. Associative symbioses in tropical grasses: characterization of microorganisms and dinitrogen-fixing sites. *In Proceedings of the first International Symposium on Nitrogen Fixation*. Vol. 2, W.E. Newton and C.J. Nyman (eds.), pp. 518-538, Washington State University Press, Pullman, USA.
- Dobermann, A., Cassman, K.G. **2004**. Environmental dimensions of fertilizer nitrogen: What can be done to increase nitrogen use efficiency and ensure global food security? In ‘‘Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertilizer Use on Food Production and the Environment’’ (A. R. Mosier, J. K. Syers, and J. R. Freney, Eds.), pp. 261–278. *SCOPE 65, Paris, France*.
- Don, C., Mann, G., Bekes, F., Hamer, R.J. **2006**. HMW-GS affect the properties of glutenin particles in GMP and thus flour quality. *Journal of Cereal Science* 44, 127-136.
- Dong, W.Y., Zhang, X., Dai, X., Fu, X., Yang, F., Liu, X., Sun, X., Wen, X., Schaeffer, S. **2014**. Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China. *Appl. Soil Ecol.* 84, 140-147.
- Duan, Y., Xu, M., Gao, S., Yang, X., Huang, S., Liu, H., Wang, B. **2014**. Nitrogen use efficiency in a wheat-corn cropping system from 15years of manure and fertilizer applications. *Field Crop Res.* 157, 47-56.

- Duangpaeng, A., Phetcharat, P., Chanthapho, S., Boonkantong, N., Okuda, N., **2012**. The Study and Development of Endophytic Bacteria for Enhancing Organic Rice Growth. *Procedia Engineering* 32, 172-176.
- Dwivedi, D.K., Thakur, S.S. **2000**. Effect of organic and inorganic fertilizers levels on productivity of rice (*Oryza sativa*) crop. *Indian Journal of Agronomy* 45:568–574.
- Effect of Organic Manures on Growth, Seed Yield and Quality of Wheat. *Karnataka J. Agric. Sci.* 21, 3 (366-368)
- Egamberdieva, D., Berg, G., Lindström, K., Räsänen, L.A. **2010**. Co-inoculation of *Pseudomonas* spp. with Rhizobium improves growth and symbiotic performance of fodder galega (*Galega orientalis* L.). *European Journal of Soil Biology* 46: 269-272.
- Egamberdiyeva, D., Hoflich, G. **2003**. Influence of growth-promoting bacteria on the growth of wheat in different soils and temperatures. *Soil Biology & Biochemistry* 35, 973–978.
- Ehdaie, B., merhaut, D.J., Ahmadian, s., Hoops, A.S., Khuong, T., Layne, A.P., WAines, J.G. **2010**. Root system size influence water- nutrient uptake and nitrate leaching potential of wheat. *J. Agron. Crop Sci.* 196, 455-466.
- El- Gizawy, **2009**. Effect of planting date and fertilizer application on yield of wheat under no till system. *World Journal of agricultural sciences* 5(6): 777-783.
- El-Komy, M.H., Hamdia, M.A., Abd El-Baki, G.K. **2003**. A. Nitrate reductase in wheat plants grown under water stress and inoculated with *Azospirillum* spp. *Biologia Plantarum* 46, 281–287.
- El-Sirafy, Z.M., Woodard, H.J., El-Norjar, E.M. **2006**. Contribution of Biofertilizers and fertilizer nitrogen to nutrient uptake and yield of Egyptian winter wheat. *J. Plant Nut.* 29, 587-599.
- Emilsson, T., Berndtsson, J. C., Mattsson, J. E., Rolf, K. 2007. Effect of using conventional and controlled release fertilizer on nutrient runoff from various vegetated roof systems. *Ecol. Eng.* 29: 260-271.
- Emrani, A., Davoodnia, A., Tavakoli-Hoseini, N. **2011**. Alumina supported Ammonium dihydrogen phosphate: Preparation, Characterization and its application as catalyst in the synthesis of 1, 2, 4, 5- tetra substituted Imidazols. *ChemInform.* 42(46), 1122-1140.

- Emtiazi, G., Naderi, A., Etemadifar, Z. **2004**. Effect of Nitrogen fixing bacteria on growth of potato tubers. *Adv. Food Sci.* 26 (2), 56–58.
- Entry, J.A., Sojka, R.E. **2010**. Matrix based fertilizers reduce nutrient leaching while maintaining Kentucky Bluegrass Growth. *Water Air Soil Poll.* 181-193.
- Esen Sekmen, A.H., Ozgur, R., Uzilday, B., Tanyolac, Z.O., Dinc, A. 2012. The response of the xerophytic plant *Gypsophila aucheri* to salt and drought stresses: the role of the antioxidant defence system. *Turk J Bot* 36, 697-706
- Evans, A.M. **1976**. In: Evolution of crop plants, Simmonds, N. W. (ed.), Lorgman, London, UK. pp.168-72.
- Eyvazi, J., Irannejad, H., Kianmehr, M.H., Esmaeili, M., Akbari, Q.A., Richard, N.O. **2010**. The effect of pellet fertilizer application on wheat yield and its components. *Int. Res. J. Plant Sci.* 1, 163- 171.
- Fan, X.H., Li, Y.C., Alva, A.K. **2011**. Effects of temperature and soil type on ammonia volatilization from slow release nitrogen fertilizers. *Comm. Soil Sci. Plant Anal.* 42, 1111-1122.
- FAO (Food and Agriculture, Organization). **2004**. FAO agricultural database (<http://www.fao.org>). FAO, Rome, Italy.
- FAO, **2012**. Agricultural Development Economics Division Food and Agriculture Organization of the United Nations. www.fao.org/economic/esa.
- FAOSTAT. **2013**. Food and agriculture organization of the United Nations. <http://faostat.fao.org/site/291/default.aspx>.
- Farhangfar, S., Bannayan, M., Khazaei, H.R., Baygi, M.M. **2015**. Vulnerability assessment of wheat and maize production affected by drought and climate change. *International journal of disaster risk reduction* 13, 37-51.
- Feldman, Kislev, M., Mordechai, E. **2007**. Domestication of emmer wheat and evolution of freethreshing tetraploid wheat in "A Century of Wheat Research From Wild Emmer Discovery to Genome Analysis", *Israel Journal of Plant Sciences*. 55, 207 221,
- Feldman, Moshe and Kislev, Mordechai, E. **2008**. Domestication of emmer wheat and evolution of freethreshing tetraploid wheat in "A Century of Wheat Research From Wild Emmer Discovery to Genome Analysis", *Israel Journal of Plant Sciences*, Volume 55, Number 3 4 / 2007, pp. 207 221, (<http://www.sciencefromisrael.com/app/home/contribution.asp?referrer=parent&backto=issue,2,14;journal,9,41;linkingpublicationresults,1:300170,1>).

- Feldman, M. **2001**. Origin of cultivated wheat. In: Bonjean AP, Angus WJ, editors. The world wheat book: a history of wheat breeding. Paris, France: Lavoisier Publishing;. p. 3-56.
- Felici, C., Vettori, L., Giraldi, E., Forino, L.M.C, Toffanon, A., Tagliasacchi, A.M, Nuti, M. **2008**. Single and co-inoculation of *Bacillus subtilis* and *Asospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. *Appl. Soil Ecol.* 40:260–270.
- Ferreira, A.S., Pires, R.R., Rabelo, P.G., Oliveira, R.C., Luz, J.M.Q., Brito, C.H. **2013**. Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. *Applied Soil Ecol.* 72, 103-108.
- Fischer, K.S. **2000**. Frontier project on nitrogen fixation in rice: Looking ahead. In “The Quest for Nitrogen Fixation in Rice” (J. K. Ladha and P. M. Reddy, Eds.), pp. 25–31.
- Fischer, S.E., Jofre, E.C., Cordero, P.V., Gutierrez-Manero, F.J., Mori, G.B. **2010**. Survival of native *Pseudomonas* in soil and wheat rhizosphere and antagonist activity against plant pathogenic fungi. *Anton. Van Leeuwen.* 97, 241–251.
- Flexas, J., Bota J., Loreto, F., Cornic, G., Sharkey, T.D. **2004**. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology.* 6, 269-279.
- Francisco, J.C., Luz, E.B., Yoav, B. **2012**. Enhanced accumulation of starch and total carbohydrates in alginate-immobilized *Chlorella* spp. induced by *Azospirillum Brasilense*. *Enzyme and Microbial Technology* 51, 300– 309.
- Gaggia, F., Baffoni, L., Gioia, D., Accorsi, M., Bosi, S., Marotti, I., Biavati, B., Dinelli, G. **2013**. Inoculation with micro-organism of *Lolium perenne* L.: Evaluation of plant growth parameters and endophytic colonization of roots. *New Biotechnology*, Volume 30 Number 6.
- Gajalakshmi, S. and Abbasi, S.A. **2004**. Neem leaves as a source of fertilizer-cum-pesticide vermicompost. *Bioresource Technology* 92, 291-296.
- Galloway, J. N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, S.P., Seitzinger, S.P., Sutton, M.A. **2008**. Transformation of nitrogen cycle: Recent trends, questions, and potential solutions. *Science* (Washington, DC) 320, 889-892.

- Garland, G.M., Suddick, E., Burger, M., Horwath, W.R., Six, J. **2011**. Direct N₂O emissions following transition from conventional till to no-till in a cover cropped Mediterranean vineyard (*Vitis vinifera*). *Agric. Ecosyst. Environ.* 141, 234–239.
- Gelinas, P., McKinnon, M.C. **2006**. Effect of wheat variety, farming site, and bread baking on total phenolics. *International Journal of Food Science & Technology*. 41, 329–332.
- Gerber, P., Chilonda, P., Franceschini, G., Menzi, H. **2005**. Geographical determinants and environmental implications of livestock production intensification in Asia. *Bioresour. Technol.* 96, 263–276.
- Ghormade, V., Deshpande, V. Mukund , Paknikar, M.K. **2011**. Perspectives for nano-biotechnology enabled protection and nutrition of plants. *Biotechnology Advances* 29 792–803.
- Ghost, B.C., Bhat, R. **1998**. Environmental hazards of nitrogen loading in wetland rice fields. *Environ. Pollut.* 102, 123– 126.
- Glick, B.R. **1995**. The enhancement of plant growth by free-living bacteria, *Can. J. Microbiol.* 4, 109–117.
- Godinot, O., Carof, M., Vertes, F., Leterme, P. **2014**. SYNE: An improved indicator to assess nitrogen efficiency of farming systems. *Agricult. Syst.* 127, 41-52.
- Gogoi, B., Baruah, K.K., 2012. Nitrous oxide emission from fields with different wheat and rice varieties. *Pedosphere* 22 (1), 112 - 121.
- Golloway, J., Raghuram, N., Abrol, Y. P. **2008**. A perspective on reactive nitrogen in a global, Asian and Indian context. *Current Science* 94:1375–1381.
- Gong, H., Zhu, X., Chen, K., Wang, S., Zhang, C. **2005**. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci* 169:313–321.
- Gong, W., Yan, X., Wang, J., Hu, T., Gong, Y. **2009**. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat–maize cropping system in northern China. *Geoderma* 149, 318-324.
- Gooding, A.R., Christopher D. G. Harley, C.D.G., Tang, E. 2009. Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *PNAS* vol-106, 9316–9321.
- Gopinath, K.A., Saha, S., Mina, B.L., Pande, H., Kundu, S., Gupta, H.S. **2008**. Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. *Nutr. Cycling Agro.* 82, 51-60.

- Gornall, J. **2010**. Implications of climate change for agricultural productivity in the early twenty-first century. *Philos Trans R Soc Lond B* 365:2973–2989
- Grant, C. A., Wub, R., Selles, F., Harker, K. N., Claydon, G. W., Bittman, S., Zebbarth, B. J. and Lupwayi, N. Z. **2012**. Crop yield and nitrogen concentration with controlled release urea and split applications of nitrogen as compared to non-coated urea applied at seeding. *Field Crop Res.*, 127, 170-180.
- Grichko, V.P., Glick, B.R. **2001**. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiol. Biochem.* 39, 11–17.
- Gu, J., Nicoulaud, B., Rochette, P., Pennock, D.J., Henault, C., Cellier, P., Richard, G. **2011**. Effect of topography on nitrous oxide emissions from winter wheat fields in central France. *Environ Poll.* 159, 3149-3155.
- Gudadhe, N.N., Mankar, P.S., Khawale, V.S., Dongarkar, K.P. **2005**. Effect of biofertilizers on growth and yield of mustard (*Brassica juncea* L.). *J. Soil Crop.* 15(1), 160-162.
- Guinazu, L.B., Andres, J.A., Rovera, M., Balzarini, M., Rosas, S.B. **2013**. Evaluation of rhizobacterial isolates from Argentina, Uruguay and Chile for plant growth-promoting characteristics and antagonistic activity towards *Rhizoctonia sp.* and *Macrophomina sp.* in vitro. *European Journal of Soil Biology* 54: 69-77.
- Guo, Z., Zhang, Y., Zhao, J., Shi, Y., Yu, Z. **2014**. Nitrogen use by winter wheat and changes in soil nitrate nitrogen levels with supplemental irrigation based on measurement of moisture content in various soil layers. *Field Crop Res.* 164, 117-125.
- Guo, Z.L., Cai, C.F., Li, Z.X., Wang, T.W., Zheng, M.J. **2009**. Crop residue effect on crop performance, soil N₂O and CO₂ emissions in alley cropping systems in subtropical China. *Agroforest. Syst.* 76, 67-80.
- Gupta, P.K., Sharma, C., Bhattacharya S., Mitra, A.P. **2002**. Scientific basis for establishing country greenhouse gas estimates for rice-based agriculture: An Indian case study, *Nutr. Cycling Agroecosys.* 64: 19–31.
- Gupta, S. K., Gupta, R. C., Chhabra, S. K., Eskiocak, S., Gupta, A. B., Gupta, R. **2008**. Health issues related to N pollution in water and air. *Current Science* 94:1469–1477.

- Gyaneshwar, P., Kumar, G.N., Parekh, L.J., Poole, P.S. **2002**. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil*. 245, 83-93.
- Halsall, D.M. **1993**. Inoculation of wheat straw to enhance lignocellulose breakdown and associated nitrogenase activity. *Soil Biol. Biochem.* 25: 419-429.
- Halvorson, A.D., Del-Grosso, S.J., Alluvione, F. **2010**. Nitrogen source effects on nitrous oxide emissions from irrigated no-till corn. *J. Environment Qual.* 39, 1554-1562.
- Hamdia, A.B.E., Shaddad, M.A.K., Doaa, M.M. **2004**. Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. *Plant Growth Regul* 44, 165-174
- Hamdia, M.A., El-Komy, H.M. **1997**. Effect of salinity, gibberellic acid and *Azospirillum* inoculation on growth and nitrogen uptake of *Zea mays*. *Biol Plant*;40:109–20.
- Hanson, R.L., **2003**. Climate Variability: Evapotranspiration and Droughts. U.S. Department of the Interior, U.S. Geological Survey. Available from: <<http://geochange.er.usgs.gov/sw/changes/natural/et/>>.
- Hariprasad, P., Divakara, S.T., Niranjana, S.R. **2011**. Isolation and characterization of chitinolytic rhizobacteria for the management of Fusarium wilt in tomato. *Crop Protection* 30, 1606-1612.
- Harris, J.N., New, P.B., Martin, P.M. **2006**. Laboratory tests can predict beneficial effects of phosphate-solubilising bacteria on plants. *Soil Biol Biochem* 38, 1521–1526
- Hartemink, A.E. **2005**. Nutrient stocks, nutrient cycling, and soil changes in cocoa ecosystems: a review. *Adv. Argon.* 86, 227–253.
- Hartmann, T.E., Yue, S., Schulz, R., Chen, X., Zhang, F., Muller, T. **2014**. Nitrogen dynamics, apparent mineralization and balance calculations in a maize-wheat double cropping system of the North China Plain. *Field Crop Res.* 160, 22-30.
- Hasanuzzaman, M., Hossain, M.A., Teixeira da Silva, J.A., Fujita, M. **2012**. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: Bandi, V., Shanker, A.K., Shanker, C., Mandapaka, M. (Eds.), *Crop Stress and its Management: Perspectives and Strategies*. Springer, The Netherlands, pp. 261–315.

- Hasegawa, P.M., Baressan, R.A., Zhu, J.K., Bohnert, H.J. **2000**. Plant cellular and molecular response to high salinity. *Annu. Rev. Plant Physiol. Plant Mol Biol.* 51, 463-499.
- Hawkesford, M.J. **2014**. Reducing the reliance on nitrogen fertilizer for wheat production. *J. Cereal Sci.* 59, 276-283.
- Hegde, S.V. Brahma Prakash, G.P. **1992**. A dry granular inoculant of *Rhizobium* for soil application. *Plant Soil* 144: 309-311.
- Heijnen, C.E., Hok-A-Hin, C.H and Van-Veen, J.A., 1992. Improvements to the use of bentonite clay as a protective agent, increasing survival levels of bacteria introduced into soil. *Soil Biol. Biochem.* 24: 533-538.
- Hejcman, M., Kunzova, E., Srek, P. **2012**. Sustainability of winter wheat production over 50 years of crop rotation and N, P and K fertilizer application on illimerized luvisol in the Czech Republic. *Field Crop Res.* 139, 30-38.
- Heller, H., Bar-Tal, A., Tamir, G., Bloom, P., Venterea, R.T., Chen, D., Zhang, Y., Clapp, C.E., Fine, P. **2010**. Effects of manure and cultivation on carbon dioxide and nitrous oxide emissions from a corn field under Mediterranean conditions. *J. Environ. Qual.* 39, 437-448.
- Henault, C., Gorssel, A., Mary, B., Roussel, M., Leonard, J. **2012**. Nitrous oxide emission by agricultural soils: A review of spatial and temporal for mitigation. *Pedosphere* 22(4), 426-433.
- Herbert, D., Phipps, P.J., Strange, R.E. **1971**. Chemical analysis of microbial cells. In: *Methods in microbiology*. (Norris, J.R. and D.W. Ribbons eds.). Academic press, London 209-234.
- Hernandez, J.P.L., de-Bashan, E., Rodriguez, D.J., Rodriguez, Y., Bashan, Y. **2009**. Growth promotion of the freshwater microalga *Chlorella vulgaris* by the nitrogen-fixing, plant growth-promoting bacterium *Bacillus pumilus* from arid zone soils. *European Journal of soil biology* 45, 88-93.
- Hernandez, L.E., Carpena-Ruiz, R., Garate, A. **1996**. Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J. Plant Nutr.* 19:1581-1598.
- Hoffmann, A., Hercus, M.J. **2000**. Environmental stress as an evolutionary force. *Bio-Science*, 50 (3).
- Holzinger, D., Nagendra-Prasad, Huys, G. **2011**. Plant protection potential and ultrastructure of *Bacillus subtilis* strain 3A25. *Crop Protection* 30, 739-744

- Hossain, A., Lozovskaya, M.V., Zvolinsky, V.P., Teixeira da Silva, J.A. **2012**. Effect of soil and climatic conditions on phenology of spring wheat varieties in the northern Bangladesh. *Nat. Sci.: J. Fund. Appl. Sci.* 2 (39), 78–86.
- Hou, X., Hua, J., Liang, W., Yang, D. **2006**. Effect of combined application of urease and Nitrification inhibitors on yield and quality of wheat. *Agricult. J.* 1(2), 109-112.
- Hu, Y., Schmidhalter, U. **2005**. Drought and salinity: A comparison of their effects on mineral nutrition of plants. *J. Plant Nutr. Soil Sci.* 168, 541–549.
- Huang, M, Liang, T., Yang, Z., Wang, L., Zhang, C., Zhou, C. **2011**. Leaching losses of nitrate nitrogen and dissolved organic nitrogen from a yearly two crops system, wheat-maize, under monsoon situations. *Nutr. Cycl. Agroecosyst.* 91, 77-89.
- Ilyas, N. and Bano, A. **2010**. *Azospirillum* strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol. Fertil. Soil.* 46, 393-406.
- Improvement of soil physical and chemical properties with compost application in Rice- Wheat cropping system. *Pakistan Journal of Botany* 40, 275-282.
- IPCC. **2006**. Agriculture, forestry and other land use. In: 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, (eds Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K), 66 pp. Institute for Global Environmental Strategies, Hayama, Japan.
- IPCC. **2007**. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (eds) *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change.* Cambridge University Press, Cambridge, United Kingdom, p 996
- Jackson, M.L. **1967**. Soil chemical analysis. Prentice Hall India, Pvt. Ltd., New Delhi, 498.
- Jadia, C.D., Fulekar, M. H. **2008**. Vermicomposting of vegetable waste: A biophysicochemical process based on hydro-operating bioreactor. *African Journal of Biotechnology* Vol. 7 (20), pp. 3723-3730.

- Jagadeeswaran, R., Murugappan, V., Govindasamy, M. **2005**, 'Effect of Slow Release NPK Fertilizer Sources on the Nutrient Use Efficiency in Turmeric (*Curcuma longa* L.) *World Journal of Agricultural Sciences* 1 (1): 65-69.
- Jalali, M. **2005**. Nitrates leaching from agricultural land in Hamadan, Western Iran. *Agric. Ecosyst. Environ.* 110, 210-218.
- Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram R, Vam R.P. **2009**. Drought stress in plants: a review on morphological characteris.
- Jaleel, C. A., Manivannan, P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram, R., Panneerselvam, R. **2007**. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. *Colloids and Surfaces B: Biointerfaces* 60, 7–11
- Jannoura., R., Joergensena, R.G., Bruns, C., 2014. Organic fertilizer effects on growth, crop yield, and soil microbial biomass indices in sole and intercropped peas and oats under organic farming conditions. *Europ. J. Agronomy* 52 259–270.
- Jayadeva, H.M., Prabhakara, T.K., Gowda, R.C., Devendra, R., Mallikarjun, G.B., Bandi, A.G. **2009**. Methane emission as influenced by different crop establishment techniques and organic manures. *Agric. Sci. Digest.* 29(4), 241- 245.
- Jeevanandam, M., Kannan, R., Srinivaslu, S., Rammohan, **2007**. Hydrogeochemistry and Groundwater Quality Assessment of Lower Part of the Ponnaiyar River Basin, Cuddalore District, South India. *Environ. Monit. Asses.* 132, 263-274.
- Jetten, M.S.M. **2008**. The microbial nitrogen cycle. *Environ Microbiol.* 10(11), 2903-2909.
- Jha, Y. and Subramanian, R.B. **2014**. PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. *Physiol Mol Biol Plants* 20(2):201–207
- Jia, X., Shao, L., Liu, P., Zhao, B., Gu, L., Dong, S., Bing, S. H., Zhang, J., Zhao, B. **2014**. Effect of different nitrogen and irrigation treatments on yield and nitrate leaching of summer maize (*Zea mays* L.) under lysimeter conditions. *Agric. Water Manag.* 137, 92-103.

- Jian-Bin, Z., Jin-Gen, X., Zhu-Jun, C., Sheng-Xiu, L. **2006**. Leaching and Transformation of Nitrogen Fertilizers in Soil After Application of N with Irrigation: A Soil Column Method. *Pedosphere* 16(2), 245-252.
- Jiang, D., Yue, H., Wollenweber, B., Tan, W., Mu, H., Bo, Y., Dai, T., Jing, Q., Cao, W. **2009**. Effects of post-anthesis drought and waterlogging on accumulation of high-molecular-weight glutenin subunits and glutenin macropolymers content in wheat grain. *Journal of Agronomy and Crop Science* 195, 89-97.
- Jiang, J., Hu, Z., Sun, W., Huang, Y. **2010**. Nitrous oxide emissions from Chinese cropland fertilized with a range of slow release nitrogen compounds. *Agric. Ecosyst. Environ.* 135, 216-225.
- Jim, V and Robert, H. **2001**. Comparison of liquid binders for limestone pelletizing. *Institute for Briquetting and Agglomeration 27th Biennial Conference*.
- Joe, M.M., Karthikeyan, B., Chauhan, P.S., Charlotte Shagol, C., Islam, M.R., Deiveekasundarama, M.S., T. **2012**. Survival of *Azospirillum brasilense* flocculated cells in alginate and its inoculation effect on growth and yield of maize under water deficit conditions. *European Journal of Soil Biology* 50, 198-206.
- Joshi, A.K., Mishra, E.B., Chatrath, E.R., Ferrara, G.O., Singh, R.P. **2007**. Wheat improvement in India: present status, emerging challenges and future prospects *Euphytica*. 157: 431-446.
- Kamara, A.Y., Menkir1, A., Kureh, I., Omoigui, L.O. **2006**. Response to low soil nitrogen stress of S1 maize breeding lines, selected for high vertical root-pulling resistance. *Maydica* 51, 425-433
- Kandil, A.A., El-Hindi, M.H., Badawi, M.A., El- Morarsy, Kalboush, F.A.H.M. **2011**. Response of wheat to rates of nitrogen, biofertilizers and land leveling. *Crop Environ.* 2(1), 46-51.
- Karivaradharajan, S., Radha P., Kumar, A., Pattnaik, S., Chakravarty, K., Shivay, Y.S., Rajendra Singh, R., Saxena, A.K. **2013**. Evaluating the influence of novel cyanobacterial biofilmed biofertilizers on soil fertility and plant nutrition in wheat. *European Journal of Soil Biology* 55, 107-116
- Kasim, W.A., Osman, M.E. Omar, M.N., Abd El-Daim, I.A. Bejai, S., Meijer, J. **2013**. Control of Drought Stress in Wheat Using Plant-Growth- Promoting Bacteria. *J Plant Growth Regul* 32:122–130.

- Kaur, G., Reddy, M.S. **2014**. Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. *App. Soil Ecol.* 72, 103–108.
- Kazmi, S.S. and Khan, S.A. **2005**. Level of nitrate and nitrite contents in drinking water of selected samples received at AFGMI, Rawalpindi. *Pak. J. Physiol.* 1, 1-2.
- Khaled, H., Fawy, H.A. **2011**. Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil Water Res.* 6, 21–29.
- Khanfar, A.R. **2010**. Dissolved nitrogen in drinking water resources in Al-Mahareth village of Assir-Saudi Arabia. *Saudi J. Biol. Sci.* 17, 265-268.
- Kirk, D.E and Othmer, D.F., 1967. Molasses. Encyclopedia of chemical technology, vol. 13, 2nd ed. New York: John Wiley and Sons; p. 613–32.
- Kizilkaya, R. **2008**. Yield response and nitrogen concentrations of spring wheat (*Triticum aestivum*) inoculated with *Azotobacter chroococcum* strains. *Ecological engineering* 33(2), 150-156.
- Kizilkaya, R., Turkay, F.S.H., Turkmen, C., Durmus, M. **2012**. Vermicompost effect on wheat yield and nutrient content in soil and plant. *Agronomy and Soil Science* 58, S175- S179
- Kloepper, J.W. and Schroth, M.N. **1981**. Plant growth-promoting rhizobacteria and plant growth under gnotobiotic conditions. *Phytopathology* 71: 642-644.
- Kloepper, J.W., Lifshitz, R., Zablutowicz, R.M. **1989**. Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol.* 7, 39–44.
- Kloepper, J.W., Ryu, C.M., Zhang, S. **2004**. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology.* 94 (11), 1259–1266
- Kłosowska K., **2010**. Plant responses to salinity, *Kosmos* 59, 539-549.
- Kohler, J., Hernandez, J.A., Caravaca, F., Roldan, A. **2009**. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environmental and Experimental Botany* 65, 245–252.
- Kong, L., Wang, F., Lopez-bellido, L. **2013**. Agronomic improvements through the genetic and physiological regulation of nitrogen uptake in wheat (*Triticum aestivum* L.). *Plant Biotechnol. Rep.* 7, 129-139.

- Kraiser, T., Gras, D.E., Gutierrez, A.G., Gonzalez, B., Gutierrez, R.A. **2011**. A holistic view of nitrogen acquisition in plants. *J. Exp. Bot.* 62, 1455-1466.
- Krauss, A., Isherwood, K., Heffer, P. **2007**. Fertilizers best management practices: General principles, Strategy for adaptation and voluntary initiatives vs regulations. Inter. Fertilizer Industry Association, Paris, France.
- Krey, T., Caus, M., Baum, C., Ruppel, S., Eichler-Löbermann, B. **2011**. Interactive effects of plant growth promoting rhizobacteria and organic fertilization on P nutrition of *Zea mays* L. and *Brassica napus* L. *J. Plant Nutr. Soil Sci.* 174, 602-613.
- Krey, T., Vassiley, N., Baum, C., Eichler-Löbermann, B. **2013**. Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions. *Europ. J. Soil Biol.* 55, 124-130.
- Kreye, C., Dittert, K., Zheng, X., Zhang, X., Lin, S., Tao, H., Sattelmacher, B. **2007**. Fluxes of methane and nitrous oxide in water-saving rice production in north China. *Nut. Cycl. Agroecosyst.* 77, 293-304.
- Krzyzaniak, M., Lemanowicz, J. **2013**. Enzymatic activity of the Kuyavia Mollic Gleysols (Poland) against their chemical properties. *Plant Soil Environ* 59, 359–365
- Kucukasik, F., H. Kazak, D. Guney, I. Finore, A.Y. Poli, N.B. Toksoy and Oner, E., **2011**. Molasses as fermentation substrate for levan production by *Halomonas* sp. *Appl. Microbiol. Biotechnol.*, 89:1729-1740.
- Kumar, B., Dubey, R.C., Maheshwari, D.K. **2005**. Biocontrol of *Macrophomina phaseolina*: prospects and constraints, in: T. Satyanarayana, B.N. Johri (Eds.), *Microbial Diversity: Current Perspectives and Potential Applications*, IK International Pvt Ltd, New Delhi, pp. 471–492.
- Kumar, B., Trivedi, P., Pandey, A. **2007**. *Pseudomonas corrugata*: A suitable bacterial inoculant for maize grown under rainfed conditions of Himalayan region. *Soil Biology & Biochemistry* 39, 3093–3100.
- Kumar, M., Bauddh, K., Kumar, S., Sainger, M., Sainger, P.A., Singh, R.P. **2013b**. Increase in growth, productivity and nutritional status of wheat (*Triticum aestivum* L. cv. Wh-711) and enrichment in soil fertility applied with organic matrix entrapped urea. *J. Environ. Biol.* 34, 1-9.
- Kumar, M., Bauddh, K., Sainger, M., Sainger, P.A., Singh, J.S., Singh, R.P. **2012**. Increase in growth, productivity and nutritional status of rice (*Oryza sativa*

- L. cv Bastmati) and enrichment in soil fertility applied with an organic matrix entrapped urea. *J. Crop Sci. Biotechnol.* 15(2), 137-144.
- Kumar, M., Bauddh, K., Sainger, M., Sainger, P.A., Singh, R.P. **2015a**. Enhancing efficacy of *Azotobacter* and *Bacillus* by entrapping in organic matrix for rice cultivation. *Agroecology and sustainable food system.* 39: 907-923.
- Kumar, M., Bauddh, K., Sainger, M., Sainger, P.A., Singh, R.P. **2015b**. Increase in growth, productivity and nutritional status of wheat (*Triticum aestivum*) and enrichment in soil microbial population applied with biofertilizers entrapped with organic matrix. *Journal of plant nutrition.* 38: 260-276.
- Kumar, P., Nanwal, R.K. **2006**. Effect of integrated nutrient management on productivity and uptake of N & P in pearl millet-wheat cropping system. *Ind. J. Fert.* 2(4), 49-53.
- Kumar, S., Bauddh, K., Barman, S.C., Singh, R.P. **2013a**. Evaluation of conventional and organic matrix entrapped urea and di-ammonium phosphate for growth and productivity of *Triticum aestivum* L. and mobilization of NO_3^- , NO_2^+ , NH_4^+ and PO_4^{-3} from soil to plant leaves. *Int. J. Agro. Plant Prod.* 4(6), 1357- 1368.
- Kumar, S., Bauddh, K., Barman, S.C., Singh, R.P. **2014a**. Organic matrix entrapped bio fertilizers increase growth, productivity and yield of *Triticum aestivum* L. and mobilization of NO_3^- , NO_2^- , NH_4^+ and PO_4^{-3} from soil to plant leaves. *J. Agri. Sci. Tech.* 16, 315-329.
- Kumar, S., Bauddh, K., Barman, S.C., Singh, R.P. **2014b**. Amendments of microbial biofertilizers and organic substances reduces requirement of urea and DAP with enhanced nutrient availability and productivity of wheat (*Triticum aestivum* L.). *Ecol. Eng.* 71, 432-437.
- Kumar, S., Pandey , P., Maheshwari, D.K. **2009**. Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. *European Journal of Soil Biology* 45, 334–340.
- Kumar, V., Behl, R. K., Narula, N. **2001**. Establishment of phosphate solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under greenhouse conditions. *Micro. Res.* 156, 87-93.
- Kundu, B.S., Nehra, K., Yadav, R., Tomar, M. **2009**. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different region of Haryana. *Ind. J. Microbiol.* 49, 120-127.

- Lafitte, H.R., Yongsheng, G., Yan, S., Lil, Z.K. **2007**. Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *J Exp Bot* 58:169–175.
- Laik, R., Sharma, S., Idris, M.m Singh, A.K., Singh, S.S., Bhatt, B.P., Saharwat, Y., Humphreys, E., Ladha, J.K. **2014**. Integration of conservation agriculture with best management practices for improving system performance of the rice-wheat rotation in the eastern Indo-gangetic plains of India. *Agric. Ecosyst. Environ.* 195, 68-82.
- Lavakush, Yadav, J., Verma, J.P., Jaiswal, J.P., Kumar, A. **2014**. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). *Ecol. Eng.* 62 (2014) 123–128.
- Lazcano, C., Dominguez, J. **2011**. The use of vermicompost sustainable agriculture: Impact on plant growth and soil fertility. *Soil Nutrient* 978-1-61327-785-3.
- Lee, J., Six, J., King, A.P., Van Kessel, C., Rolston, D.E. **2006**. Tillage and field scale controls on greenhouse gas emissions. *J. Environ. Qual.* 35, 714-725.
- Leopold, A.C. 1990. Coping with desiccation, In: stress responses in plants: adaptation and acclimation mechanism pp. 37-56, Alsher R.G, Cumming, J.R.eds, wiley-Lis, New york.
- Liew, Y.A., Omar, S.R.S. Husni, M.H.A. Abidin, M.A.Z. Abdullah. N.A.P. **2010**. Effects of micronutrient fertilizers on the production of MR 219 Rice (*Oryza sativa* L.). *Malaysian Journal of Soil Science* 14:71–82.
- Linquist, B., Groenigen, K.V., Adviento, M.A., Borbe, M.A., Pittelkow, C., Kessel, C. **2012**. An agronomic assessment of greenhouse gas emission from major cereal crops. *Glob. Chan. Biol.* 18, 194-209.
- Liu, C., Wang, K., Meng, S., Zheng, X., Zhou, Z., han, S., Chen, D., Yang, Z. **2011**. Effects of irrigation, fertilization and crop straw management on nitrous oxide and nitric oxide emissions from a wheat-maize rotation field in northern china. *Agric. Ecosyst. Environ.* 140, 226-233.
- Liu, J., Zhu, L., Luo, S., Bu, L., Chen, X., Yue, S. **2014**. Response of nitrous oxide emission to soil mulching and nitrogen fertilization in semi-arid farmland. *Agric.Ecosyst. Environ.* 188, 20-28.
- Lobell, D.B., Monasterio, J.I.O., Sibley, A.M., Sohu, V.S. **2013**. Satellite detection of earlier wheat sowing in India and implications for yield trends. *Agricult. Syst.* 115, 137-143.

- Lopez, C.G., Banowetz, G.M., Peterson, C.J., Kronstad, W.E. **2003**. Dehydrin expression and drought tolerance in seven wheat cultivars. *Crop Sci.* 43, 577–582.
- Lopez-Fernandez, S., Diez, J.A., Hernaiz, P., Arce, A., Garcia-Torres, L., Vallejo, A. **2007**. Effects of fertilizer type and the presence or absence of plants on nitrous oxide emissions from irrigated soils. *Nutr. Cycl. Agroecosyst.* 78, 279–289.
- Lucy, M., Reed, E., Glick, B.R. **2004**. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek* 86:1–25.
- Luo, L. Itoh, S. Zhang, Q. Yang, S. Zhang, Q. Yang, Z.2011. Leaching behavior of nitrogen in a long-term experiment on rice under different N management systems. *Environ Monit Assess* 177:141–150.
- Lupwayi, N.Z., Grant, C.A., Soon, Y.K., Clayton, G.W., Bittman, S., Malhi, S.S., Zebarth, B.J. **2010**. Soil microbial community response to controlled-release urea fertilizer under zero tillage and conventional tillage. *Appl. Soil Ecol.* 45, 254-261.
- Ma, J., Li, X.L., Xu, H., Han, Y., Cai, Z.C., Yagi, K. **2007**. Effects of nitrogen fertilizer and wheat straw application on CH₄ and N₂O emissions from a paddy rice field. *Aust. J. Soil Res.* 45, 359-367.
- Ma, J., Ma, E., Xu, H., Yagi, K., Cai, Z. **2009**. Wheat straw management affects CH₄ and N₂ emissions from rice fields. *Soil Biol. Biochem.* 41, 1022-1028.
- Maene, L. M. **2010**. The role of fertilizer of the global level. In: W. Payne, J. Ryan, eds. *The International dimension of the American society of agronomy: Past and future*, ASA, Madison, WI, USA. p. 75-82.
- Maene, L. M. **2000**. 10th World Food Prize Congress, Washington DC, pp. 169–171.
- Mahajan, A., Chaudhary, A.K., Jaggi, R.C., Dogra, R.K. **2003**. Importance of biofertilizers in sustainable agriculture. *Farm Forum.* 17-20.
- Mahanta, D., Rai, R.K., Mishra, S.D., Raja, A., Purakayastha, T. J., Varghese, E. **2014**. Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. *Field Crop. Res.* 166, 1-9.
- Mahdy, A.M. **2011**. Comparative effects of different soil amendments on amelioration of saline-sodic soils. *Soil Water Res* 6, 205–216.

- Majumdar, K., Jat, M.L., Pampolno, M., Dutta, S., Kumar, A. **2013**. Nutrient management in wheat: current scenario, improved strategies and future research needs in India. *J. Wheat Res.* 4, 1-10.
- Malik, S., Chaurasia, P., Lahudkar, S., Uprety, B., Bhaumik, S.R. **2012**. Rad26p regulates the occupancy of histone H2A-H2B dimer at the active genes in vivo. *Nucleic Acids Res* 40(8):3348-3363
- Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T., Samiyappan, R., **2010**. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. *Biological Control* 54, 83–89.
- Marcelino, C.M., Roberto, U.P., Rodrigo, O.B., Stanley, B.S., Natalia, V.V. **2011**. Quantifying nitrate leaching in irrigated wheat with different nitrogen fertilization strategies in an alfisol. *Chilean J. Agricult. Res.* 71(1), 148-156.
- Marenya, P., Nkonya, E., Xiong, W., Deustua, J., Kato, E. **2012**. Which policy would work better for improved soil fertility management in sub-Saharan Africa, fertilizer subsidies or carbon credits? *Agricult. Syst.* 110, 162-172.
- Maria P., Susana, B., Gallego, M., Tomaro, M.L. **2005**. Cadmium toxicity in plants. *Braz. J. Plant Physiol.* vol.17 no.1 Londrina Jan./Mar.
- Marques, P.G.C.A., Pires, C., Moreira, H., Rangel, O.S.S.A., Castro, M.L.P. **2010**. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biology & Biochemistry* 42, 1229-1235.
- Martin, S.J., Medeiros, F.H.V., de-Souza, R.M.S., De- Resende, M.L.V., Ribeiro, P.M.J. **2013**. Biological control of bacterial wilt of common bean by plant growth-promoting Rhizobacteria. *Biological Control* 66, 65–71.
- Martiniello, P., Da-Silva, T.J.A. **2011**. Physiological and bio– agronomical aspects involved in growth and yield components of cultivated forage species in Mediterranean environments: A review. *European J. Plant Sci. Biotech* 5 (Special Issue 2), 64–98.
- Marulanda, A., Porcel, R., Barea, J.M., Azcon, R. **2007**. Drought tolerance and antioxidant activities in lavender plants colonized by native drought-tolerant or drought-sensitive *Glomus* species. *Microb Ecol* 54:543–552
- Masclaux-Daubresse, C., Daniel-Vedel, F., Dechorgnat, J., Chardon, F., Gaufichon, L. **2010**. Nitrogen uptake, assimilation and remobilization in plants:

- challenges for sustainable and productive agriculture. *Ann. Bot.* 105, 1141-1157.
- Maxwell, O., Mile, I., Obeta, M.C. **2010**. Seasonal variation in nitrate levels in hand dug wells in makurdi metropolis. *Pak. J. Nut.* 9(6), 539-542.
- Mayak, S., Tirosh, T., Glick, B.R. **2004**. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science* 166, 525–530
- McAllister, C.H., Beatty, P.H., Good, A.G. **2012**. Engineering nitrogen use efficient crop plants: the current status. *Plant Biotechnol. J.* 10, 1001-1025.
- Meada, M. **2003**. Nitrate leaching in an Andisol treated with different type of fertilizers. *Environmental pollution* 121, 477-487.
- Menendez, S., Lopez-Bellido, R.J., Benitez-vega, J., Gonzalez-Murua, C., Lopez-Bellido, L., Estavillo, J.M. **2008**. Long-term effect of tillage, crop rotation and N fertilization to wheat on gaseous emissions under rainfed Mediterranean conditions. *European J. Agro.* 28, 559-569.
- Merrington, G., Winder, L., Parkinson, R., Redman, M. **2002**. Agricultural Pollution: Environmental problems and Practical Solution. Spon's Environmental Science and Engineering Series, Spon Press, Taylor and Francis group, London and New York, pp. 11-40.
- Migliorati, M.D.A., Scheer, C., Grace, P.R., Rowlings, D.W., Bell, M., McGree, J. **2014**. Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N₂O emissions from a subtropical wheat-maize cropping system. *Agric. Ecosyst. Environ.* 186, 33-43.
- Miller, M.N., Zebarth, B.J., Dandie, C.E., Button, D.L., Goyer, C., Trevors, J.T. **2008**. Crop residue influence on denitrification, N₂O emissions and denitrifier community abundance in soil. *Soil Boil Biochem.* 40, 2553-2562.
- Minaxi, Saxena, J., S. Chandra, S., Nain, L. **2013**. Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *Journal of Soil Science and Plant Nutrition*, 13 (2), 511-525.
- Minj, R.P., Singh, R.P., **2015**. Enhanced dose of *Azotobacter chroococcum* and *Bacillus subtilis* co-immobilized in vermicompost based organic granules increase biomass yield and harvest index of wheat (*Triticum aestivum*.L). *Climate Change and Environmental Sustainability*, 3(2), 157-162.

- Mitsch, W.J., Day, J.W. **2006**. Restoration of wetlands in the Mississippi–Ohio–Missouri (MOM) river basin: experience and needed research. *Ecol. Eng.* 26, 55–69.
- Mittler, R. **2002**. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7: 405410.
- Mohanty, S., Patra, A.K., Chhonkar, P.K. **2008**. Neem (*Azadirachta indica*) seed kernel powder retards urease and nitrification activities in different soils at contrasting moisture and temperature regimes. *Biores. Technol.* 99, 894–899.
- Monem, M.A., Lindsay, W.L., Sommer, R., Ryan, J. **2010**. Loss of nitrogen from urea applied to rainfed wheat in varying rainfall zones in northern Syria. *Nut. Cycl. Agroecosyst.* 86, 357–366.
- Montanez, A., Blanco, A.R., Barlocco, C., Beracochea, M., Sicardi, M. **2012**. Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects in vitro. *Applied Soil Ecology* 58: 21–28.
- Morrissey, J.P., Dow, J.M., Mark, G.L., O’Gara, F. **2004**. Are microbes at the root of a solution to world food production? Rational exploitation of interactions between microbes and plants can help to transform agriculture. *EMBO Rep* 5:922–926.
- Mubeen, F., Sheikh, A.A., Iqbal, T., Hameed, S., Malik, K.A., Hafeez, F.Y. **2006**. Response of wheat yield under combined use of fungicides and biofertilizers. *Int. J. Agric. Biol.* 8, 580–582.
- Myrbeck, A., Stenberg, M., Rydberg, T. **2012**. Establishment of winter wheat strategies for reducing the risk of nitrogen leaching in a cool-temperature region. *Soil Till. Res.* 120, 25–31.
- Mzoughi, N., 2014. Do organic farmers feel happier than conventional ones? An exploratory analysis. *Ecological Economics* 103 38–43.
- Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M. **2014**. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol. Adv.* 32, 429–448.
- Nadeem, S.M., Zahir, A. Z., Naveed, M., Nawaz, S. **2013**. Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-

- promoting rhizobacteria in naturally saline conditions. *Ann Microbiol* 63, 225–232
- Naher, U.A., Othman, R., Shamsuddin, Z.H.J., Saud, H.M., Ismail, M.R., 2009. Growth Enhancement and Root Colonization of Rice Seedling by *Rhizobium* and *Corynebacterium* spp. *International Journal of Agriculture and Biology*. 11-5-586-590.
- Naik, P.R., Sakthivel, N. **2006**. Functional characterization of a novel hydrocarbonoclastic *Pseudomonas* sp. strain PUP6 with plant-growth-promoting traits and antifungal potential. *Research in Microbiology* 157, 538–546.
- Naik, P.R., Raman, G., Narayanan, K.B, Sakthivel, N. **2008**. Assessment of genetic and functional 1099 diversity of phosphate solubilizing *fluorescent pseudomonas* isolated from rhizospheric soil. 1100 *BMC Microbiol* 8:230–243.
- Naiman, A.D., Nico, A.L., Salamone, I.G.D. **2009**. Inoculation of wheat with *Azospirillum Brasilense* and *Pseudomonas fluorescens*: Impact on the production and culturable rhizosphere microflora. *European Journal of soil biology* 45, 44 – 51.
- Nain, L., Rana, A., Joshi, M., Jadhav, S.D., Kumar, D., Shivay, Y.S., Paul, S., Prasanna, R. **2010**. Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. *Plant Soil* 331, 217-230.
- Narula, N., Kumar, V., Behl, R.K., Deubel, A., Gransee, A., Merbach, W. **2000**. Effect of P-solubilizing *Azotobacter chroococcum* on N, P, K uptake in P-responsive wheat genotypes grown under greenhouse conditions. *J. Plant Nutr. Soil Sci.* 163, 393–398.
- Nasholm, T., Kielland, K., Ganeteg, U. **2009**. Uptake of organic nitrogen by plants. *New Phytol.* 182, 31-48.
- Nautiyal, C.S., Upadhyay, R.K., Mukherji, K.G., Chamola, B.P. **2000**. *Biocontrol Potential and its Exploitation in Sustainable Agriculture*, Kluwer Academic/ Plenum Publishers, New York, pp. 9–23.
- Nautiyal, C. S., Srivastava, S., Chauhan, P. S. Seem K., Mishra, A., Sopory, S.K. **2013**. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant Physiology and Biochemistry* 66 1- 9

- Nayak, A.K., Gangwar, B., Shukla, A.K., Mazumdar, S.P., Kumar, A., Raja, R., Kumar, A., Kumar, V., Rai, P.K., Mohan, U. **2012**. Long-term effect of different integrated nutrient management on soil organic carbon and its fractions and sustainability of rice–wheat system in Indo Gangetic Plains of India. *Field Crop Res.* 127, 129-139.
- Nayak, A.K., Sharma, D.K. Singh, C.S., Mishra, V.K., Singh, G., Swarup, A. **2011**. Diagnosis and recommendations integrated system approach for nitrogen, phosphorous, and zinc foliar diagnostic norms for anola in central Indo-Gangetic plains. *J. Plant Nutr.* 34, 547-556.
- Nest, T. V., Vandecasteele, B., Ruyschaert, G., Cougnon, M., Merckx, R., Reheul, D. **2014**. Effect of organic and mineral fertilizers on soil P and C levels, crop yield and P leaching in a long term trial on a silt loam soil. *Agric. Ecosyst. Environ.* 197, 309-317.
- Nobbe, F. and Hiltner, L. **1896**. U.S. Patent 570 813. Inoculation of the soil for cultivating leguminous plants.
- Nylinder, J., Stenberg, M., Jansson, P., Klemedtsson, A.K., Weslien, P., Klemedtsson, L. **2011**. Modelling uncertainty for nitrate leaching and nitrous oxide emissions based on a swedish field experiment with organic crop rotation. *Agric. Ecosyst. Environ.* 141, 167-183.
- Oaks, A. **1986**. Biochemcial aspects of nitrogen metabolism in a whole plant context. In: Fundamental, Ecological and Agricultural Aspects of Nitrogen. Fixation in Higher plants (Eds, Lambers, H., Neeteson, J.J. and Stulen, I.), Martinus Nijhoff Pub. Dordrecht, pp. 133-151.
- Oaks, A. **2005**. Conflict resolution in science: then and now. In: Biotechnological Approaches to Improve nitrogen use Efficiency in Plants (Eds. Singh, R.P. and Jaiwal, P.K.) Stadium Press LLC, Houston, USA, pp.
- Ogut, M., Akdag, C., Duzdemir, O., Sakin, M.A. **2005**. Single and double inoculation with *Azospirillum/Trichoderma*: the effects on dry bean and wheat. *Biol. Fert. Soil.* 41, 262-272.
- Olad, A., Rashidzadeh, A. **2012**. Poly (N-vinylpyrrolidone) modified polyaniline/Na⁺ cloisite nanocomposite: Synthesis and characterization. *Fiber Poly.* 13(1), 16-20.
- Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimaraes, C.T., Schaffert, R.E., Sa, N.M.H. **2009**. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated

- in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* 41, 1782–1787.
- Olsen, S.R., Cole, C.H., Wantanabe, F.S., Dean, L.A. **1954**. Estimation of available phosphorus by extraction with sodium carbonate. U.S. Deptt. of Agric. Circ. 939, Washington D.C
- Omar, M.N., Fang, P., Jia, X.M. **2000**. Effect of inoculation with *Azospirillum brasilense* NO40 isolated from Egyptian soils on rice growth in China. *Egypt J Agric Res* 78:1005–1014.
- Omar, M.N.A., Osman, M.E.H., Kasim, W.A., Abd El-Daim I.A. **2009**. Improvement of salt tolerance mechanisms of barley cultivated under salt stress using *Azospirillum brasilense*. In: Ashraf M, Ozturk M, Habib-ur-Rehman A (eds) *Salinity and water stress improving crop efficiency*. Springer, *Dordrecht*, pp 133–147.
- Osborne, S.L. **2007**. Utilization of existing technology to evaluate spring wheat growth and nitrogen nutrition in South Dakota. *Comm. soil Sci. Plant Anal.* 38: 949-958.
- Otinga, A.N., Pypers, P., Okalebo, J.R., Njoroge, R., Emongole, M., Six, L., Vanlauwe, B., Merckx, R. **2013**. Partial substitution of phosphorus fertiliser by farmyard manure and its localized application increases agronomic efficiency and profitability of maize production. *Field Crop Res.* 140, 32-43.
- Ouahmane, L., Thioulouse, J., Hafidi, M., Prin, Y., Ducousso, M., Galiana, A., Plenchette, C., Kisa, M., Duponnois, R., 2007. Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochthonous arbuscular mycorrhizal fungi. *For. Ecol. Manage.* 241, 200–208.
- Ovrutskaya, I. 2014. Callose content in cell walls of leaf epidermis and mesophyll in *Alisma plantago aquatica* L. plants growing in different conditions of water supply. *Cytology and Genetics*. ISSN 0095-4527
- Owens, L.B. and Shipitalo, M.J. **2006**. Surface and subsurface phosphorous losses from fertilized pasture systems in Ohio. *J. Environm. Qual.* 35, 1101- 1109.
- Ozkan, H., Brandolini, A., Schafer-Preg, R., Salamini, F. **2002**. "AFLP analysis of a collection of tetraploid wheats indicates the origin of emmer and hard wheat domestication in southeast Turkey". *Molecular Biology and Evolution* 19 (10): 1797–801.

- Packowski, M.E. and Berryhill, D.L. **1979**. Survival of *Rhizobium phaseoli* in coal based inoculants. *Appl. Environ. Microbiol.* 38: 612-615.
- Padilla, F.M., Fleitas, M.T.P., Gallardo, M., Thompson, R.B. **2014**. Evaluation of optical sensor measurements of canopy reflectance and of leaf flavonols and chlorophyll contents to assess crop nitrogen status of muskmelon. *Europ. J. Agron.* 58, 39-52.
- Palsova, L., Schwarczovaa, L., Schwarcza, P., Bandlerova, A., 2014. The support of implementation of organic farming in the Slovak Republic in the context of sustainable development. *Procedia - Social and Behavioral Sciences* 110, 520 – 529.
- Pandey, A., Durgapa, A., Joshi, M., Lok Man S. Palni, L.M.S. **1999**. Influence of *Pseudomonas corrugata* inoculation on root colonization and growth promotion of two important hill crops. *Microbiological Research.* 154, 259-266.
- Pandey, R., Singh, B., Nair, T.V.R. **2005**. Impact of Arbuscular Mycorrhizal Fungi on Phosphorus efficiency of wheat, rye and triticale. *J. Plant Nut.* 28, 1867-1876.
- Panhwar, Q.A., Radziah, O., a, U.A., Zaharah, A.R., Sariah, M., Mohd- Razi, I., 2012. Root colonization and association of phosphate-solubilizing bacteria at various at various levels of triple super phosphate in aerobic rice seedlings. *African Journal of Microbiology Research* Vol. 6 (10), 2277-2286.
- Park, J., Bolan, N., Megharaj, M., Naidu, R. **2010b**. Isolation of phosphate-solubilizing bacteria and characterization of their effects on lead immobilization. *Pedologist* 53, 67–75.
- Park, K.H., Lee, O.M., Jung, H.I., Jeong, J.H., Jeon, Y.D., Hwang, D.Y., Lee, C.Y., Son, H.J. **2010a**. Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamensis* M6 isolated from ginseng rhizospheric soil. *Appl. Microbiol. Biotechnol.* 86, 947–955.
- Parkin, T.B. and Hatfield, J.L. **2010**. Influence of nitrapyrin on N₂O losses from soil receiving fall-applied anhydrous ammonia. *Agric Ecosyst. Environ.* 136, 81-86.
- Patel, D., Saraf, M. **2013**. Influence of soil ameliorants and microflora on induction of antioxidant enzymes and growth promotion of *Jatropha curcas* L. under saline condition. *European Journal of Soil Biology* 55 47-54

- Patel, K.J., Singh, A.K., Nareshkumar, G., Archana, G. **2010**. Organic-acid-producing, phytate-mineralizing rhizobacteria and their effect on growth of pigeon pea (*Cajanus cajan*). *Applied Soil Ecology* 44, 252–261.
- Pathak, H., Bhatia, A., Jain, N and Aggarwal, P.K.. **2010**. Greenhouse gas and mitigation in Indian agriculture –A review. ISBN: 81-85992-32-0.
- Patra, A.K., Chonkar, P.K., Khan, M.A. **2006**. Effect of green manure *Sesbania sesban* and nitrification inhibitor encapsulated calcium carbide (ECC) on soil mineral-N, enzyme activity and nitrifying organisms in a enzyme activity and nitrifying organisms in a rice-wheat cropping system. *European J. Soil Biol.* 42, 173-180.
- Patra, D.D., Chand, S., Anwar, M. **2014**. Organic C dynamics and its conservation under wheat (*Triticum aestivum*), Mint (*Mentha arvensis*)-*Sesbania rostrata* cropping in sub-tropical condition of northern Indo-Gangetic plains. *J. Environ. Manag.* 135,118-125.
- Patra, D.D., Kiran, U., Chand, S. **2009**. Use of urea coated with natural products to inhibit urea hydrolysis and nitrification in soil. *Biol. Fertil. Soil.* 45, 617-621.
- Peng, S., Yang, S., Xu, J., Luo, Y., Hou, H. **2011**. Nitrogen and phosphorus leaching losses from paddy fields with different water and nitrogen managements. *Paddy Water Environ.* 9, 333-342.
- Perego, A., Basile, A., Bonfante, A., De Mascellis, R., Terribile, F., Brenna, S., Acutis, M. **2012**. Nitrate leaching under maize cropping systems in Po Valle (Italy). *Agric. Ecosyst. Environ.* 147, 57-65.
- Pereyra MA, Garcia P, Colabelli MN, Barassi CA, Creus CM. **2012**. A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. *Appl Soil Ecol* 53:94–97.
- Pereyra, M.A., Zalazar, C.A., Barassi, C.A., **2006**. Root phospholipids in *Azospirillum* inoculated wheat seedlings exposed to water stress. *Plant Physiol. Biochem.* 44, 873–879.
- Pereyra, R.T., Bergström, L., Kautsky, L., Johannesson, K. **2009**. Rapid speciation in a newly opened postglacial marine environment, the Baltic Sea. *BMC Evol. Biol.* 9, 70. <http://dx.doi.org/10.1186/1471-2148-9-70>.
- Perez-Montano, F., Alias-Villegas, C., Bellogin, R.A., Cerro, P. D., Espuny, M.R., Jimenez Guerrero, I., Lopez-Baena, F.J., Ollero, F.J., Cubo, T. **2014**. Plant

- growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. *Microbiol. Res.* 169, 325- 336
- Phillips, R.L., Tanaka, D.L., Archer, D.W., Hanson, J.D. **2009**. Fertilizer application timing influences greenhouse gas fluxes over a growing season. *J Environ.Qual.* 38, 1569-1579.
- Pinto AP, Mota AM, de Varennes A, Pinto FC., **2004**. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Sci. Tot. Environ* 326:239–247.
- Piromyou, P., Buranabanyat, B., Tantasawat, P., Tittabutr, P., Boonkerd, N., Teaumroong, N., 2011. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. *European Journal of Soil Biology* 47, 44-54
- Pirozi, M.R., Margiotta, B., Lafiandra, D., MacRitchie, F. **2008**. Composition of polymeric proteins and bread-making quality of wheat lines with allelic HMWGS differing in number of cysteines. *Journal of Cereal Science* 48, 117-122.
- Plaza-Bonilla, D., Alvaro-Fuentes, J., Luis, J., Arrue, Cantero-Martinez, C. **2014**. Tillage and nitrogen fertilization effects on nitrous oxide yield-scaled emissions in a rainfed Mediterranean area. *Agric. Ecosyst. Environ.* 189, 43- 52.
- Pourjavadi, A., Doulabi, M., Soleyman, R., Sharif, S., Eghtesadi, S.A. **2012**. Synthesis and characterization of a novel (salep phosphate)-based hydrogel as a carrier matrix for fertilizer release. *Reactive Function. Poly.* 72, 667-672.
- Povilaitis, A., Sileika, A., Deelstra, J., Gaigalis, K., Baigys, G. **2014**. Nitrogen losses from small agricultural catchments in Lithuania. *Agric. Ecosyst. Environ.* DOI/ <http://dx.doi.org/10.1016/j.agee.2014.02.002>.
- Prabha, M.L., Jayraay, I.A., Jayraay, R. and Rao, D.S. 2007. Effect of vermicompost on growth parameters of selected vegetable and medicinal plants. *Asian Journal of microbiology, Biotechnology and Environmental Sciences*, 9(2), 321326.
- Prather, M.R., Derwent, D., Erhalt, P., Fraser, P., Sanhueza, E., Zhou, X. **1995**. Other trace gases and atmospheric chemistry. In: Houghton JT et al (ed)

- Climate change 1994: radiative forcing of climate change and an evaluation of the IPCC IS92 emission scenarios. Cambridge University Press, Cambridge, UK, pp 73 -126.
- Probanza, A., Garcia, L.J.A., Ruiz-Palomino, M., Ramos, B., Gutierrez- Maero, F.J. **2002**. *Pinus pinea* L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT5106 and *B. pumilus* CECT 5105). *Appl. Soil Ecol.*, 20: 75-84.
- Probanza, A., Mateos, J.L., Lucas Garcia, J.A., Ramos, B., De-Felipe, M.R., Gutierrez-Manero, F.J. **2001**. Effects of inoculation with PGPR *Bacillus* and *Pisolithus tinctorius* on *Pinus pinea* L.: growth, bacterial rhizosphere colonization, and mycorrhizal infection. *Microbial Ecology* 41, 140–148.
- Qadir, M., Ghafoor, A., Murtaza, G. **2000**. Amelioration strategies for saline soils: a review. *Land Degrad Dev* 11:501–521.
- Qin, Y., Liu, S., Guo, Y., Liu, Q., Zou, J. **2010**. Methane and nitrous oxide emissions from organic and conventional rice cropping systems in Southeast China. *Biol. Fertil Soil.* 46, 825-834.
- Quyên, N.V., Sharma, S.N., Gautam, R.C. **2002**. Comparative study of organic and traditional farming for sustainable rice production. *Omonrice* 10:74–78.
- Radha, T.K., Rao, D.L.N. **2014**. Plant Growth Promoting Bacteria from Cow Dung Based Biodynamic Preparations. *Ind. J. Microbiol.* DOI 10.1007/s12088-014- 0468-6.
- Rajpar, I, Khanif, Y.N., Ahmed, O.H., Sial, N.B., Korejo, M.A. **2006**. Effect of source and time of application of phosphatic fertilizer on wheat (*Triticum aestivum* L.). *American-Eurassian J. Agric. Environ. Sci.* 1(2), 83-85.
- Ram, H., Dadthwal, V., Vashist, K.K., Kaur, H. **2013**. Grain yield and water use efficiency of wheat (*Triticum aestivum* L.) in relation to irrigation levels and rice straw mulching in North West India. *Agric. Water Manag.* 128, 92-101.
- Ram, M., Davari, M.R., Sharma, S.N. **2014**. Direct, residual and cumulative effects of organic manures and biofertilizers on yields, NPK uptake, grain quality and economics of wheat (*Triticum aestivum* L.) under organic farming of rice-wheat cropping system. *Journal of Organic Systems* 9, 16-30.
- Ramesh , A., Sharma, S.K., Sharma, M.P., Yadav, N., Joshi, O.P. **2014**. Inoculation of zinc solubilizing *Bacillus aryabhatai* strains for improved growth,

- mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Appl. Soil Ecol.* 73, 87-96.
- Ramos, H.J.O., Roncato-Maccari, L.D.B., Souza, E.M., Soares-Ramos, J.R.L., Hungria, M., Pedrosa, F.O. **2002**. Monitoring *Azospirillum*-wheat interactions using the *gfp* and *gusA* genes constitutively expressed from a new broad-host range vector. *Journal of Biotechnology* 97, 243-252.
- Ramos, M.C. and Martinez-Casasnovas, J.A. **2006**. Erosion rates and nutrient losses affected by composted cattle manure application in vineyard soils of NE Spain. *Catena* 68, 177–185.
- Rampino, P., Pataleo, S., Gerardi, C., Perotta, C. **2006**. Drought stress responses in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ* 29:2143–2152.
- Rana, A., Joshi, M., Prasanna, R., Singh, Y., Shivay, Y.S., Nain, L. **2012**. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *European Journal of Soil Biology* 50, 118-126.
- Rashidzadeh, A., Olad, A. **2014**. Slow-Released NPK Fertilizer Encapsulated by NaAlg-g-Poly(AA-co-AAm)/MMT 9 Superabsorbent Nanocomposite. *Carbohydrate Poly.* <http://dx.doi.org/10.1016/j.carbpol.2014.08.010>
- Rawat, S.K., Singh, R.K., Singh, R.P. **2010**. Seasonal variation of nitrate level in ground and surface waters of Lucknow and its remediation using certain aquatic macrophytes. *Int. J. Lake Riv.* 3(1), 25-35.
- Rawat, S.K., Singh, R.K., Singh, R.P. **2012**. Remediation of nitrite in ground and surface waters using aquatic macrophytes. *J. Environ. Biol.* 33: 51-56.
- Reddy, M.S., Kumar, S., Babita, K., Reddy, M.S. **2002**. Biosolubilization of poorly soluble rock phosphates by *Aspergillus stubingensis* and *Aspergillus niger*. *Bioresour. Technol.* 84, 187–189.
- Reed, M.L.E. and Glick, B.R. **2004**. Applications of free living plant growth-promoting rhizobacteria. *Anton. Van Leeuwenhoek* 86, 1-25.
- Remus, R., Ruppel, S., Jacob, H.J., Hecht-Buchholz, ch., Merbach, W. **2000**. Colonization behaviour of two enterobacterial strains on cereals. *Biol. Fertil. Soils.* 30, 550–557.
- Ren, N.Q., Tang, J., Liu, B.F., Guo, W.Q., 2010. Biological hydrogen production in continuous stirred tank reactor systems with suspended and attached microbial growth. *Int. J. Hydrogen Energy* 35, 2807–2813.

- Rentsch, D., Schmidt, S., Tegeder, M. **2007**. Transporters for uptake and allocation of organic nitrogen compounds in plants. *FEBS Lett.* 581, 2281-2289.
- Ridzwan, K., Ibrahim, M., Babadi, F.E., Yunus, R. **2014**. Comparative performance of different urea coating materials for slow release. *Particology* DOI: 10.1016/j.partic.2014.03.009.
- Riley, W.J., Monasterio, O., Matson, P.A. **2001**. Nitrogen leaching and soil nitrate, nitrite, and ammonium levels under irrigated wheat in Northern Mexico. *Nut. Cycl. Agroecosyst.* 61, 223-236.
- Rivera-Cruz, M.C., Narcia, A.T., Ballona, G.C., Kohler, J. Caravaca, F., Roldain, A. **2008**. Poultry manure and banana waste are effective biofertilizer carriers for promoting plant growth and soil sustainability in banana crops. *Soil Biology & Biochemistry* 40, 3092–3095.
- Rodriguez, H. and Fraga, R. **1999**. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotech. Advan.* 17: 319–339.
- Rodriguez-Navarro, D.N., Temprano, F and Orive, R. **1991**. Survival of *Rhizobium* sp. (*Hedysarum coronarium* L.) on peat-based inoculants and inoculated seeds. *Soil Biol. Biochem.* 23:375-379.
- Russo, A., Vettori, L., Felici, C., Fiaschi, G., Morini, S., Toffanin, A. **2008**. Enhanced micropropagation response and biocontrol effect of *Azospirillum brasilense* Sp245 on *Prunus cerasifera* L. clone Mr.S 2/5 plants. *J Biotechnol* 134:312–319.
- Ryan, J., Sommer, R., Ibrikci, H. **2012**. Fertilizer best management practices: A perspective from dry land west Asia- North Africa region. *J. Agron. Crop Sci.* 198, 57-67.
- Ryder, M.H., Yana, Z., Terrace, E.T., Rovira, A.D., Wenhua, T., Correll, R.L. **1999**. Use of strains of *Bacillus* isolated in China to suppress take-all and rhizoctonia root rot, and promote seedling growth of glasshouse-grown wheat in Australian soils. *Soil Biology and Biochemistry* 3, 19- 29.
- Ryu, C.M., Kim, J., Choi, O., Kim, S.H., Park, C.S. **2006**. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biol Control* 39:282–289
- Saad A. Alamri, Yasser S. Mostafa, **2009**. Effect of nitrogen supply and *Azospirillum brasilense* Sp-248 on the response of wheat to seawater irrigation Saudi Journal of *Biological Sciences* 16, 101–107.

- Sahni, S., Sarma, B.K., Singh, D.P., Singh, H.B., Singh, K.P. **2008**. Vermicompost enhances performance of plant growth-promoting rhizobacteria in *Cicer arietinum* rhizosphere against *Sclerotium rolfsii*. *Crop Protection* 27, 369–376.
- Sahu, S.N., Jana, B.B. **2000**. Enhancement of the fertilizer value of rock phosphate engineered through phosphate-solubilizing bacteria. *Ecol. Eng.* 15, 27–39.
- Saigusa, M. **1999**. Slow release nitrogen fertilizer and plant nutrition. In: Nitrogen Nutrition and Plant Growth (Eds. Srivastava, H.S. and Singh, R.P.), Science Pub; Enfield, USA, pp. 305-335.
- Samarah , N.H. **2005**. Effects of drought stress on growth and yield of barley. *Agron Sustain Dev* 25:145–149.
- Sanchez-Martin, L., Sanz-Cobena, A., Meijide, A., Quemada, M., Vallejo, A. **2010b**. The importance of the fallow period for N₂O and CH₄ fluxes and nitrate leaching in a Mediterranean irrigated agroecosystem. *Eur. J. Soil Sci.* 61, 710- 720.
- Sanita di –Toppi, L., Gabbrielli, R. **1999**. Response to cadmium in higher plants. *Environ. Exp. Bot.* 41:105– 130.
- Santoro, M.V, Zygadlo, U., Giordano, W., Banchio, E. **2011**. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). *Plant Physiology and Biochemistry.* 49, 1177-1182.
- Sanz-Cobena, A., Sanchez-Martin, L., Garcia-Torres, L., Vallejo, A. **2012**. Gaseous emissions of N₂O and NO and NO₃⁻ leaching from urea applied with urease and nitrification inhibitors to a maize (*Zea mays*) crop. *Agric. Ecosyst. Environ.* 149, 64-73.
- Sapkota, T.B., Majumdar, K., Jat, M.L., Kumar, A., Bishnoi, D.K., McDonald, A.J., Pampolino, M. **2014**. Precision nutrient management in conservation agriculture based wheat production of Northwest India: Profitability, nutrient use efficiency and environment footprint. *Field crop Res.* 155, 233-244.
- Saravanakumar, D., Vijayakumar, C., Kumar, N., Samiyappan, R. **2007**. PGPR-induced defense responses in the tea plant against blister blight disease. *Crop Protection* 26 556–565.
- Sarwar, G., Schmeisky, H., Hussain, N., Muhammad, S., Ibrahim, M., Safdar, E. **2008**. Improvement of soil physical and chemical properties with compost

- application in Rice- Wheat cropping system. *Pakistan Journal of Botany* 40(1): 275-282.
- Shah, S.A., Shah, S.M., Mohammad, W., Shafi, M., Nawaz, H., 2009. N uptake and yield of wheat as influenced by integrated use of organic and mineral nitrogen. *International Journal of Plant Production* 3(3), 1735- 8043.
- Sary, G.A., El-Naggar, H.M., Kabesh, M.O., El-kramany, M.F., Gehan., Bakhom, S.H., 2009. Effect of Bio-organic fertilization and some weed control treatments on yield and yield components of wheat. *World J Agric Sci.* 5(1): 55-62
- Satyanarayana, V., P. V. V. Prasad, V. R. K. Murthy, K. J. Boote. **2002**. Influence of integrated use of farmyard manure and inorganic fertilizers on yield and yield components of irrigated lowland rice. *Journal of Plant Nutrition* 25: 2081–2090.
- Saxena, M.J. **2011**. Efficacy of rhizobacterial strains encapsulated in nontoxic biodegradable gel matrices to promote growth and yield of wheat plants. *Applied Soil Ecology* 48, 301– 308.
- Schlöter, M., Wiehe, W., Assmus, B., Steindl, H., Becke, H., Hoflich, G., Hartmann, A. **1997**. Root colonization of different plants by plant-growth –promoting *Rhizobium Leguminosarum* by. *Trifolii R39* studied with Monospecific Polyclonal Antisera. *Environmental Microbiology*, p. 2038-2046.
- Seoud, A.E., Megeed, A.A. **2012**. Impact of rock materials and biofertilizations on P and K availability for maize (*Zea Maize*) under calcareous soil conditions. *Saudi Journal of Biological Sciences* 19: 55–63
- Sepaskhah, A.R., Tafteh, A. **2012**. Yield and nitrogen leaching in rapeseed field under different nitrogen rates and water saving irrigation. *Agric Water Manag* 112:55–62
- Setien, I., Fuertes-Mendizabal, T., Gonzalez, A., Aparicio-Tejo, P.M., González-Murua, C., González-Moro, M.B., Estavillo, J.M. **2013**. High irradiance improves ammonium tolerance in wheat plants by increasing N assimilation. *J. Plant Physiol.* 170,758-771.
- Shaharoon, B., Naveed, M., Arshad, M., Zahir, Z.A. **2008**. Fertilizer-dependent efficiency of *Pseudomonas* for improving growth, yield and nutrients use efficiency of wheat (*Triticum aestivum* L.). *Appl. Microbiol. Biotechnol.* 79, 147-155.

- Shahzad, S.M., Khalid, A., Arshad, M., Tahir, J., Mahmood, T., **2010**. Improving nodulation, growth and yield of *Cicer arietinum* L. through bacterial ACC-deaminase induced changes in root architecture. *European Journal of Soil Biology* 46: 342-347.
- Shang, Q., Yang, X., Gao, C. **2011**. Net annual global warming potential and greenhouse gas intensity in Chinese double rice-cropping systems: a 3-year field measurement in long-term fertilizer experiments. *Glob. Chan. Biol.* 17, 2196-2210.
- Sharma, C., M. Tiwari, K. Pathak, H. **2008**. Estimate of emission and deposition of reactive nitrogenous species for India. *Current Science* 94:1439–1446.
- Sharma, P., Dubey, R.S. **2005**. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant Growth Regulation* 46, 209-221.
- Sharma, P., Singh, G., Singh, R.P. **2011b**. Conservation tillage, optimal water and organic nutrient supply enhance soil microbial activities during wheat (*Triticum aestivum* L.) cultivation. *Brazilian J. Microbiol.* 42, 531-542.
- Sharma, P., Singh, G., Singh, R.P. **2012**. Conservation tillage, optimal water supply enhance microbial enzyme (glucosidase, urease and phosphatase) activities in field under wheat cultivation during various nitrogen management practices. *Arch. Agrn. Soil Sci.* In press, <http://dx.doi.org/10.1080/03650340.2012.690143>.
- Sharma, S.N., Prasad, R. **1999**. Effects of *Sesbania* green manuring and mungbean residue incorporation of productivity and nitrogen uptake of a rice-wheat cropping system. *Biores. Technol.* 67, 171-175.
- Sharma, V.K. Singh, R.P. **2011a**. Organic matrix based slow release fertilizers enhances plant growth, nitrate assimilation and seed yield of Indian mustard (*Brassica juncea* L.). *J. Environ. Biol.* 32, 619-624.
- Shaukat, K., Affrasayab, S., Hasnain, S. **2006**. Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a biofertilizers. *Res. J. Microbiol.* 1(4): 330-338.
- Sheng, X., Sun, L., Huang, Z., He, L., Zhang, W., Chen, Z. **2012**. Promotion of growth and Cu accumulation of bio-energy crop (*Zea mays*) by bacteria: Implications for energy plant biomass production and phytoremediation. *Journal of Environmental Management.* 103, 58-64.

- Sheng, X.F. and Gong, J.X. **2006**. Increased degradation of phenanthrene in soil by *Pseudomonas* sp. GF3 in the presence of wheat. *Soil Biology & Biochemistry* 38: 2587–2592.
- Shenoy, V.V., Kalagudi, G.M. **2005**. Enhancing plant phosphorus use efficiency for sustainable cropping. *Biotechnol. Adv.* 23, 501–513.
- Shewry, P.R., M.J., Hawkesford, M.J., Barraclough, P.B., Holdsworth, M.J., Kerr, S., Kightley, S., **2009**. Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects. *Field Crop. Res.* 114, 329–324.
- Shewry, P.R., Popineau, Y., Lafandra, D., Belton, P. **2000**. Wheat glutenin subunits and dough elasticity: findings of the EUROWHEAT project. *Trends in Food Science and Technology* 11, 433–441.
- Shi, Y., Yu, Z. **2008**. Effects of nitrogen fertilizer rates and ratios of base and topdressing on wheat yield, soil nitrate content and nitrogen balance. *Front. Agric. China*, 2(2), 181–189.
- Shigaki, F., Sharpley, A.N., Prochnow, L.I. **2006**. Animal-based agriculture, phosphorus and management and water quality in Brazil: options for the future. *Sci. Agric.* 63, 194–209.
- Shimokawa, S. **2010**. Nutrient Intake of the Poor and its Implications for the Nutritional Effect of Cereal Price Subsidies: Evidence from China. *World Develop.* 38(7), 1001–1011.
- Siddiqui, I.A., Shaukat, S.S., Khan, G.H., Ali, N.I. **2003**. Suppression of *Meloidogyne javanica* by *Pseudomonas aeruginosa* IE-6S ϕ in tomato: the influence of NaCl, oxygen and iron levels. *Soil Biology & Biochemistry* 35, 1625–1634
- Siddiqui, Z.A. **2004**. Effects of plant growth promoting bacteria and composed organic fertilizers on the reproduction of *Meloidogyne incognita* and tomato growth. *Bioresource Technology* 95 223–227.
- Sieling, K., Brase, T., Svib, V. **2006**. Residual effects of different N fertilizer treatments on growth N uptake and yield of oilseed rape, wheat and barley. *Europ. J. Agronomy.* 25, 40–48.
- Sindhu, S., Suneja, S., Goel, A.K., Parmar, N., Dadarwal, K.R. **2002**. Plant growth promoting effects of *Pseudomonas* sp. On coinoculation with *Mesorhizobium* sp. Cicer strain under sterile and “wilt sick” soil conditions. *Applied Soil Ecology* 19, 57–64.

- Singh, A.K., Ram, H., Maurya, B.D. and Prasad, J. **2006**. Influence of Neem products on urease activity, urea transformation in soils and wheat yield. *Ind. J. Fertil.* 2(2), 45-48.
- Singh, C.M., Sharma, P.K., Kishor, P.K., Singh, A.P., Verma, R. V., Raha, P. **2011**. Impact of integrated nutrient management on growth, yield and Nutrient uptake by wheat (*Triticum aestivum* L.). *Asian J. Agric. Sci.* 5(1), 76-82.
- Singh, R., Singh, R., Sonid, S.K., Singh, S.P., Chauhan, U.K., Kalra, A. **2013**. Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of pogostemon cabin (patchouli) benth. *Applied Soil Ecology* 70: 48-56.
- Singh, R.P., Kumar, M., Jaiwal, P.K. **2008a**. Improvement in nitrogen use efficiency and yield of crop plants by sustained nutrient supply and enhanced nitrogen assimilation. In: Development in Physiology, Biochemistry and Molecular Biology of Plants Vol. 2. (Eds Bose, B and Hemantranjan, A.), New India Publishing Agency, New Delhi, pp. 1-31.
- Singh, R.P., Sainger, M., Baudh, K., Sengar, R.S., Jaiwal, P.K. **2010**. Sustained nutrient supply reduced nutrient loss and high plant productivity with slow release fertilizers, In: Stable Food Production and Sustainable Agriculture; A challenge ahead 21st century (Eds. Sengar, R.S. and Sharma, A.K.) Studium Press (India) Pvt Ltd., pp. 62-79.
- Singh, R.P., Sengar, M, Singh, D.P., Jaiwal, P.K. **2008b**. Nitrate and ammonia transporters. In: Membrane and vacuolar transporters in plants (Eds Jaiwal, P.K., Singh, R.P. and Dhankher, O.P.) CAB International. pp: 345-371.
- Singh, S., Ladha, J.K., Gupta, R.K., Bhushan, L., Rao, A.N., Sivaprasad, B., Singh, P.P. **2007**. Evaluation of mulching, intercropping with Sesbania and herbicide use for weed management in dry-seeded rice (*Oryza sativa* L.). *Crop Protect.* 26, 518-524.
- Singh, V.K., Dwivedi, B.S., Tiwari, K.N., Majumdar, K., Rani, M., Sing, S.K., Timsina, J. **2014**. Optimizing nutrient management strategies for rice-wheat system in the Indo-Gangetic Plains of India and adjacent region for higher productivity, nutrient use efficiency and profits. *Field Crop Res.* 164, 30-44.
- Siviero, M.A., Motta, A.M., Lima, D.D., Birolli, R.R., Huh, S.Y., Santinoni, I.A., Murate, L.S., de-Castro, C.M.A., Miyauchi, M.Y.H.M., Zangaro, W., Nogueira, M.A., Andrade, G. **2008**. Interaction among N-fixing bacteria

- and AM fungi in Amazonian legume tree (*Schizolobium amazonicum*) in field conditions. *Applied soil ecology* 39:144-152.
- Smith, R.S. **1997**. The potential for feedback effects induced by global warming on emissions of nitrous oxide by soils. *Global change biol.* 3, 327-338.
- Smith, R.S. **1995**. Inoculant formulations and applications to meet changing needs. In: Tikhonovich IA, Provorov NA, Romanov VI, Newton WE, editors. Nitrogen Fixation: Fundamentals and Applications. Dordrecht, The Netherlands: Kluwer Academic; 1995. pp. 653–657.
- Soltani, A., Gholipour, M., Zeinali, E. **2006**. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. *Environmental and Experimental Botany* 55 195–200.
- Sorensen, P., Rubaek, G.H. **2012**. Leaching of nitrate and phosphorus after autumn and spring application of separated solid animal manures to winter wheat. *Soil Use Manag.* 28, 1-11.
- Spaepen, S., Dobbelaere, S., Croonenborgs, A., Vanderleyden, J. **2008**. Effects of *Azospirillum brasilense* indole-3-acetic acid production on inoculated wheat plants. *Plant Soil* 312, 15–23.
- Spaepen, S., Vanderleyden, J., Okon, Y. **2009**. Plant growth promoting actions of rhizobacteria. *Advances Bot Res* 51:283–320
- Spaepen, S., Versees, W., Gocke, D., Pohl, M., Steyaert, J., Vanderleyden, J. **2007**. Characterization of phenylpyruvate decarboxylase, involved in auxin production of *Azospirillum brasilense*. *J Bacteriol* 189:7626–7633.
- Srivastava, H.S. **1980**. Regulation of nitrate reductase activity in higher plants. *Phytochem*, 19, 995-999.
- Srivastava, H.S. **1992**. Multiple functions and forms of nitrate reductase in higher plants, *Phytochem.* 31, 2941-2947.
- Srivastava, H.S. **1995**. Nitrate reductase, In: Nitrogen Nutrition in Higher Plants (Eds, Srivastava, H.S. and Singh, R.P.), Associated Publishing Co., New Delhi, India pp. 145-164.
- Srivastava, H.S., Singh, R.P. **1987**. Role and regulation of L-glutamate dehydrogenase in higher plants. *Phytochem.* 26, 597-610.
- Stanojkovic, A., Dukic, D.A., Mandic, L., Pivic, R., Stanojkovic, A., Josic, D. **2012**. Evaluation of the chemical composition and yield of crops as influenced by bacterial and mineral fertilization. *Romanian Biotech. Lett.* 17(2), 7136-7144.

- Steng, V., Popelkova, D., Grygar, T. M. **2014**. Composite pigments based on surface coated kaolin and metakaolin. *Appl Clay Science*. DOI: 10.1016/j.clay.2014.07.030.
- Stevens, D.L. and Oaks, A. **1973**. The influence of nitrate in the induction of nitrate reductase in the maize roots. *Canadian J. Bot.* 51, 1255-1258.
- Stewart, W.M., Dibb, D.W., Johnston, A.E., Smyth, T.J. **2005**. The contribution of commercial fertilizer nutrients to food production. *Agron. J.* 97, 1-6.
- Stolze, M., and Lampkin, N., 2009. Policy for organic farming: Rationale and concepts. *Food Policy* 34 237–244.
- Subbiah, B.V. and Asija, G.L. **1956**. A rapid procedure for estimation of available nitrogen in soil. *Current Sci* 25, 259-260.
- Sundara, B., Natarajan, V., Hari, K. **2002**. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crops Res* 77, 43–49
- Swarnalakshmi, K., Prasanna, R., Kumar, A., Pattnaik, S., Chakravarty, K., Shivay, Y.S., Singh, R., Saxena, A.K. **2013**. Evaluating the influence of novel cyanobacterial biofilmed biofertilizers on soil fertility and plant nutrition in wheat. *European J. Soil Boil.* 55, 107-116.
- Tabatabai, M.A., Bremner, J.M. **1969**. Use of p-nitrophenylphosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* 1, 301-307.
- Tafteh, A., Sepaskhah, A.R. **2012**. Yield and nitrogen leaching in maize field under different nitrogen rates and partial root drying irrigation. *Int. J. Plant Prod.* 6, 93- 113.
- Tamilselvi, S.M., Chinnadurai, C., Ilamurugu, K., Arulmozhiselvan, K., Balachandar, D. **2015**. Effect of long-term nutrient managements on biological and biochemical properties of semi-arid tropical Alfisol during maize crop development stages. *Ecol. Indicat.* 48, 76-87.
- Tan, X., Shao, D., Liu, H., Yang, H., Xiao, C., Yang, H. **2013**. Effects of alternate wetting and drying irrigation on percolation and nitrogen leaching in paddy fields. *Paddy Water Environ.* 11, 381-395.
- Tang, S.H., Yang, S.H., Chen, J.S., Xu, P.Z., Zhang, F.B., Ai, S.Y., Huang, X. **2006**. Studies on the mechanism of single basal application of controlled-release fertilizers for increasing yields of rice (*Oryza sativa* L.). *Sci. Agri. Sinica.* 39, 2511-2520.

- Tapias, D.R. Galvan, A.M. Diaz, S.P. Rivera, M.O.D. Bonilla, R. **2012**. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). *Applied Soil Ecology* 61 264–272
- Tarabily, K.A.E., Nassar, A.H., Sivasithamparam, K. **2008**. Promotion of growth of bean (*Phaseolus vulgaris L.*) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of *Micromonospora endolithica*. *Applied soil ecology* 39: 161 – 171.
- Tausz, M., Sircelj, H., Grill, D. **2004**. The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? *Journal of Experimental Botany*, 55 (404), 1955-1962.
- Tawfik, M., Bahr, A., Salem, A.K. **2006**. Response of kallar grass (*Leptochloafusca L*) to biofertilizer inoculation under different levels of seawater irrigation. *J. Appl. Sci. Res.* 2, 1203–1211.
- Thind, H. S., Singh, B., Pannu, R.P.S., Singh, Y, Singh, V., Gupta, R.K., Vashistha, M., Singh, J., Kumar, A. **2010**. Relative performance of neem (*Azadirachta indica*) coated urea vis-a-vis ordinary urea applied to rice on the basis of soil test or following need based nitrogen management using leaf colour chart. *Nutr. Cycl. Agroecosyst.* 87:1-8.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S. **2002**. Agricultural sustainability and intensive production practices. *Nature*, 418, 671-677.
- Tiwari, S., Singh, P., Tiwari, R., Meena, K.K., Yandigeri, M., Singh, D.P., and Arora, D.K. **2011**. Salt tolerance rhizobacteria- mediated induced tolerance in wheat (*Triticum aestivum L.*) and chemical diversity in rhizosphere enhances plant growth. *Biol-fertilizer soil*.
- Trabelsi, D., Ammarb, H.B., Mengoni, A., Mhamdi, R. **2012**. Appraisal of the crop-rotation effect of rhizobial inoculation on potato cropping systems in relation to soil bacterial communities. *Soil Biology & Biochemistry* 54 1-6.
- Trethowan, R. and Pfeiffer, W.H. **1999**. Challenges and future strategies in breeding wheat for adaptation to drought stressed environments: A CIMMYT wheat program perspective. In: Ribaut, J.M., Poland, D. (Eds.), *Molecular approaches for the genetic improvement of cereals for stable production in water-limited environments. A strategic planning workshop held at CIMMYT, El Batan, Mexico. Jun 21–25, 1999. CIMMYT, Mexico, DF, Mexico.* pp. 45–48.

- Tripathy, P.P., Ayyappan, S. **2005**. Evaluation of *Azotobacter* and *Azospirillum* as Biofertilizers in aquaculture. *World J. Microbiol. Biotechnol.* 21, 1339-1343.
- Trivedi S and Shah, S., 2014. The Effect of Cane molasses on Cow Milk Productivity. *International Journal of Current Engineering and Technology*, Vol.4, No.6: 4157-4161.
- Tugba-Eren, A., Aksu, Z. 2005. Carotenoids production by the yeast *Rhodotorula mucilaginosa*: Use of agricultural wastes as a carbon source. *Process Biochemistry* 40 2985–2991
- Uzilday, B., Turkan, I., Sekmen, A.H., Ozgur, R., Karakaya, H.C. **2012**. Comparison of RO formation and antioxidant enzymes in *Cleome gynandra* (C₄) and *Cleome spinosa* (C₃) under drought stress. *Plant Sci.* 182: 59-70.
- Uzilday, B., Turkan, I., Sekmen, A.H., Ozgur, R., Karakaya, H.C. **2012**. Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C₄) and *Cleome spinosa* (C₃) under drought stress. *Plant Sci.* 182: 59-70.
- Van-Elsas, J.D., Kijkstra, A.F., Govaert, J.M and Van-Veen, J.A. 1986. Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into two soils of different texture in field microplots. *FEMS Microbiol. Ecol.* 38: 151-160.
- Vassilev, N., Medina, A., Azcon, R., Vassileva, M., **2006a**. Microbial solubilization of rock phosphate on media containing agro-industrial wastes and effect of the resulting products on plant growth and P uptake. *Plant Soil* 287, 77–84.
- Vassilev, N., Vassileva, M., Nikolaeva, I., **2006b**. Simultaneous Psolubilizing and biocontrol activity of microorganisms: potentials and future trends. *Appl. Microbiol. Biotechnol.* 71, 137–144.
- Velumurugan, A., Dadhwal, V.K., Abrol, Y.P. **2008**. Regional nitrogen cycle: An Indian perspective. *Current Science* 94:1455–1468.
- Venterea, R.T., Dolan, M.S., Ochsner, T.E. **2010**. Urea decreases nitrous oxide emissions compared with anhydrous ammonia in a Minnesota corn cropping system. *Soil Sci. Soc. America J.* 74, 407-418.
- Vessey, J.K. **2003**. Plant growth promoting rhizobacteria as biofertilizer. *Plant Soil* 255, 571–586.
- Vinocur, B., Altman, A. **2005**. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr Opin Biotechnol* 16:123–132.
- Vivas, A., Moreno, B., GarciaRodriguez, S. and Benitez, E. 2009. Assessing the

- impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive mill waste, *Bioresource Technology*, 100, 1319-1326.
- Wagner-Riddle, C., Furon, A., McLaughlin, N.L. **2007**. Intensive measurement of nitrous oxide emissions from a corn-soybean-wheat rotation under two contrasting management systems over 5 years. *Glob. Chan. Biol.* 13, 1722-1736.
- Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R. **2007**. Heat tolerance in plants: an overview. *Enviro*
- Wang, J., Wang, D., Zhang, G., Wang, Y., Wang, C., Teng, Y., Christie, P. **2014**. Nitrogen and phosphorus leaching losses from intensively managed paddy fields with straw retention. *Agric. Water Manag.* 141, 66-73.
- Wang, Q., Li, F., Zhao, L., Zhang, E., Shi, S., Zhao, W., Song, W., Vance, M.M. **2010**. Effect of irrigation and nitrogen application rates on nitrate nitrogen distribution and fertilizer nitrogen loss, wheat yield and nitrogen uptake on a recently reclaimed sandy farmland. *Plant Soil* 337, 325-339.
- Wang, Q., Li, F., Zhao, L., Zhang, E., Shi, S., Zhao, W., Song, W., Vance, M.M. **2010**. Effect of irrigation and nitrogen application rates on nitrate nitrogen distribution and fertilizer nitrogen loss, wheat yield and nitrogen uptake on a recently reclaimed sandy farmland. *Plant Soil.* 337, 325-339.
- Wang, X., Zhu, J., Gao, R., Yasukazu, H., Feng, K. **2007**. Nitrogen cycling and losses under rice-wheat rotations with coated urea and urea in the Taihu lake region. *Pedosphere* 17(1), 62- 69.
- Wang, Z., Li, J., Li, Y. **2014**. Effects of drip irrigation system uniformity and nitrogen applied on deep percolation and nitrate leaching during growing seasons of spring maize in semi-humid region. *Irrig. Sci.* 32, 221-236.
- Wang, D., Shi, Q., Wang, X., Wei, M., Hu, J., Liu, J. and Yang, F. 2010. Influence of cow manure vermicompost on the growth, metabolite contents, and antioxidant activities of Chinese cabbage (*Brassica campestris* ssp. *chinensis*). *Biology and Fertility of Soils*, 46, 689-696.
- Warman, P.R. and Anglopez, M.J. 2010. Vermicompost derived from different feed stocks as a plant growth medium. *Bioresource Technology* 101, 4479-4483.
- Weatherburn, M.W. **1967**. Phenol-hypo chlorite reaction for determination of Ammonia. *Anal. Chem.* 39, 971-974.

- Weber, A., Gutser, R., Schmidhalter, **2001**. Field emission of NH₃ and NO_x following urea application to wheat. *Plant Nut. Food Sec. Sust. Agro-Ecosyst.* 92, 884- 885.
- Wegglar, K.B., Mclaughin, M.J., Graham, R.D., **2000**. Salinity increases cadmium uptake by wheat and swiss chard from soil amended with biosolids. *Aust.J. Soil Res.* 38, 37-45.
- Wei, X.R., Hao, M.D., Xue, X.H., Shi, P., Horton, R., Wang, A., Zang, Y.F. **2010**. Nitrous oxide emission from highland winter wheat field after long-term fertilization. *Biogeosci.* 7, 3301-3310.
- Weiske, A., Benckiser, G, Herbert, T, Ottow, J.C.G. **2001**. Influence of the nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. *Biol. Fertil. Soil.* 34, 109-117.
- Wei-xin, D., Lei, M., Zu-cong, C., Feng- xiang, **2007**. Effects of long-term amendment of organic manure and nitrogen fertilizer on nitrous oxide emission in a sandy loam soil. *J. Environ. Sci.* 19, 185-193.
- Weligama, C., Sale, P. W. G., Conyers, M. K., Liu, D.L., Tang, C. **2010**. Nitrate leaching stimulates subsurface root growth of wheat and increase rhizosphere alkalization in a highly acidic soil. *Plant soil* 328, 119-132.
- Weller, D.M. **1998**. Biological control of soil borne plant pathogens in the rhizosphere with bacteria, *Ann. Phytopathol.* 26, 379–407.
- White J.W. **2006**. From genome to wheat: Emerging opportunities for modelling wheat growth and development. *Europ. J. Agronomy* 25 79–88.
- Wild, B., Schneckler, J., Alves, R.J.E., Barsukov, P., Barta, P., Capek, P., Gentsch, N., Gittel, A., Guggenberger, G., Lashchinskiy, N., Mikutta, N., Rusalimova, O., Ckova, S., Shibistova, O., Urich, T., Watzka, M., Zrazhevskaya, G., Richter, A. **2014**. Input of easily available organic C and N stimulates microbial decomposition of soil organic matter in arctic permafrost soil. *Soil Biol. Biochem.* 75, 143-151.
- Wollenweber, B., Porter, J.R., Lubberstedt, T. **2005**. Need for multidisciplinary research towards a second green revolution. *Curr. Opin. Plant Biol.* 8, 337–341.

- Wollenweber, B., Porter, J.R., Schellberg, J. **2003**. Lack of interaction between extreme high temperature events at vegetative and reproductive growth stages in wheat. *J. Agron. Crop Sci.* 189, 142–150.
- Wu, L., Liu, M. **2008**. Preparation and properties of chitosan-coated NPK compound fertilizer with controlled-release and water-retention. *Carbohydrate Poly.* 72, 240-247.
- Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C., Wong, M.H. **2005**. Effects of biofertilizers containing N-fixer, P and K solubilizers and AM fungi on maize growth: A greenhouse trial. *Geoderma.* 125, 155-166.
- Xiaoyu, N., Yuejin, W., Zhengyan, W., Lin, W., Guannan, Q., Lixiang, Y. **2013**. A novel slow-release urea fertiliser: Physical and chemical analysis of its structure and study of its release mechanism. *Biosyst. Eng.* 115, 274-282.
- Xiao-Zhi, W., Jian-Gao, Z., Ren, G., Yasukazu, H., Ke, F. **2007**. Nitrogen cycling and losses under rice-wheat rotations with coated urea and urea in Taihu Lake region. *Pedosphere* 17(1), 62-69.
- Xin-kai, Z., Chun-yan, L.I., Zong-qing, J., Lian-Lian, H., Chao-nian, F., Wen-shan, G., Yong-xin, P. **2012**. Responses of phosphorous use efficiency, grain yield, and quality to phosphorous application amount of weak- gluten wheat. *J. Integrat. Agric.* 11(7), 1103-1110.
- Xu, J., Peng, S., Yang, S., Wang, W. **2012**. Ammonia volatilization losses from a rice paddy with different irrigation and nitrogen managements. *Agri. Water Manag.* 104, 184-192.
- Yadav, J., Yadav, S., Singh, S.G. **2011**. Plant growth promotion in wheat crop under environmental condition by PSB as biofertilizers. *Research journal of agricultural sciences* 2(1): 76-78.
- Yadav, R.L. **2003**. Assessing on farm- efficiency and economics of fertilizers N, P and K in rice- wheat systems of India. *Field Crop. Res.*, 81, 39-51.
- Yadav, R.L. **2004**. Enhancing efficiency of fertilizer N use in rice-wheat systems of Indo-Gangetic Plains by intercropping *Sesbania aculeate* in direct seeded upland rice for green manuring. *Biores. Technol.* 93, 213-215.
- Yadav, S.K., Babu, S., Yadav, M.K., Singh, K., Yadav, G.S., Pal, S. **2013**. A review of organic farming for sustainable agriculture in Northern India. *Inter J. Agro.* 1-8.
- Yan, J., Kloepper, J.W., Ryu, C. **2009**. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci* 14:1–4.

- Yan, P., Yue, S., Qiu, M., Chen, X., Cui, Z., Chen, F. **2014**. Using maize hybrids and in season nitrogen management to improve grain yield and grain nitrogen concentrations. *Field Crop Res.* 166, 38-45.
- Yan, X., Hosen, Y., Yagi, K. **2001**. Nitrous oxide and nitric oxide emissions from maize field plots as affected by N fertilizer type and application method. *Biol. Fertil. Soil* 34, 297-303.
- Yang, B., Xiong, Z., Wang, J., Xu, X., Huang, Q and Shen, Q. **2015**. Mitigating net global warming potential and greenhouse gas intensities by substituting chemical nitrogen fertilizers with organic fertilization strategies in rice-wheat annual rotation systems in China: A 3-year field experiment. *Ecological Engineering*, 81, 289–297.
- Yang, C.W., Xu, H.H., Wang, L.L., Liu, J., Shi, D.C., Wang, D.L. **2009**. Comparative effects of salt-stress and alkali-stress on the growth, photosynthesis, solute accumulation, and ion balance of barley plants. *Photosynthetica* 4, 79-86.
- Yang, M.G., Lin, X.Y., Yang, X.E. **1998**. Impact of Cd on growth and nutrient accumulation of different plant species. *Chin. J. Appl. Ecol.* 19:8994.
- Yang, S.M., Malhi, S.S., Song, J.R., Xiong, Y.C., Yue, W.Y., Lu, L.L., Wang, J.G., Guo, T.W. **2006**. Crop yield, nitrogen uptake and nitrate- nitrogen accumulation in soil as affected by 23 annual applications of fertilizer and manure in the rain fed region of northwestern china. *Nutr. Cycl. Agroecosyst.* 76:81-94.
- Yang, S.S. **2003**. Application of microbial fertilizers on the three objectives agriculture. In: Chou, C.H., Yang, S.S. (Eds.), *Challenge of Three Objectives in Agriculture*. Council of Agriculture, Southern Taiwan Joint Services Center of Executive Yuan, Institute of Biotechnology of National Pingtung University of Science and Technology, Department of Biochemical Science and Technology of National Taiwan University, *Taiwan*, pp. 265–292.
- Yang, S.S., Chen, K.S. **2003**. Application of thermophilic microbes for preparing biofertilizers. *Plant Prot. Bull. Spe. Publ. New* 5, 267–291.
- Yang, X., Lu, Y., Tong, Y., Yin, X. **2014a**. A 5-year lysimeter monitoring of nitrate leaching from wheat–maize rotation system: Comparison between optimum N fertilization and conventional farmer N fertilization. *Agric. Ecosyst. Environ.* 199, 34-42.

- Yang, Y. C., Zhang, M., Zheng, L., Cheng, D.D., Liu, M., Geng, Y.Q. **2011**. Controlled release urea improved nitrogen use efficiency, yield and quality of wheat. *Agron. J.* 103(2), 479-485.
- Yang, Z.C., Zhao, N., Huang, F., Lv, Y.Z. **2014b**. Long-term effects of different organic and inorganic fertilizer treatments on soil organic carbon sequestration and crop yields on the North China Plain. *Soil Till Res.* <http://dx.doi.org/10.1016/j.still.2014.06.011>.
- Yao, L., Wu, Z., YImdad, Z., Chun, L.K. **2010**. Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. *European Journal of Soil Biology* 46, 49-54.
- Yao, X., Ren, H., Cao, Z. Tian, Y., Cao, W., Zhu, Y., Cheng, T. **2014**. Detecting leaf nitrogen content in wheat with canopy hyperspectrum under different soil backgrounds. *Inter. J. Appl. Earth Observat. Geoinformat.* 32, 114-124.
- Yao, Y., Gao, B., Zhang, B., Inyang, M., Zimmerman, A.R. **2012**. Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere.* 89 (11), 1467-1471.
- Yao, Z.S., Zheng, X.H., Xie, B.H., Mei, B.L., Wang, R., Butterbach- Bahl, K., Zhu, J.G., Yin, R. **2009**. Tillage and crop residue management significantly affects N trace gas emissions during the non-rice season of a subtropical rice wheat rotation. *Soil Biol. Biochem.* 41, 2131-2140.
- Yin, F., Fu, B., Mao, R. **2007**. Effects of nitrogen fertilizer application rates on nitrate nitrogen distribution in saline soil in the Hai River basin, China. *J. soil Sediment.* 7, 1-7.
- Yue, H.T., Mo, W. P., Li, C. **2007**. The salt stress relief and growth promotion effect of Rs-5 on cotton. *Plant Soil* 297, 139-145.
- Yunhai, Z., Xu H., Nianpeng H., Min L., Jianhui H., Guangming Z., Qibing W. Xingguo H. **2014**. Increase in ammonia volatilization from soil in response to Ndeposition in Inner Mongolia grassland. *Atmos. Environ.* 84, 156-162.
- Yushi, Y., Xingiang, L., Yingxu, C., Jin, L., Jiatao, G., Ru, G., Liang, L. **2013**. Alternate wetting and drying irrigation and controlled-release nitrogen fertilizer in late-season rice. *Field Crop Res.* 144, 212-224.
- Zabihi, H.R., Savaghebi, G.R., Khavazi, K., Ganjali, A., Miransari, M. **2011**. *Pseudomonas* bacteria and phosphorous fertilization, affecting wheat (*Triticum aestivum* L.) yield and P uptake under greenhouse and field conditions. *Soil Biol. Biochem* 33, 145-152.

- Zafar, A., Schjodt-Thomsen, J., Sodhi, R., Goacher, R., Kubbe, D.D. **2013**. Investigation of the ageing effects on phenol-urea-formaldehyde binder and alkanol amine-acid anhydride binder coated mineral fibres. *Poly. Degrad. Stabil.* 98, 339-347.
- Zahir, Z.A., Arshad, M., Frankenberger, W.T. **2004**. Plant growth-promoting rhizobacteria: perspectives and applications in agriculture. *Adv. Agron.*, 81: 97-168.
- Zaidi, A., Khan, M.S., Ahemad, M., Oves, M., Wani, P.A. **2009**. Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: Khan MS (ed) *Microbial strategies for crop improvement*. Springer, Berlin, p 23 1280-1281
- Zaman, M., Nguyen, M.L., Blennerhasset, J.D., Quin, B.F. **2008**. Reducing NH₃, N₂O, and NO₃-N losses from a pasture soil with urease or nitrification inhibitors and elemental S- amended nitrogenous fertilizers. *Biol. Fertil. Soil* 44, 693-705. Zhang, H., Rong, H., Pillbeam, D. **2007**. Signaling mechanisms underlying the morphological responses of the root system to nitrogen in *Arabidopsis thaliana*. *J. Exp. Bot.* 58, 2329-2338.
- Zaman, M., Nguyen, M.L., Blennerhasset, J.D. **2010**. The effect of different rates of urea with or without urease inhibitor (NBPT) on wheat yield and quality. *Agricul. J.* 5(5), 309-312.
- Zamen, M., Blennerhasset, J.D. **2010**. Effects of the different rates of urease and nitrification inhibitors on gaseous emissions of ammonia and nitrous oxide, nitrate leaching and pasture production from urine patches in an intensive grazed pasture system. *Agric. Ecosyst. Environ.* 136, 236-246.
- Zarea, M.J, Hajinia, S., Karimi, N., Goltapeh, E.M, Rejali, F., Varma, A. **2012**. Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl *Soil Biol Biochem.* 45 139-146.
- Zayed, G., Abdel-Motaal, H. **2005a**. Bio-active composts from rice straw enriched with rock phosphate and their effect on the phosphorous nutrition and microbial community in rhizosphere of cowpea. *Bioresour. Technol.* 96, 929– 935.
- Zayed, G., Abdel-Motaal, H. **2005b**. Bio-production of compost with low pH and high soluble phosphorus from sugar cane bagasse enriched with rock phosphate. *World J. Microbiol. Biotechnol.* 21, 747–752.

- Zemrany, H.E., Cortet, J., Lutz, M.P., Chabert, A., Baudoin, E.K., Haurat, J., Maughan, N., lix, D., fago, G.V., Bally, R., Loccoz, B.R. **2006**. Field survival of the phytostimulator *Azospirillum lipoferum* CRT1 and functional impact on maize crop, biodegradation of crop residues, and soil faunal indicators in a context of decreasing nitrogen fertilisation. *Soil Biology & Biochemistry* 38, 1712–1726.
- Zhang, A., Cui, L., Pan, G. **2010**. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agric Ecosyst Environ.*139, 469-475.
- Zhang, J., Liu, J., Zhang, J., Cheng, Y., Wang, W. **2013**. Nitrate-Nitrogen Dynamics and Nitrogen Budgets in Rice-Wheat Rotations in Taihu Lake Region, Chin. *Pedosphere* 23(1), 59-69.
- Zhang, J.S., Zhang, F.P., Yang, J.H., Wang, J.P., Cai, M.L., Li, C.F., Cao, C.G. **2011**. Emissions of N₂O and NH₃, and nitrogen leaching from direct seeded rice under different tillage practices in central China. *Agric Ecosyst Environ* 140:164–173.
- Zhang, M., Li, R., Cao, L., Shi, J., Liu, H., Huang, Y., Shen, Q. **2014b**. Algal sludge from Taihu Lake can be utilized to create novel PGPR-containing bio-organic fertilizers. *J. Environ Manag.* 132, 230-236.
- Zhang, Q.C., Shamsi, I.H., Xu, D.T., Wang, G.H., Lin, X.Y., Jilani, G., Hussai, N., Chaudhry, A.N. **2012**. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Appl. Soil Ecol.* 57, 1-8.
- Zhang, X., a, Cai, J., Wollenweber, B., Liu, F., Dai, T., Cao, W., Jiang, D. **2013**. Multiple heat and drought events affect grain yield and accumulations of high molecular weight glutenin subunits and glutenin macropolymers in wheat. *Journal of Cereal Science* 57, 134-140
- Zhang, Y., Li, Z., Feng, J., Zhang, X., Jiang, Y., Chen, J., Zhang, M., Deng, M., Zhang, W. **2014a**. Differences in CH₄ and N₂O emissions between rice nurseries in Chinese major rice cropping areas. *Atmos. Environ.* 96, 222-228.
- Zhao, G.Z., Liu, Y.Q., Tian, Y., Sun, Y.Y., Cao, Y. **2010**. Preparation and properties of macromolecular slow-release fertilizers containing nitrogen, phosphorus and potassium. *J. Polymer Res.* 17, 119-125.

- Zhao, X., Wang, S., Xing, G. **2014**. Nitrification, acidification, and nitrogen leaching from subtropical cropland soils as affected by rice straw-based biochar: laboratory incubation and column leaching studies. *J. Soil Sedim.* 14, 471-482.
- Zhao, X., Chang, A.Y, Toh-E A., Arvan, P. **2007**. A role for Lte1p (a low temperature essential protein involved in mitosis) in proprotein processing in the yeast secretory pathway. *J Biol Chem.* 3, 1670-8
- Zhengchao, Z., Zhuoting, G., Zhouping, S., Fuping, Z. **2013**. Effects of long-term repeated mineral and organic fertilizer applications on soil organic carbon and total nitrogen in a semi-arid cropland. *Europ. J. Agron.* 45, 20-26.
- Zhou, M., Bahl, K. **2014**. Assessment of nitrate leaching loss on a yield-scaled basis from maize and wheat cropping systems. *Plant Soil.* 374, 977-991.
- Zhou, M., Zhu, B., Bahl, K.B., Wang, T., Bergmann, J., Bruggemann, N., Wang, Z., Li, T., Kuang, F. **2012**. Nitrate leaching, direct and indirect nitrous oxide fluxes from sloping cropland in the purple soil area, southwestern china. *Environ. Poll.* 162, 361-368.
- Zou, H.T., Wang, Y.S., Song, H.W., Han, Y.Y., Yu, N., Zhang, Y.L., Dang, X.L., Huang, Y., Zhang, Y.L. **2009**. The production of organic-inorganic compound film- coated urea and the characteristics of its nutrient release. *Agric. Sci. China* 8(6), 703-708.



PUBLICATIONS



Enhanced Dose of *Azotobacter chroococcum* and *Bacillus subtilis*, Co-immobilised in Vermicompost Based Organic Granules, Increase Biomass Yield and Harvest Index of Wheat (*Triticum aestivum* L)

Kose P. Minj¹ • Rana Pratap Singh^{2*}

Abstract It has recently been reported that optimised dose of *Azotobacter chroococcum* and *Bacillus subtilis* (Hindustan Bioenergy Ltd.) supplied as charcoal based mixture co-immobilised in cow dung, clay soil and neem leaves in the ratio of 1:1:1 and 15% (w/v) acacia gum significantly enhanced growth and yield of wheat (*Triticum aestivum* L.) (Kumar *et al.*, 2013; 2014a,b; 2015a,b). Due to limitations of the availability of cow dung, we replaced it with vermicompost and Farm Yard Manure (FYM) in similar ratio and the organic matrix based granules were applied in the same amount. Our results reveal that the biofertilisers co-immobilised with vermicompost to prepare organic matrix based super granules enhance the efficacy of *A. chroococcum* and *B. subtilis* over cow dung and FYM for the biomass production, seed yield and harvest index (HI) of wheat in earthen pot.

Keywords Biofertilisers, Organic super granules, Plant productivity, Sustainable agriculture, Climate resilience

1. Introduction

The aim of fertiliser application to crops is to obtain higher grain yield by providing nutrients to the growing and maturing plants (Adesemoye *et al.*, 2009). Most of the agricultural soil exhibit nitrogen (N) and phosphorus (P) deficiency, which become a yield limiting factor for the crop production that reduce the grain yield, and effect harvest index (HI) (Kemanian *et al.*, 2007; Abedi *et al.*, 2010; Cerny *et al.*, 2010; Pennington, 2013; Laik *et al.*, 2014; Sapkota *et al.*, 2014). The excessive use of inorganic fertilisers, however, has become a common practice of green revolution agriculture, which causes many environmental, economic

and health related problems (Weber *et al.*, 2001; Abedi *et al.*, 2010; Singh *et al.*, 2010; Rawat *et al.*, 2012; Ryan *et al.*, 2012; Kong *et al.*, 2013; Laik *et al.*, 2014).

Sustainable agriculture aims at long-term maintenance of the natural resources and agricultural productivity with minimal adverse impacts on the environment. It emphasises upon optimal crop production with minimal external inputs, reducing dependence on chemical inputs (fertilisers and pesticides) and substituting them with the internal and natural resources (Crecchio *et al.*, 2007; Cerny *et al.*, 2010; Singh *et al.*, 2010; Nayak *et al.*, 2012; Sapkota *et al.*, 2014; Kumar *et al.*, 2014a,b, 2015b; Laik *et al.*, 2014).

Biofertilisers have been identified as alternative to chemical fertilisers to increase soil fertility and crop production in sustainable manner in sustainable farming systems (Kundu *et al.*, 2009; Nayak *et al.*, 2012; Otinga *et al.*, 2013; Kumar *et al.*, 2013; 2014a,b; 2015a,b). It reduces the load of chemical fertiliser in agricultural crop field (Kumar *et al.*, 2013; 2014a). Biofertilisers are products containing living cells which have an ability to convert nutritionally important elements (N, P and K) from unavailable to available form through biological processes such as N₂ fixation and solubilisation from insoluble sources etc. (Ardakani *et al.*, 2011; Yadav *et al.*, 2011), however, their efficacy is not at par to the chemical fertilisers, and hence they are unable to replace it in the main agricultural systems.

Organic manures are better supplement of inorganic fertilisers for sustainable crop production and soil fertility, which also enhance the efficacy of biofertilisers (Gopinath *et al.*, 2008; Shaharoona *et al.*, 2008; Hasanuzzaman *et al.*, 2010; Sharma *et al.*, 2012; Kumar *et al.*, 2015b). The vermicompost has been reported to be a better nutrient

¹Ph.D. Scholar, ²Professor, Department of Environmental Science, Babasaheb Bhimrao Ambedkar (Central) University, Rae Bareilly Road, Lucknow-226025, Uttar Pradesh, India

*Corresponding author Email Id: cceseditor@gmail.com

supplier as per built up of shoot biomass, dry matter and grain yield in comparison to the other organic wastes (Minaxi *et al.*, 2013; Singh *et al.*, 2013).

Different measures have been adopted to increase the efficacy of microbial biofertilisers which include bio-prospecting new strains (genetic improvement in the existing microbial strains) and improved novel carriers for their storage and application (Kumar *et al.*, 2013; 2014a; 2015a,b). In this paper, we report the effect of various organic manures on the efficacy of consortia of *Azotobacter chroococcum* and *Bacillus subtilis*, co-immobilised in the optimised dose in the organic super granules of vermicompost, cow dung and Farm Yard Manure (FYM) as a major component; and its efficacy for wheat cultivation in earthen pots has also been investigated.

2. Materials and Methods

2.1 Site Description

The experiments were conducted in the field research station of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India which is located at height of 123 m above the sea level and is situated on 26.30° and 27.10° north latitude, and 80.30° and 81.13° east longitudes.

2.2 Experimental Design and Co-Immobilisation of *A. chroococcum* and *B. subtilis* in Organic Matrix Based Super Granules

The experiments were established in two consequent winter/Rabi seasons, 2011–2012 and 2012–2013. The organic matrix materials like cow dung, vermicompost, FYM, neem (*Azadirachta indica*) leaves, clay soil (diameter of particles <0.002 mm) and charcoal based biofertilisers were collected locally. All the collected materials of organic matrix were dried separately in oven at 60–70°C for 3 days and powdered in a grinder. These were mixed in 1:1:1 ratio and 15% (w/v) saresh (*Acacia gum*) was also added to

enhance the binding of the matrix materials during the super granule formation. The granules of approximately 4–5 mm diameter were prepared manually and dried at room temperature. The certified seeds of wheat (*Triticum aestivum* L. cv. PBW-502) were obtained from a local dealer of the Lucknow city. The experiments were laid out with three replicates with duplicate determinations of each replicate (i.e., $n=6$). Five plants were maintained in each pot with three replicate pots for each treatment. net house temperature ranged between 20°C and 25°C during the period of experiment in 2011–2012 and 2012–2013. Details of various nutrient treatments are as below.

2.3 Measurement of Fresh and Dry Weight of Shoot and Grain

Fresh shoot was collected at 120 days after sowing (DAS) and fresh weight was determined using single pan electrical balance. The same were oven dried at 70°C, till constant dry weight was recorded.

2.4 Grain Yield and Harvest Index

Six plants were harvested for each treatment and grains were separated from the spikes from 120 DAS old plants. The grain weight (g/plant) was measured and averaged for mean value and was statistically analysed to determine grain yield.

HI is defined as the ratio of grain yield (Y) and total biomass (dry weight of plant) at maturity (X)

The HI was calculated using the following equation (Huehn, 1993):

$$HI = \frac{Y}{X} = \frac{Y}{Y + S}$$

where X indicates sum of grain yield (Y)

And straw yield (S)

The HI is closely related to the grain straw ratio (R)

$$(R) = \frac{Y}{S}$$

Table 1 The various organic manures and other materials used to co-immobilise *A. chroococcum* and *B. subtilis* to prepare organic matrix based super granules

Treatment	Explanation
T0	No Fertiliser (control-1)
T1	Chemical fertilisers: Urea– 384.62 mg/10kg soil and DAP – 192.31 mg/10kg soil(control-2)
T2	Charcoal based <i>A. chroococcum</i> – 0.93 mg/10kg soil and <i>Bacillus subtilis</i> – 0.93 mg/10kg soil(control-3)
T3	The same biofertiliser consortium co-immobilised with cow dung, clay soil, neem leaves and saresh
T4	The same biofertiliser consortium co-immobilised with vermicompost, clay soil, neem leaves and saresh
T5	The same biofertiliser consortium co-immobilised with farm yard manure, clay soil, neem leaves and saresh
T6	The same biofertiliser consortium co-immobilised with clay soil, neem leaves and saresh (control-4)
T7	The same biofertiliser consortium co-immobilised with clay soil and saresh (control-5)

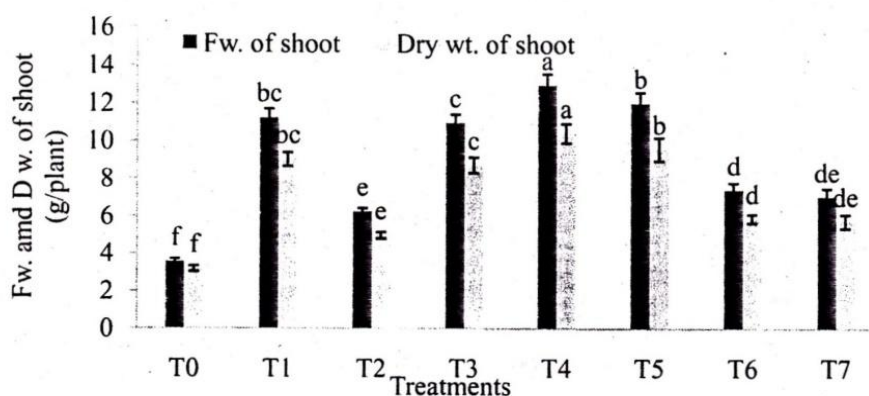


Figure 1 Fresh weight (Fw) and dry weight (Dw) of shoot (g/plant) of wheat (*Triticumaestivum* L.) plant applied with various organic matrix based granular immobilised biofertiliser consortium and other treatments at 120 DAS. The values are means of three replicates with two determinations ($n = 6$) \pm S.E. (where, T0– No fertiliser, T1 – Recommended dose of Urea and DAP, T2 – Free form of charcoal based biofertiliser, T3 – T2 immobilised with cow dung, T4 – T2 immobilised with vermicompost, T5 – T2 immobilised with farm yard, T6 – T2 immobilised with neem leaves and clay soil, T7 – T2 immobilised with clay soil)

2.5 Statistical Analysis

The data were calculated as Mean \pm S.D.($n=6$) and analysed using analysis of variance(ANOVA). Probability of 0.05 or less was considered significant according to Duncan's Multiple Range Test (DMRT). DMRT was applied to compare the means within the treatments at $p<0.05$.

3. Result

3.1 Fresh and Dry Weights of Vegetative and Reproductive Shoot at 120 DAS

The results presented in Figure 1 clearly indicate that the enhanced doses of *A. chroococcum* and *B. subtilis* (triple to the doses recommended on its commercial packet) can

produce similar biomass (fresh and dry weights) obtained with the recommended doses of urea and DAP in the earthen pot by co-immobilising these biofertilisers in the organic manure based organic super granules. The shoot biomass is increased significantly by the use of vermicompost as a supergranule constituent (10.44g/plant) over urea and DAP (9.03g/plant) applied pots, and the same amount of charcoal based commercial biofertilisers in the consortium of *A. chroococcum* and *B. subtilis* (Figure 1, Table 2). This enhanced biomass product was followed by FYM as organic manure in the super granules. Addition of cow dung as organic manure constituent of the super granules could enhance shoot biomass accumulation over its commercial charcoal based form but not over urea and DAP application.

Table 2 Effect of organic matrix based immobilised biofertiliser on grain yield, biomass and harvest index at 120 (DAS)

Treatments	Grain (Dw of grain yield g/plant)	Biomass (Dw of plant, g/plant)	Harvest Index (g/plant)
T0	0.67 \pm 0.06 ^a	3.19 \pm 0.27 ^f	0.21 \pm 0.01 ^d
T1	2.89 \pm 0.05 ^c	9.03 \pm 0.63 ^{bc}	0.32 \pm 0.02 ^{ab}
T2	1.14 \pm 0.03 ^f	4.98 \pm 0.30 ^e	0.23 \pm 0.02 ^{cd}
T3	2.62 \pm 0.09 ^d	8.74 \pm 0.72 ^c	0.30 \pm 0.03 ^b
T4	3.60 \pm 0.16 ^a	10.44 \pm 0.89 ^a	0.35 \pm 0.03 ^a
T5	3.23 \pm 0.10 ^b	9.59 \pm 1.07 ^b	0.34 \pm 0.03 ^a
T6	1.44 \pm 0.02 ^e	5.89 \pm 0.40 ^d	0.25 \pm 0.02 ^c
T7	1.33 \pm 0.04 ^e	5.75 \pm 0.63 ^{de}	0.24 \pm 0.02 ^c
F-value	974.87	89.57	40.33
CV%	4.03	9.29	7.17

a,b,c,d,e,f,g represent different variations in different treatments.

All treatments were replicated for three times with two determinations ($n=6$) \pm S.D. The samples were collected randomly at 120 DAS and analysed by using mean, standard deviation and one way analysis of variance (ANOVA), DMRT was applied to compare the means between the treatments at $p<0.05$. Values followed by different letters are significantly different between the treatments at 5% level.

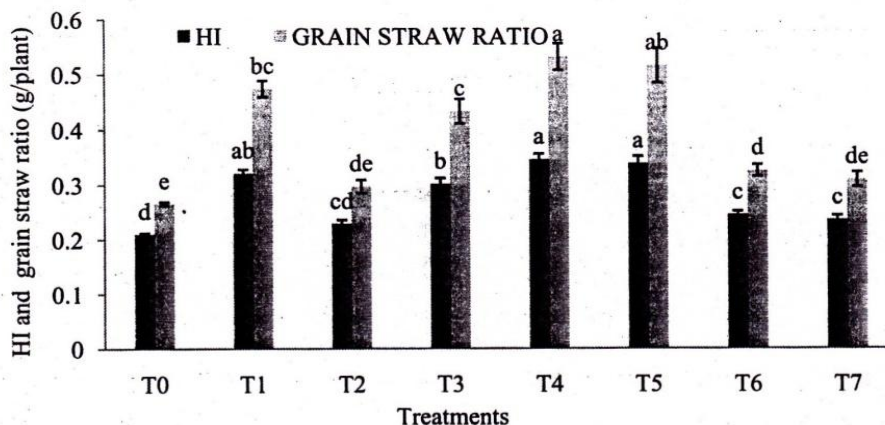


Figure 2: The grain straw ratio and harvest index (g/plant) of wheat (*Triticum aestivum* L.) applied with various form of nutrients as mentioned in Table 1. All the values are means of three replicates with two determinations ($n = 6$) \pm S.E. Other details are as mention in Figure 1

The organic matrix based immobilised biofertiliser granules increases the grain yield and HI of *T. aestivum* L. The plants' dry biomass productions were recorded as 69.44, 13.50, 52.39% and grain yields by 81.66, 19.72 and 68.61% by the application of T4 at 120 DAS, over controls T0, T1 and T3, respectively. Similarly, the application of T5 produces biomass of 66.73, 5.83, 48.17% and gain yield by 79.50, 10.24, 64.90% over T0, T1 and T2. The application of T3 has increased the biomass by 63.50 and 43.13% and grain yield by 74.80 and 56.87% over T0 and T2 only but not over T2 (urea and DAP applied pots). By the application of T4 grain yield has also recorded over the application of T3 by 27.22 and T5 16.28%.

3.2 Grain Yield and Harvest Index

Similar patterns for the various nutrient treatments were observed for grain yield per plant and HI also which indicate that the biomass of wheat produced in the earthen pot is directly related to crop yield in this variety atleast in earthen pot and net house condition.

The HI was increased by the application of T4, T5 and T3, respectively (Table 2). The straw weight and grain yield increased significantly with these treatments. The grain straw ratio was significantly higher on the application of T4 granules and HI and grain straw ratio both increased by T4 as compared to the application of T1, T3, T7, and T6 (Figure 2).

4. Discussion

The findings of this paper indicate that vermicompost is a better organic manure than FYM and cow dung as a constituent of organic matrix to co-immobilised *A. chroococcum* and *B. subtilis* biofertilisers to produce organic

matrix based super granules (Figures 1 and 2, Table 2). Though in the previous reports from the same group we have demonstrated that co-immobilising reduced quantity of urea and DAP, *A. chroococcum*, *B. subtilis* and BGA in cow dung based organic super granules increase efficacy of both the microbial biofertilisers and the organic manures over their recommended and commercially available farms (Dahiya *et al.*, 2004; Kumar *et al.*, 2013; 2014a,b; 2015a,b). The clay soil and neem leaves added to prepare the super granules (without a manure: T6 and T7) also add to the performance of these biofertilisers over their charcoal based commercially available form. However, it is the organic manure which adds a real value to it, possibly by providing easy nutrients for the metabolism and multiplication of the biofertiliser microbes.

The enhanced efficacy of the nutrients in organic matrix based super granules may be related to its increased population and providing the nutrient as a slow control release fertiliser synchronised with the requirements of wheat plants during its different vegetative and reproductive phases (Channabasanagowda *et al.*, 2008; Hasanuzzaman *et al.*, 2010; Singh *et al.*, 2010; Kumar *et al.*, 2013). The better performance of vermicompost as an organic constituent of the organic super granules over FYM and cow dung may be attributed to its high nutrient value especially in terms of NPK, micronutrients and rich microbial populations (Channabasanagowda *et al.*, 2008; Lazcano and Dominguez, 2011).

5. Conclusion

This study indicates that we can further improve the efficacy of microbial biofertilisers *A. chroococcum* and *B. subtilis*, which was already enhanced by their co-

immobilisation in cow dung based organic super granules previously by us (Kumar *et al.*, 2013; 2014a,b; 2015a,b), replacing cow dung with vermicompost as more obvious and new generation organic manure. It is a very significant preliminary finding to develop new generation organic plant nutrients to replace synthetic fertilisers in the era of climate change for sustainable agricultural productivity of wheat. The detailed investigations with these super granules are in process.

Acknowledgements

Rose Minj is grateful to UGC, New Delhi for providing UGC Non-Net fellowship, RPS is thankful to UGC, New Delhi for MRP, FN-43-320/2014 (SR).

References

- Abedi T, Alemzadeh A and Kazemeini SA (2010). Effect of organic and inorganic fertilizers on grain yield and protein banding pattern of wheat. *Aust. J. Crop Sci.* 4(6): 384–389.
- Adesemoye AO, Torbert HA, and Kloepper JW (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microb. Ecol.* 58: 921–929.
- Ardakani MR, Mazaheri D, Mafakheri S and Moghaddam A (2011). Absorption efficiency of NPK through triple inoculation of wheat (*Triticumaestivum* L.) by *Azospirillum brasilense*, streptomycetes sp. *Glomus intrardices* and manure application. *Physiol. Mol. Biol. Plants* 17(2): 181–192.
- Cerny J, Balik J, Kulhanek M, Casova K and Nedved V (2010). Mineral and organic fertilization efficiency in long-term stationary experiments. *Plant Soil Environ.* 56(1): 28–36.
- Channabasanagowda, Patil BN, Awaknavar JS, Ninganur BT and Hunje R(2008). Effect of organic manures on growth, seed yield and quality of wheat. *Karnataka J. Agric. Sci.* 21(3):366–368.
- Crecchio C, Curci M, Antonella P, Ricciuti P, Tursi N and Ruggiero P(2007). Soil microbial dynamics and genetic diversity in soil under monoculture wheat grown in different long-term management systems. *Soil Biol. Biochem.* 39: 1391–1400.
- Dahiya S, Jaiwal PK and Singh RP (2004). Efficient nitrogen assimilation and high productivity in rice (*Oryzasativa* L.) applied with organic matrix based slow release nitrogen fertilizers. *Physiol. Mol. Biol. Plant.* 10, 83-92.
- Gopinath KA, Saha S, Mina BL, Pande H, Kundu S and Gupta HS (2008). Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. *Nut. Cycl. Agroecosyst.* 82: 51–60.
- Hasanuzzaman M, Ahamed KU, Nahar K and Akhter N (2010). Plant growth pattern, tiller dynamics and dry matter accumulation of wetland rice (*Oryzasativa* L.) as influenced by application of different manures. *Nat. Sci.* 8:1-10.
- Huehn M (1993). Harvest index versus grain/straw-ratio. Theoretical comments and experimental results on the comparison of variation. *Euphytica* 68: 27–32
- Kemarian RA, Stockle OR, Huggins RD and Viega ML (2007). Field crop research. *Sci. Dir.* 103: 208–216.
- Kong L, Wang F and Lopez-bellido L (2013). Agronomic improvements through the genetic and physiological regulation of nitrogen uptake in wheat (*Triticumaestivum* L.). *Plant Biotechnol.* 7: 129–139.
- Kundu BS, Nehra K, Yadav R and Tomar M (2009). Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different region of Haryana. *Indian J. Microbiol.* 49: 120–127.
- Kumar M, Baudhdh K, Sainger M, Sainger PA and Singh RP(2015a). Enhancing efficacy of *Azotobactor* and *Bacillus* by entrapping in organic matrix for rice cultivation. *Agroecol. Sustain. Food Syst.* 39: 907–923.
- Kumar M, Baudhdh K, Sainger M, Sainger PA, and Singh RP, (2015b). Increase in growth, productivity and nutritional status of wheat (*Triticumaestivum*) and enrichment in soil microbial population applied with biofertilizers entrapped with organic matrix. *J. Plant Nutr.* 38: 260–276.
- Kumar S, Baudhdh K, Barman SC and Singh RP(2014a). Amendments of microbial biofertilizers and organic substances reduces requirement of urea and DAP with enhanced nutrient availability and productivity of wheat (*Triticumaestivum* L.). *Ecol. Eng.* 71: 432–437.
- Kumar S, Baudhdh K, Barman SC and Singh RP (2014b). Organic matrix entrapped bio-fertilizers increase growth, productivity and yield of *Triticumaestivum* L. and mobilization of NO₃⁻, NO₂⁻, NH₄⁺ and PO₄³⁻ from soil to plant leaves. *J. Agric. Sci. Technol.* 16(2): 315–329.
- Kumar M, Baudhdh K, Kumar S, Sainger M, Sainger PA and Singh RP (2013). Increase in growth, productivity and nutritional status of wheat (*Triticumaestivum* L. cv. Wh-711) and enrichment in soil fertility applied with organic matrix entrapped urea. *J. Environ. Biol.* 34:1–9.
- Kumar M, Baudhdh K, Sainger M, Sainger PA, Singh JS and Singh RP (2012). Increase in growth, productivity and nutritional status of rice (*Oryzasativa* L. cv Bastmati) and enrichment in soil fertility applied with an organic matrix entrapped urea. *J. Crop Sci. Biotechnol.* 15(2): 137–144.
- Laik R, Sharma S, Idris MM, Singh AK, Singh SS, Bhatt BP, Saharwat Y, Humphreys E, and Ladha JK (2014). Integration of conservation agriculture with best management practices for improving system performance of the rice wheat rotation in the eastern Indo-gangetic plains of India. *Agric. Ecosyst. Environ.* 195: 68–82.
- Lazcano C and Dominguez J (2011). The use of vermicompost sustainable agriculture: impact on plant growth and soil fertility. Soil nutrients ISBN 978-1-61327-785-3.
- Minaxi S, Chandra JS and Nain L (2013). Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants. *J. Sci. Plant Nutr.* 13(2): 511–525.
- Nayak AK, Gangwar B, Shukla AK, Mazumdar SP, Kumar A, Raja R, Kumar A, Kumar V, Rai PK and Mohan U (2012). Long-term effect of different integrated nutrient management on soil organic carbon and its fractions and sustainability of rice-wheat system in Indo Gangetic Plains of India. *Field Crop Res.* 127: 129–139.
- Otinga AN, Pypers P, Okalebo JR, Njoroge R, Emongole M, Six L, Vanlauwe B and Merckx R (2013). Partial substitution of phosphorus fertiliser by farmyard manure and its localized application increases agronomic efficiency and profitability of maize production. *Field Crop Res.* 140: 32–43.
- Rawat SK, Singh RK and Singh RP (2012). Remediation of nitrite in ground and surface waters using aquatic macrophytes. *J. Environ. Biol.* 33:51–56.
- Pennington D (2013). Harvest index: A predictor of corn stover yield. <http://www.msue.msu.edu>.
- Ryan J, Sommer R and Ibrkci H (2012). Fertilizer best management practices: A perspective from dry land west Asia–North A1 region. *J. Agron. Crop Sci.* 198: 57–67.
- Sapkota TB, Majumdar K, Jat ML, Kumar A, Bishnoi DK, McDonald AJ and Pampolino M (2014). Precision nutrient management in

- conservation agriculture based wheat production of Northwest India: Profitability, nutrient use efficiency and environment footprint. *Field Crop Res.* 155: 233–244.
- Shaharoona B, Naveed M, Arshad M and Zahir AZ (2008). Fertilizer-dependent efficiency of *Pseudomonas* for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). *Appl. Microb. Biotechnol.* 79: 147–155.
- Sharma P, Singh G and Singh RP (2012). Conservation tillage, optimal water supply enhance microbial enzyme (glucosidase, urease and phosphatase) activities in field under wheat cultivation during various nitrogen management practices. *Arch. Agr. Soil Sci.* doi.org/10.1080/03650340-690143.
- Singh R, Singh R, Sonid SK, Singh SP, Chauhan UK and Kalra A (2013). Vermicompost from biodegraded distillation waste improves soil properties and essential oil yield of pogostemon cabin (patchouli) benth. *Appl. Soil Ecol.* 70: 48–56.
- Singh RP, Sainger M, Baudhh K, Sengar RS and Jaiwal PK (2010). Sustained nutrient supply reduced nutrient loss and high plant productivity with slow release fertilizers, In: *Stable Food Production and Sustainable Agriculture: A challenge ahead 21st century* (Eds. Sengar, R.S. and Sharma, A.K.) Studium Press (India) Pvt Ltd. New Delhi, pp. 62–79.
- Weber A, Gutser R and Schmidhalter U (2001). Field emission of NH₃ and NO_x following urea application to wheat. *Plant Nut. Food Sec. Sust. Of Agro-Ecosyst.*, 92, 884–885.
- Yadav J, Yadav S, and Singh SG (2011). Plant growth promotion in wheat crop under environmental condition by PSB as biofertilizers. *Res. J. Agric. Sci.* 2(1): 76–78.

प्रोफेसर राणा प्रताप सिंह,
पीएच. डी., फेलो, एई.वी., फेलो, आई.एस.पी.पी.
प्रोफेसर प्रभारी, शैक्षणिक
संकायाध्यक्ष (i/c), प्रबन्ध अध्ययन विद्यापीठ
संयोजक, उद्योग संस्थान भागीदारी कार्यक्रम केन्द्र

Professor Rana Pratap Singh,
Ph.D., FAEB, FISPP
Professor-in-Charge, Academic
Co-ordinator Centre for Industry Institution Partnership
Program (CIIPP)
Dean (i/c), School for Management Studies
Former Dean, School for Environmental Sciences
Former Head, Department of Environmental Science
Vice-President, Academy of Environmental Biology, India
Editor-in-Chief : *Physiol. & Mol. Biol. of Plants*
www.springer.com/journal/12298; www.phssfoundation.org.in
Editor-in-Chief : *International Journal of Science, Technology & Society*
www.bbau.ac.in/researchjournal/IJSTS
Editor : *Climate Change and Environmental Sustainability*
www.ssceindia.org
<http://www.indianjournals.com/ijor.aspx?target=ijor.cces&type=home>



बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय
(केन्द्रीय विश्वविद्यालय)
विद्या विहार, रायबरेली रोड, लखनऊ-226 025, भारत

Babasaheb Bhimrao Ambedkar University
(A Central University)
Vidya Vihar, Raebareilly Road, Lucknow-226 025, India
Tel. : +91-522-2995606, Mob.: +91-9889121823
Fax : +91-0522-2441888
Emails: cceseditor@gmail.com, ranapratap@bbau.ac.in

www.ranapratap.in
www.bbau.ac.in/faculty/DES/RPSingh

15 Dec., 2015

Acceptance Letter

Ms. Rose P. Minj
Research Scholar
Department of Environmental Science
BBA University, Lucknow

Dear Ms Minj,

I am pleased to inform you that your research paper entitled "Differential Increase in Efficacy of *Azotobacter choroococcum* and *Bacillus subtilis* Co-immobilized with Different Organic Manure in Relation to Plant Growth, Nutritional Status and Grain Yield of Wheat Crop (*Triticum aestivum* L)" (Authors: Rose P. Minj and Rana Pratap Singh) has been finally accepted in its revised form for publication in *International Journal of Science, Technology and Society (IJSTS)*. It will appear in *IJSTS* Vol 1(2), July-December, 2015 issue, shortly going to press.

Thanking you,

Yours Sincerely

Rana Pratap

Rana Pratap Singh
Editor-in-chief, IJSTS