Efficacy of *Trichoderma* spp. and plant growth promoting rhizobacteria (PGPR) as biofertilizer for wheat (*Triticum aestivum* L.) cultivation

THESIS

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CERTIFICATE

This is to certify that the thesis entitled "**Efficacy of *Trichoderma* spp. and plant growth promoting rhizobacteria (PGPR) as biofertilizer for wheat (*Triticum aestivum* L.) cultivation**" submitted by **Mr. Mahesh Kumar** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) Regulations – 1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: **Supervisor**

Head of Department

DECLARATION

This is to certify that the material embodies in the present Ph.D. work entitled "**Efficacy of *Trichoderma* spp. and plant growth promoting rhizobacteria (PGPR) as biofertilizer for wheat (*Triticum aestivum* L.) cultivation**" is original research work done in partial fulfillment of requirements for the award of the degree of Doctor of Philosophy under the supervision of Prof. Rana Pratap Singh, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow-226025, India. It has not been submitted in part or full for any other diploma or degree in any other University or institute. It is also declared that the thesis is essentially free from all kinds of plagiarism. In this thesis, matter written, data presented and plagiarism, if any, is the sole responsibility of the student Mr. Mahesh Kumar. If any allegations/query/question arises regarding the thesis, I, Mr. **Mahesh Kumar** will be solely responsible and answerable.

Date:

(Mahesh Kumar)
Department of Environmental Science,
Babasaheb Bhimrao Ambedkar University
Lucknow, U. P., India.

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Preface

The world's population is increasing gradually and is about to reach to the numeric value of 9.8 billion in 2050. The problem of food security is increasingly being threatened in many countries especially in the developing countries of the tropics with huge population. Wheat (*Triticum aestivum* L.) is the most widely grown and major food crop all across the world including India. It provides about 20% of the daily proteins of the food calories for 4.5 billion people worldwide. The modern agricultural practices utilize fertilizers, pesticides, fungicides and insecticides to artificially fulfill the nutritional requirements of crops with the primary objective of enhancement in the productivity. Application of agro-chemicals usually improved crop yield but over fertilization has degraded soil quality of single crop system. There is no doubt that application of chemical fertilizers increased the productivity of crop, but initially the negative impacts of using such NPK fertilizers and other chemicals to the environment. The advancements made in field of agricultural have initiated the application of beneficial microorganisms that are not only eco-friendly and sustainable but also inexpensive than chemical fertilizers. These microorganisms are able to easily accelerate the absorption of nutrients from the soil and promote growth as well as productivities. These are technically termed as 'bio-fertilizers'. Further, due to their potential to enhance growth and development of plant these are also termed as plant growth promoting microorganisms (PGPMs). These helps in improving the uptake of nitrogen (N), phosphorus (P), iron (Fe), zinc (Zn) & manganese (Mn) like essential nutrients, enhances the activity of vital phytohormones like indole acetic acid (IAA), gibberellins, cytokinins which help wheat plants to uptake, translocate and accumulate nutrients in a sustainable way.

The present study was planned to isolate plant growth promoting microorganisms (PGPMs) from the native wheat fields and to characterize their affectivity to be used as biofertilizers for wheat cultivation in the similar agro-climatic conditions. The isolated bacterial and fungal strains will be studied for their morphological, biochemical and plant growth promoting activities e.g. phosphate solubilization, IAA production, nitrogen fixation, siderophore production and ammonia production were differentially shown by all the isolates. Further, these PGPMs will be studies individually and in consortium to optimize the best

formulations for enhancing the growth and productivity of wheat in earthen pots. Efficacy of best performing formulation of PGPMs will be studied on growth and yield of wheat in experimental field. The availability of nutrients in soil and its distribution in plant parts during application of biofertilizers will also be studied with estimation of cost benefit analysis of newly developed eco-friendly consortium in comparison to the conventional chemical fertilizers.

The present study reported that plant growth promoting rhizobacteria and soil fungi isolated from the rhizosphere of native wheat field were found to be more effective than the commercially available biofertilizers. The consortium of compatible microbes (*Microbacterium phyllosphaerae, Alcaligenes faecalis* and *Trichoderma virens*) were most effective to promote growth and productivity of wheat than single isolates of the same microbial strains. In-vitro plant growth promoting activities were effective in in-vivo conditions in earthen pots and experimental field based experiments for cultivation of wheat. These microbes enhanced the availability of nitrate, nitrite, ammonium and phosphate in soil and plant parts which were subsequently increased the growth, productivity and yield of wheat in pot and field conditions. The results of present study indicated that different microbial species are available in agricultural fields which can be isolated and characterized for various uses. New generation biofertilizers can be prepared by making consortia of microbes with multiple activities and use of chemical can be avoided to maintain the agricultural sustainability.

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Abbreviations & Symbols

%	Percent
°C	Degree Celsius
°E	Degree East
°N	Degree North
ACC	1-Aminocyclopropane-1-Carboxylate
ANNOVA	Analysis of variance
BLAST	Basic Local Alignment Sequence Tool
Bp	Base Pairs
CAS	Chrome Azurol S
CFU	Colony forming unit
Cm	Centimeter
DAS	Days After Sowing
DMRT	Duncan's Multiplicity Range Test
G	Gram
Ha	Hectare
HCN	Hydrogen cyanide
IAA	Indole 3 acetic acid
K	Potassium
kg	Kilogram
N	Nitrogen
NA	Nutrient Agar
NO_3	Nitrite
NOx	Nitrogen oxide
OD	Optical density
P	Phosphorus
PDA	Potato Dextrose Agar
PGPA	Plant growth promoting activities
PGPF	Plant growth promoting fungi
PGPM	Plant growth promoting microorganism
PGPR	Plant growth promoting rhizobacteria
FAO	Food and Agricultural organization
PSB	Phosphate solubilization
psi	Phosphate Solubilization Index
RBD	Random block design
S.D	Standard deviation
WHO	World Health Organization
µl	Micro Liters

Chapter 1
Introduction

1. Introduction

The current world population of 7.6 billion and is predictable to touch the digits of 8.6, 9.8 and 11.2 billion in 2030, 2050 and 2100, respectively (Unites Nations 2017). Millions of people are being added to the world's population and it has become a major issue to satisfy the nutritional needs of such a huge population with safe food and drinking water (Godfray et al. 2010). The problem of food security is increasingly being threatened in many countries especially in the developing countries of the tropics. Here traditional agricultural systems have become unsustainable due to reduction of arable fertile lands, demographic pressure and increased usage of fertile lands for non-agricultural practices e.g. for industries, factories, mining and urbanization (Mensah et al. 2018). In addition to this, food anxiety will also intensify because of rising temperature and drought stress persuaded by climate change and anthropogenic pollution, in 21st century (Mäder et al. 2011). These circumstances have led to the excessive usage of chemical fertilizers, pesticides, herbicides, fungicides, insecticides etc. to enhance crop productivity to fulfill diffrent nutritional necessities of such a huge population all over the world. Agriculture is major sector that not only provide food to a huge population but also contribute towards the national income and export earnings to any country ensuing the food security and employments (Meena et al. 2017). Thus, there need to develop such technology that augments with the needs of sustainable agriculture with special care towards minimal usage of chemicals and ecological sustainability.

1.1 Environmental degradation by chemical fertilizers

Since the dawn of civilization, humans are unmindfully using natural resources to satisfy their greed of so called development in all sectors including agriculture. The estimated present population growth rate is 1.8% which requires production of a shortfall of 20 million tons of cereal grains till the year 2020 (Singh and Sing 2001). This shows that to application of chemical fertilizers and pesticides will also increase to gain such a high amount of cereals accordingly. Now days, various chemical fertilizers, pesticides etc. are being used to enhance the productivity of food crops and to avoid losses by different pest, herbs, insects and harmful microorganisms, respectively (Meena et al. 2017). These chemicals are manufactured by combination

of various harmful substances that have the ability to persist in the environment. With the advent of 'green revolution' in 20th century, excessive and unmindful application of agrochemicals including urea and DAP fertilizers, pesticides, herbicides, fungicides etc. started. Urea and DAP are common nitrogen fertilizers that are widely applied by farmers and occupy first two positions in the consumption of fertilizers and feed additives. These contain higher percentage of N and P content, cheaper and easy to handle, so farmer are conveniently using these. Urea and DAP both endure a series of reactions after applying to the field and finally resulted in N and P losses and NH_3 volatilization that ultimately pollute the environmental systems (Wang et al. 2018). The basic idea or thought of farmers in using these chemical was to achieve better performances and more productivity of crops (Azizullah et al. 2011). But, in spite of different improvements in food grain production with arrival of green revolt loss of these chemicals in the atmosphere, its persistence in the soil and leaching in ground deteriorated the most essential natural assets i.e. air, water and soil causing serious threat to the mankind (Saritha and Tollamadugu 2019). It is a renowned fact that agricultural practices including numerous traditional methods of farming e.g. burning of crop residues, spraying of pesticides and herbicides etc. have increased the concentration of greenhouse gases in the ambient environment aiding in climate change and global warming (Ali et al. 2016). Greenhouse gases (GHGs) attributes towards change in weather conditions which is the most concern issue all across the world. Oxides of nitrogen (NO_x), phosphate, ammonia and other reactive chemicals emitted by the agricultural fields used to participate in global warming as these are persistence in nature and have higher warming potential, usually 298 times higher than carbon dioxide (IPCC 2007). About 60–100 Tg N year^{-1} of reactive N have been found to get lost in the environment due low nitrogen use efficiencies (NUE) of agriculture (Yao et al. 2018). Likewise, 10.7 Tg NH_3 year^{-1} have been found to be emitted from the agricultural fields, about 42 % of which came from synthetic chemical fertilizers (Wang et al. 2018). Further, there is also the likelihood of these chemical pollutants to enter the fresh water bodies causing a number of alterations in the physicochemical characteristics of water and can also leach into the ground water (Singh et al. 2014; Kantachote et al. 2016). Agriculture system is single largest consumer of fresh water in the whole world. These chemicals also have the tendency to get bio accumulated in the plant system and may reach to the living beings by transfer through the food chain. Thus, these chemicals pollute soil, surface water as

and ground water through agricultural run-off which may result in eutrophication of water bodies, ambient air through greenhouse gas emission and ultimately reaches the highest trophic level by contamination of the food chain (Jian et al. 2016; Tejada et al. 2016) (Table 1.1). In developing countries like India these is a lack of public awareness as users and sellers both do not have appropriate ideas about application of actual quantities, time of application and correct application methods for fruitful usage of all chemical fertilizers, pesticides and herbicides etc. There is another major issue regarding auditing and investigation of chemical products concerning the impacts, life cycles and nature of persistence of these chemicals into the environments.

Table 1.1 Environmental degradation due to application of conventional chemical fertilizers and their causative mechanisms

Environmental consequences	**Causative Mechanisms**	**References**
Air and water pollution	Ammonia (NH_3) volatilization and nitrogen (N_2) surface runoff	Li et al. (2018)
Greenhouse gases (GHG) emission and soil pollution	soil ammonia (NH_3) and nitrous oxide (N_2O) emission	Liu et al. (2017)
Nitrate pollution in water and emission of NO_x	Chemical fertilizers runoff and emission from soil	Duan et al. (2014); Wang et al. (2016)
Ground water contamination	Nitrate leaching from soil	Delin and Stenberg (2014);
Eutrophication	Erosion, runoff, and leaching of N and P from cultivated fields	Zhou and Bahl (2014); Jian et al. (2016); Dari et al. (2017)
Stratospheric ozone depletion	Nitrous oxide and nitric oxide emissions from soil	Migliorati et al. (2014)
Global warming by stratospheric ozone destruction	Nitrous oxide and nitric oxide emissions from a wheat–maize cropping system	Cui et al. (2012)

1.2 Role of nitrogen and phosphorus in plants

N and P are major macronutrients and often limiting factor for enhancing growth and development of plants (Yasin et al. 2012; Solangi et al. 2016). The excess and deficiency of these two important macronutrients may adversely affect growth and developments of plants (Table 1.2). These are utilized by plants in various metabolic activities as well as in the proper functioning of their vegetative and reproductive cycles. These are major constituents of vital macromolecules e.g. amino acids, nucleic acids, chlorophyll and metabolites etc. that aid in translocation of nutrients, flood and water in various metabolic, catabolic and biochemical mechanisms like cell division, transpiration and photosynthesis, energy transfer, signal transduction etc. (Khan et al. 2009; Mukhtar et al. 2017). Nitrogen and phosphorus are found in two forms i.e. organic and inorganic forms in soil. In organic form, nitrogen is confined to soil in the form of manure produced by plant and animal wastes, whereas, inorganic form includes ammonium (NH_4^+) ions and nitrate (NO_3^-) ions. Phosphorus is present in relatively large quantities but less poorly available as found in either less soluble organic forms or soluble inorganic forms (Richardson 1994; Mahanta et al. 2014; Munda et al. 2018). Plants take up nitrogen in the form of nitrates of ammonium, calcium and potassium and ammonium sulfate and phosphorus as orthophosphate which are maintained by natural N and P cycles (Cevallos et al. 2015). The deficiency and excess of both these elements are harmful for the growth and development of plants (Table 1.1). On the other hand, utilization of various N and P fertilizers has increased significantly since the middle of the 20^{th} century due to the impact of the "green revolution". Chemical fertilizers e.g. urea, di-ammonium phosphate (DAP), ammonium nitrate, etc. have been profoundly used by farmers for enhancing the productivity during this period (Paulson and Babcock 2010). About 550-600 kg Ha^{-1} of high input have been found to be associated with rice production in China which increased yield significantly on the other hand also caused two times loss of N in the environment (Ju et al. 2009). Such an indiscriminate and unmindful use of N and P fertilizers have adversely affected the health of the environment including all forms i.e. soil, air and water. Thus, healthy and green agriculture systems for food and crop production without excessive use of chemicals fertilizers have become the need of today.

It has been estimated that crop production need to be doubled till 2050 to fulfill the needs of escalating population (Tilman et al. 2011). Likewise, the usage of N containing chemical fertilizers is expected to increase by many folds in upcoming years (Meena et al. 2017). Although this will possibly increase the crop productivity but will surely damage soil quality as well as environmental sustainability. Accordingly, main concern of the workers is to minimize the harmful effects of such chemical fertilizers with alternatives that not only reduces soil and water pollution level but also increases the growth and productivity of the food crops (Savci 2012). In this context, organic farming has emerged as an effective and suitable substitute of traditional farming that extensively uses chemicals to enhance the productivity (Munda et al. 2018). Organic farming involves usage of compost & manure, organic fertilizers, microorganisms that produces crops with sustaining the health of soils, ecosystems and human beings. Among these, consumption of microorganism's living or latent cells come under the term 'bio-fertilizers'. These are the most effective one due to their positive impacts on to the health of plant and the agro-system as well as onto the health of living beings. Some of the bio-fertilizers have been found to produce some environmental stress (salt and drought) reducing compounds like 5-aminolevulinic acid (ALA) that help plants to sustain their lives (Nunkaew et al. 2014). Thus, application of 'bio-fertilizers' has gained significant place as these are ecofriendly and aids in maintaining long term ecological stability (Khan et al. 2007). It has appeared as an effective and cost efficient substitute of chemical fertilizers for greener and sustainable agriculture.

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Table 1.2 Consequences of deficiency and excess of nitrogen and phosphorus on plants and environment

Role in plants	Deficiency	Excess	References
Vital component of nucleic acids, amino acids coenzymes, phospholipids etc. that aid in metabolic activities	Stunned and abnormal growth, pigmentation of leaves	Interfere in the uptake of other vital elements e.g. iron, manganese and zinc that are essential for different metabolic and catabolic activities	Khan et al. (2009)
Encourage root development in plants and enhance nitrogen fixing capacity of legumes	Slow germination rate of seeds	Leaching of N and P may contaminate ground water system	McMcKague (2005); Wang et al. (2018)
Increases shoot or stalk length	Growth and development of plants are stunned	Agricultural runoff may cause surface water pollution e.g. eutrophication that may degrade water quality by minimizing the amount of oxygen and enhancing the growth of macrophytes	Tucker (1999); Khan et al. (2009)
Improves flowering and seed production	Flowering and fruiting are late	It may also harm air quality by emission of different harmful gases like ammonia by volatilization process	Tucker (1999); Khan et al. (2009)
Resistance to bacterial and fungal diseases	Emergence of various bacterial (leaf and sheath blight, blast) or insects (leaffolder) or and fungal diseases	It may aid in acid rain that again harm our historical monuments and buildings	Tucker (1999); Khan et al. (2009)
Improve quality of fruits and cereals	Death of plants, flowers or fruits may occur	May increase acidification and denitrification of soil and water	Tucker (1999); Khan et al. (2009)

1.3 Biofertilizers

Growing demands for increase in crop and food production for nourishing vast population in an ecofriendly and cost effective way have led to a keen interest and requirement towards the usage of organic fertilizers that not only improve productivity but also retain soil quality (Nishita and Joshi 2010). As an estimate 11.2 and 1 kWh of energy is required to produce one kilogram of N and P fertilizers that shows high energy consumption (Saritha and Tollamadugu 2019). Further, price of conventional fertilizers in regards of the declining petroleum based merchandises and feedstock are once again prohibits and discourage farmers to apply these chemicals. To overcome such limitations, application of 'bio-fertilizers' have emerged as an effective alternative to accomplish these requirements without causing severe ill effects onto the environment (Kantachote et al. 2016).

History of bio-fertilizers started in starting of 19th century by discovery of a laboratory culture of *Rhizobium* followed by various other microorganisms (Chatterjee et al. 2017). These are biologically active single or multiple living microorganisms or microbial inoculants that are used to enhance productivity of crop by means of nitrogen fixation, phosphate solubilization, or cellulolytic activities (Arriola et al. 2015). These inbuilt processes are considered to encourage growth and production by upgrading accessibility of soil nutrients in the rhizospheric region (Mazid et al. 2011). Plant growth promoting bacteria, blue-green algae, and arbuscular mycorrhizal fungi are very frequently applied as biofertilizers. Biofertilizers are normally applied directly to seeds or plant surfaces or soil after that microbes used to colonize or the interior of plants. These help to increase availability of principal nutrients to host plants, by defending them from phyto-pathogens either by regulating or hindering them which promotes the growth and productivity (Ahemad and Kibret 2014). These also help in degradation and decomposition of origin matter, mineralization, nitrogen fixation and denitrification of soil system.

Among bacteria and fungus, most commonly used biofertilizers are the plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF). PGPR include different species of genera *Rhizobium*, *Azotobacter*, *Azospirillum*, *Acinitobacter*, *Acetobacter*, *Azolla*, *Bacillus*, *Frankia*, *Pseudomonas*, *Serratia*, *Thiobacillus*, Blue green algae (*Anabaena*, *Nostoc*, *Plectonema*), Mycorrhiza, potassium solubilizing bacteria (*Aspergillus*, *Bacillus*, and *Clostridium*) etc. whereas, PGPF embrace different species of genera *Trichoderma*, *Verticillium*, *Aspergillus*, *Penicillium* etc. Further, the properties of mostly commonly used biofertilizers with specified genera and species have been summarized in Table 1.3.

Table 1.3 Commonly used biofertilizers and their plant growth enhancing properties

Biofertilizers	Strain	Properties	References
***Rhizobium* sp.**	*R. trifoli, R. meliloti, R. phaseoli, R. japonicum, R. leguminosarum* etc.	These may fix N at 50-100 kg ha^{-1} in pulse crops.	Saikia and Jain (2007)
***Herbspirillum* sp.**	*Azospirillum brasilense*, *Herbaspirillum* sp.	These improve accessibility of NPK and also stimulate production of growth hormones.	Khan et al. (2011); Lee et al. (2012)
***Azotobacter* sp.**	*A. vinelandii*, A. *beijerinckii*, *Agrionoptera insignis*, *Azomonas macrocytogenes* etc.	These may fix N at 40-200 kg ha^{-1} and can accomplish up to 80-90% of N necessities of crops with inhibition of certain root pathogens and helps in seed germination growth.	Saritha and Tollamadugu (2019); Mazid et al. (2011)
***Acetobacter* sp.**	*A. diazotrophicus* etc.	These may help in N fixation up to 15 kg ha^{-1} $year^{-1}$.	Saritha and Tollamadugu (2019)
***Azospirillum* sp.**	*A. amazonense, A. halopraeferens*, *A. brasilense* etc.	These may fix N at N 20-40 kg ha^{-1} and significantly improve grain yield in C4 plants.	Brusamarello-Santos et al. (2017)
***Azolla* sp.**	*A. pinnata* etc.	These may fix N at 45-50 kg ha^{-1} and form symbiotic relationship with blue-green algae that helps in production of rice grains.	Ghosh (2004)
Phosphate Solubilizing Bacteria (PSB)	*Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *Aspergillus* sp., *Fusarium* sp., etc.	These helps in conversion of convert insoluble inorganic phosphate to its soluble form and make it accessible to plants and may enhance the productivity up to 200-500 kg ha^{-1}.	Saritha and Tollamadugu (2019)
Blue green algae	*Anabaena* sp., *Nostoc* sp., *Plectonema* sp. etc.	These create symbiotic associatio with fungi, ferns, roots of different plants etc. with especial choice to *Azolla* sp. that aid in N fixation.	Saritha and Tollamadugu (2019)
Potassium Solubilizing Bacteria	*Aspergillus* sp., Bacillus sp., *Clostridium* sp. etc.	These help in solubilization of potassium which is an essential primary macronutrient.	Mohammadi and Sohrabi (2012)
Mycorrhiza	-	It is a symbiotically beneficial relationship with host plant with substitution of zinc (Zn) and P over carbohydrates	Saritha and Tollamadugu (2019)

1.3.1 Plant growth promoting rhizobacteria (PGPRs)

The PGPRs were first described by Kloepper and Schroth (1978). These are soil bacteria living in soil and rhizospheric region of plants, where they encourage plant growth and development of their hosts by their direct and indirect mechanism (Arrudaa et al. 2013; Mukhtar et al. 2017). PGPR favors plant growth and productivity directly or indirectly by stimulating relatively higher production of phytoharmones e.g. indole acetic acid (IAA), gibberellic acid, cytokinins ethylene, siderophores, HCN, solubilization of minerals (P and Zn) and breakdown of complex organic substances into simpler form for easy conveyance of plants and for also for their own consumption (Arshad and Frankenberger 1993; Glick 1995; Mukhtar et al. 2017; Wang et al. 2018). Generally, solubilization of minerals by PGPRs is the release of organic acids of low molecular weights. These acids contain hydroxyl and carboxyl groups that chelate cations bound to minerals and convert them into soluble forms (Panhwar et al., 2011). Now days PGPRs have been applied as biofertilizers, efficient substitute of chemical fertilizers as these are ecofriendly and reduce chances of environmental pollution and also the cost of crop production (Tahir et al. 2013). Further, PGPRs used to make available soil nutrient to crops without compromising with the soil fertility and environmental sustainability. Previous studies have shown positive outcomes of PGPR application on growth and productivity of various crops in diverse climatic conditions, soils, temperature and also under water stress as well as these PGPRs also work very efficiently in sodic and acid soils (Kloepper and Schroth 1978; Kumar and Singh 2018).

1.3.2 Plant growth promoting fungi (PGPF)

The PGPFs may be boon for sustainable development in the field of agriculture and help to reduce the nutrients losses from agricultural sector. The more efficiently used PGPF are different strains of *Trichoderma* e.g. *T. viride, T. asperellum, T. virens, T. harzianum, T. atroviride* etc. demonstrated increased growth and productivity of different plants as and tomato (Molla et al. 2012), cucumber (Akter et al. 2013), cabbage and red beet (Topolovec-Pintarić et al. 2013), lemon balm (Kowalska et al. 2014), wheat (Xue et al. 2017; Kumar and Singh 2018; Kumar et al. 2018). *Trichoderma* sp. used as plant growth promoter that converts solubilize nutrients and

make it accessible to plant. Further, these secrete various vitamins and enzymes including phytohormones and siderophore which again help in enhancing the growth and productivity of various crops (de Santiago et al. 2011; Li et al. 2015). Siderophore are produced by different bacterial and fungal species that works in iron solubilization, mobilization, transportation and/or storage (Angel et al. 2016). Fungal siderophore also participate significantly in suppression of pathogens and diseases (Mensah et al. 2018). Further, *Trichoderma* species produce multiple compound e.g. cell wall diminishing enzymes and secondary metabolites etc. which increase root development, resistance to biotic and abiotic stresses. *Trichoderma* sp. are also used as bio-control agents for various pathogens, bacterial and viral diseases in the plants e.g. black rot of pine apple (Wijesinghe et al. 2011), damping off and root rot of beans and chick peas (Shaban and El-Bramawy 2011), foot and root rots of tomatoes (Marzano et al. 2013), Bakanae disease of rice (Ng et al. 2015), root rot and cereal cyst nematode in wheat (Foroutan 2013; Zhang et al. 2014) etc. These may also be one of the reasons for acceleration of growth and productivity of different crops after application of *Trichoderma* (Hasanloo et al. 2010). Moreover, it has also been reported that biofertilizer using *Trichoderma viride* significantly reduced nitrous oxide, a potent air pollutant, emission by 33.3-71.8% at application of 225 kg N ha^{-1} $year^{-1}$ (Xu et al. 2014). Past studies showed increase in growth parameters (plant height, panicle weight, number of grains, biomass yield), grain yield over control in the wheat plant, with *Trichoderma virens and T.viridi* (Kumar and Singh 2018; Mahato et al. 2018). Thus, this *Trichoderma*-enriched biofertilizer may be applied as an alternative to chemical fertilizers and pesticides that causes environmental pollution. Therefore, usage of plant growth and development promoting *Trichoderma* sp. based biofertilizers may be supportive in the sustainable and ecofriendly agriculture without causing any negative effects over health of environment and living beings.

1.3.3 Possible mechanism of biofertilizers

Several possible mechanisms have been given to explain different reasons involved for the improvement in the growth and productivity. The mechanisms involved may be categories into two i.e. direct and indirect mechanisms. Direct mechanism involves mineral solubilization and enhanced plant nutrient uptake (Hajieghrari and

Momammadi 2016). Nitrate, phosphate and zinc solubilization are important processes that promote the plant growth in nitrogen and phosphorus deficient or in complex form in soils. Biofertilizers used to breakdown or convert insoluble or complex forms of essential nutrients specially N, P, K and Zn into soluble forms by different organic acids i.e. malic acid, acetic acid, oxalic acid, citric acid and gluconic acid produced (Mukhtar et al. 2017). This process eases uptake of the vital nutrients and enhances growth and productivity of corps. Nitrogen fixing microorganisms form symbiotic association with plants are usually present in biofertilizers and help in biological nitrogen fixation and transform N to organic forms. These microorganisms are recognized as "diazotrophs," and their roles are represented in Table 1.4. Whereas, indirect mechanisms involve siderophores and phytohormones (IAA, cytokinins, gibberellins) production that help in different metabolic activities like photosynthesis, nutrient uptake and transportation, respiration and transpiration etc. which result in improved plant growth (Abbasi et al. 2011; Lavakush et al. 2014). Additionally, production of antibiotics, exhaustion of rhizosphere iron, production of antifungal metabolites, fungal cells wall lysis enzymes and induce struggle for space in roots and, induced systemic resistance to the plants which aid in enhancement plant (Glick et al. 1999; Abbasi et al. 2011). A brief detail of possible mechanism has been summarized in Table 1.4.

Table 1.4 Mechanism of bio-fertilizers and theirs significance in growth promotion

Mechanism	Microorganisms	Significances	References
Mineral Solubilization	*Pseudomonas* sp., *Bacillus* sp.; *Rhizobium* sp., *Herbspirillum* sp., *Azolla* sp., *Azotobacter* etc.	Production of organic acids (e.g. carboxylic) that lowers pH in of the rhizosphere and subsequently solubilize complex forms of phosphate, nitrate, Zn etc.	Panhwar et al. (2011); Mohammadi and Sohrabi (2012); Kuan et al. (2016).
Siderophore production	*Trichoderma sp. e.g. T. viride, T. virens, T. harzianum; Peudomonas sp.,* e.g. *P. fluorescens, P. striata, Bacillus coagulans,* Brevibacillus brevis; *Enterobacter* sp. etc.	These are microbial iron chelating compounds with low molecular weight that help in increased Fe^{3+} supply to plants and lower the competency of other pathogenic microbes to survive.	Gupta and Gopal (2008); Angel et al. (2016)
Phyto-hormone production	*Acetobacter diazotrophicus*; *Herbaspirillum seropedicae*; *Azospirillum lipoferu*; *Trichoderma* sp., *Pseudomonas* sp. *P. putida, P. fluorescens* etc.	Increased production of phytohormones e.g. Indole-3-acetic acid (IAA), Zeatin and ethylene, Gibberellic acid (GA_3) and Abscisic acid (ABA) that enhances root, shoot length, biomass etc. and thus productivity	Lee et al. (2012); Waadt et al. (2015); Kumar and Singh (2018)
Bio-control agent	*Trichoderma* sp. e.g. *T. harzianum, T. viride* etc.; *Pseudomonas fluorescens*; *Bacillus* sp. e.g. *B. pumilus, B.s subtilis* etc.	These defend plants in contradiction of pathogenic diseases by direct antagonistic interactions between bio-control agents and pathogens and also induce host resistance.	Hasan et al. (2012); Triveni et al. (2013); Kowalska et al. (2014); Ng et al. (2015)
Enzyme production	*Pseudomonas* sp., *Bacilus* sp., *Xanthomonas* sp., *Agrobacterium* sp. etc.	Catalases, H_2O_2, ascorbate peroxidases (APX), peroxiredoxins (PRX), glutathione eroxidases (GPX), and glutathione S-transferases (GST) etc. enzymes are induced which regulates various metabolic activities of plants.	Iqbal et al. (2006); Ghodsalavi et al. (2013); Sofo et al. (2015)

1.4 Application of PGPR and PGPF to enhance Wheat (*Triticum aestivum* L.) productivity

Wheat (*Triticum aestivum*) is the foremost and major food crop and in cultivated all across the world in a huge area and provides 20% of daily dietary protein and calories to about 4.5 billion people (WHO 2018). It not only provides food security but also economic safety to the farmers who depend on it for their earnings as it is top most cereal in world trades. As an estimate, it is cultivated in 120 countries of the world including maximum parts of India. In India, wheat cultivation started 5000 years ago and at the current time India stood second after China in total production of wheat. Indian share in global wheat production was recorded to be about 15.36 percent (Fig. 1.1). About 30 million hectare of Indian agricultural land is cultivated by wheat crop in the year 2017-18 (Department of Agriculture, Cooperation & Farmers Welfare 2017-2018, Directorate of Economics & Statistics 2018). According to International Grains Council as an estimate total wheat production of India was about 758 million metric tons in 2017-18 as presented in Fig. 1.2 (Commodity Profile of Wheat for July 2018). Further, Uttar Pradesh is the chief producer of wheat trailed by Punjab, Haryana, Madhya Pradesh and Rajasthan in India. The nutrients content of wheat grains include carbohydrate (70%), protein (12%), lipid (2%), vitamins and minerals (2%) and raw fiber (2%) (Arnarson 2015).

The present scenario needs sustainable, eco-friendly and cost effective agricultural practices to enhance the productivity of major food crops including wheat to fulfill the nutritional requirements of intensively growing population. Application of biofertilizers including various rhizospheric microbes e.g. *Azotobacter chroococcum*, *Azotobacter*, *Azospirillum* and *Basillu*s sp. have emerged as best alternative in regards to such requirements. Biofertilizers have been reported to enhance wheat productivity by providing essential nutrients (Kandil et al. 2011; Bhattacharyya and Jha 2012). These microbial populations have the potential to enhance the productivity by different mechanism including mineral solubilization, N fixation, production of phytohormones, siderophores, etc. with antagonist activities and biocontrol to soil born phytpathogens (Defez et al. 2017a,b; Zhao et al. 2018).

Wheat belongs to family Poaceae (Gramineae) and tribe Triticeae which contains various grasses that are economically important like barley, rye etc. Wheat is a grass that is cultivated all over the world for its grains that are one of the major staple crops among other cereals e.g. rice (*Oryza sativa* L.), barley (*Hordeumvulgare* L.), gram (*Cicer arietinum* L.), maize (*Zea mays* L.), oat (*Avena sativa* L.), rye (*Secalecereale* L.) etc. The nutritional values of wheat grains depend upon the climatic conditions including soil type and qualities, temperature etc. On an average, it contains about 70-75% carbohydrate, 12% water and protein, 2 percent fat, 1.8 percent minerals, and 2.2 percent crude fibers including Thiamin, iboflavin, and minor volumes of vitamin A also (Encyclopædia Britannica, 2016). A summary of nutritional values of wheat have been given in Table 1.5.

Table 1.5 Nutritional values of 100 g of wheat (*Triticum aestivum* L.) grains (Source- Arnarson 2015)

Nutrients	Values
Energy	340 K Cal
Water	11.0 %
Protein	13.20 g
Carbs	72.0 g
Sugar	0.40 g
Fiber	10.70 g
Fat	2.50 g
Saturated Fat	0.43 g
Monounsaturated	0.28 g
Polyunsaturated	1.17 g
Omega-3	0.07 g
Omega-6	1.09 g
Trans fat	-

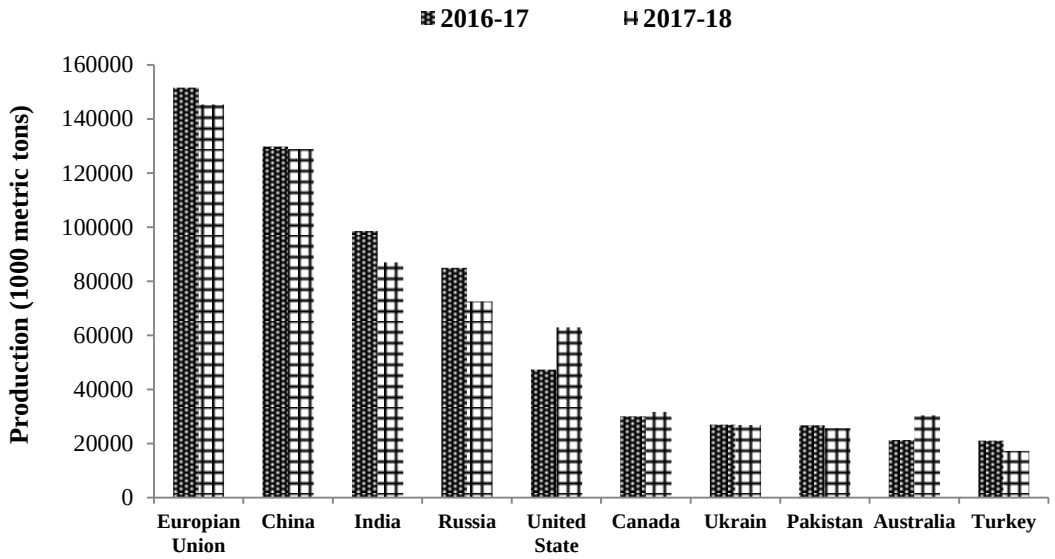

Fig. 1.1 Top ten worldwide wheat (*Triticum aestivum*) producers during 2016/2017 and 2017/2018 (in 1,000 metric tons)

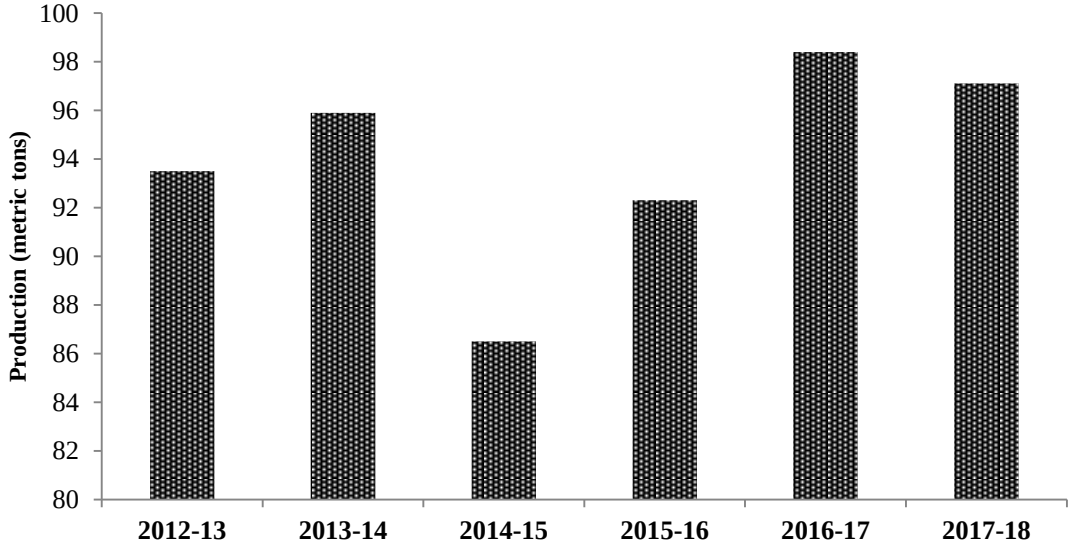

Fig. 1.2 Total wheat (*Triticum aestivum*) productions in India during 2012-2017

Data of 2017-18 is an estimation (Source = agricoop.gov.in and DES=Directorate of Economics & Statistics)

Although biofertilizers have been studies for its efficacy with different plants including wheat, but, its performance was not found to be parallel with that of chemical fertilizers. The best suited optimum dosages of different biofertilizers in different agro-climatic zones should also be considered before selection of the biofertilizers. Further, one of the most challenging parts of its application is the selection of suitable carrier. The biofertilizers are normally prepared with carrier

based inoculants and peat is the most commonly used as carrier (Hong-yuan et al. 2015). Other carriers include coal, clays, inorganic soil, organic substances e.g. composts, soybean meal, wheat bran, sawdust, etc., or vermiculite, perlite, kaolin, bentonite, silicates etc. (Smith 1992). These carriers should be easy to handle and inexpensive so that can be practically used by the farmers of lower economic background with reasonable and adequate shelf life of as a minimum of two or three months for commercial applications, at room temperature (Malusá et al. 2012). It should support microorganism growth and endurance, and must help in easily release of microorganisms into the soil. The microbial biofertilizers with efficient carriers should have a high moisture absorption capacity, proper aeration and good buffering capacity (Rivera-Cruz et al. 2008). The problems of lower efficacy of microbial biofertilizers can be overcome by isolating and characterizing new microbial strain and optimizing dose of PGPR for different agro-climatic conditions with suitable carriers. The present study was done to meet these challenges in the field of application of biofertilizers for need of bio- prospecting high efficacy PGPRs that enhance the productivity of wheat, a major food crop. More precisely, following specific objectives were drawn for the present study-

1) Isolation and characterization of *Trichoderma* spp. and plant growth promoting rhizobacteria (PGPR) from various agricultural soils.

2) Evaluation of plant growth promoting activities of the different isolates of *Trichoderma* and PGPR and its similar commercially available products in pot conditions.

3) Evaluation of best performing isolates in different combinations for growth and productivity of wheat in earthen pots.

4) Studies on availability of nutrients in soil and its distribution in plant parts during application of *Trichoderma* and PGPR.

5) Efficacy of best performing formulation of *Trichoderma* and PGPR will be studied on growth and yield of Wheat (*Triticum aestivum* L.) in experimental plots.

6) Estimation of cost benefit analysis of newly developed eco-friendly consortia vis-a-vis conventional chemical fertilizers

Chapter 2

Review of Literatures

2. Review of Literatures

The world's population is increasing gradually and is about to reach to the numeric value of 9.8 billion in 2050 (United Nations 2017). Urbanization, industrialization, development and constructional activities have been intensified in the past few decades to meet the needs of rapidly growing population. The activities have escalated the problem of contamination of the environment systems including water, air and soil (Malusá et al. 2012). Soil is a substrate for establishment of civilization, sustaining whole biome, contains water in it and maintains the proper functioning of the whole environment (Kumar et al. 2017a; Meena et al. 2017). Soil pollution is the build-up of toxic and harmful substances, chemicals, salts, radioactive materials or disease causing pathogens that adversely affects growth and development of plant communities and cause threat to the lives of living organisms. The primary sources of soil pollution are anthropogenic and are categories into two types *viz.* industrial and agricultural pollution. As the names suggest, industrial pollution is caused by the dumping off industrial solid and liquid wastes directly on the soil surfaces with no or very insignificant primary, secondary and tertiary treatment of wastes, and agricultural pollution is caused by the usages of chemical fertilizers, pesticides, herbicides, fungicides, insecticides etc. to enhance the productivity (Azizullah et al. 2011). According to Cevallos et al. (2015) in upcoming years, the consumption of NPK fertilizers will be increased to such an extent that will cause treat to the health of environment. Application of these agro-chemicals usually improved crop yield but over fertilization has degraded soil quality of single crop system (Lenka et al. 2013). These chemicals are very reactive to the ambient environment and have the potential to contaminate the food chain (Barkat 2011). Plants are the primary producers and pollutants may enter in the plant system by different mechanisms of transportation of food and water by plant cells (xylem and phloem). Here, these pollutants may be accumulated and transferred to next tropic levels of herbivores and omnivores and may finally reach to the top most trophic level that contain human beings and other higher vertebrates. In the fatty cells of living beings, the pollutants may persist for longer duration and may cause various health related. Some of the pollutants *e.g.* nitrate, phosphate, heavy metals (lead, chromium, mercury, arsenic etc.), persistent organic pollutants (POPs), volatile organic compounds (VOCs) are largely used in these chemical substance that have the potential to get absorb by the soil and leach in

to the ground water or run off may pollute the surface water (Zhao et al. 2015; Kantachote et al. 2016). In addition to this, the volatile compound may pollute the ambient air. Thus, these chemicals work as a potent source of soil, air and water pollution in spite of their ability of provide essential nutrients like N, P, K, Zn, Fe, Ca, Mg etc. to the plants (Zhao et al. 2015; Poonam et al. 2018).

2.1 Consequences of chemicals fertilizers on soil quality

Application of chemical fertilizers in agricultural practices is a very common approach of farmers as these provide essential nutrients to the soil (Song and Liu 2015). According to FAO (United Nations Organization for the Fed and the Agriculture) and IFA (International Association of the Fertilizer Industry) about 150 million tons of nutrients were consumed during 2001to 2002 that utilized about 91, 36 and 23 million tons of nitrogen, phosphorus and potassium, respectively (Cevallos et al. 2015). The excessive usage of NPK chemical fertilizers in modern agriculture have become an integral part in erroneous believes of enhancement in the productivity (Azizullah et al. 2011; Meena et al. 2017). The use of these soil amendments has not only been significantly intensified but there are widespread negligence of their harmful impacts and indiscreet applications in the agriculture (Shaharoona et al. 2008). NPK are the primary nutrients also known as 'macro-nutrients' and are required for various metabolic and physiological activities like photosynthesis, cell division, transportation of food material etc. that help in enhancing productivity of crops (Mahanta et al. 2014; Mukhtar et al. 2017; Yao et al. 2018). Whereas, secondary nutrients also called as 'micro-nutrients' like calcium, magnesium, and sulfur and few trace elements e.g. copper, chlorine, iron, manganese, molybdenum, zinc etc. are also required by plants for different physiological and metabolic activities (Bhattacharyya and Jha 2012). The modern agricultural practices utilize fertilizers, pesticides, fungicides and insecticides to artificially fulfill the nutritional requirements of crops with the primary objective of enhancement in the productivity (Kantachote et al. 2016). There is no doubt that application of chemical fertilizers increased the productivity of crop, but initially the negative impacts of using such NPK fertilizers and other chemicals to the environment were neither recognized nor fully understood by the governments and also by farmers. Present time they are familiar with the negative impacts of these on the environment. But, it is unfortunate

that farmers are still using conventional fertilizer for over production to gain profits. According to Chatterjee et al. (2017) with the arrival of 'green revolution' in 1960, use of these chemicals increased rapidly to ensure high yielding and disease resistant crop varieties. These practices were not only responsible for improving the productivity but also major source of environmental degradation and contamination of its aspects (Li et al. 2018). Air, water and soil are interlinked and their quality depends largely upon the quality of soil as all kinds of pollution originate on the land or soil. So, the maintenance of soil quality is essential for sustaining lives on the planet Earth. In the past few decades, the usage of chemicals in the agriculture has raised to enhance the food productivity to meet the nutritional requirements of increasing population as well as to give the financial profit to the farmers and at the end to the country in the form of export of food materials (Cevallos et al. 2015; Hong-yuan et al. 2015). The conventional fertilizers often contain ammonium nitrate (NH_4NO_3), amide in urea $\{CO(NH_2)_2\}$, phosphorus in diammonium phosphate $\{NH_4)_2HPO_4\}$ and potassium as K_2O. Further, arsenic (As), lead (Pb) and cadmium (Cd) are found to be present in traces in minerals phosphate rocks which are transferred to super phosphate fertilizer. These heavy metals are non-biodegradable and are accumulated into soil by excessive usage of these fertilizers which becomes an indestructible poison for crops and animals feeding on it. Besides, unmindful and excessive usages of NPK fertilizers have also been found to reduce quantity of vegetables and crops, protein, carbohydrate and mineral contents of crops grown on soil over the years (Marles 2017). According Fageria (2014) that about 40-70% N, 80-90% P, and 50-70% K of the total applied conventional chemical fertilizers used to lose in the environment due to variations in the different soil properties. The global expenditure of NPK fertilizers in 2010-11 was found to be about 104, 41 and 28 Mt and predicted that about 115. 46 and 33 Mt of NPK will be utilized in 2016-2017 (Meena et al. 2017). The details of total amount of NPK fertilizers used by farmers across the globe have been summarized in Table 2.1.

Table 2.1 Total amount of NPK fertilizers used by farmers across the globe (Source- Meena et al. 2017)

Country	NPK (kg ha^{-1})
India	156.1
Pakistan	204.9
Bangladesh	188.3
China	396.0
Korean Republic	284.0
Egypt	375.0
Sri Lanka	122.1
Indonesia	101.0
U.S.A	114.0
World average	107.0

The most important consequences of using chemical fertilizers include direct or indirect contamination of air, water and soil by nitrification, acidification, eutrophication, volatilization etc. which ultimately end up in the increase in greenhouse gases (NO_x, SO_x, CO etc.) and global temperature i.e. global warming (Gu et al. 2015). Additionally contamination food chain is also a major problem that may adversely affect the health of living beings due to tendency of these pollutants to get bio-accumulated into fatty tissues. A brief summary of these consequences have given below-

2.1.1 Leaching, soil and ground water pollution

The fertilizers and chemicals used by farmers in modern agriculture contain substances like chemicals *e.g.* nitrate, phosphate, heavy metals like cadmium (Cd), arsenic (As), lead (Pb), mercury (Hg), copper (Cu), nickel (Ni) etc., and natural radionuclide like uranium ($_{238}U$), thorium ($_{232}Th$), polonium ($_{210}Po$) etc., which are toxic to living beings (Savci 2012; Jabloun et al. 2015; Wang et al. 2016). The long term applications of chemicals facilitate these chemicals (nitrate, phosphate, heavy metals etc.) to leach out into the soil profile and possibly infiltrate into ground water (Radersma and Smit 2011; Jia et al. 2014; Cevallos et al. 2015). Leaching is the removal of soluble substances from soil by water percolating inside the soil and reaching the ground water table (Ghiberto et al. 2015). Leaching of agro-chemicals

depends upon the applied dose, time of application, solubility of fertilizer and other chemicals, water regime etc. (Blum et al. 2013; Wang et al. 2016). Consumption of such contaminated ground water may pose risk to human beings form various diseases like methemoglobinemia or 'blue baby syndrome' caused by intake of nitrate, black foot disease by arsenic, itai itai by cadmium (Cd) etc. (Basso and Ritchie 2005). Further, loss of ions like $Ca^{2+}$and Mg^{2+} have the potential to alter the pH of soil causing acidification or alkalinity of soil that adversely affect microbial and plant communities (Ghiberto et al. 2015). It also reduces soil health and texture by degrading the available nutrients and makes it unsuitable for further cultivation or reduces the yield and productivity of the soil. Thus it becomes necessary to stop the usage of chemicals in the agricultural practices to ensure the survival of flora and fauna present on the earth either by creating social awareness or by imposing judicial restrictions (Bhatt et al. 2016). Previous studies of some researchers like Huang et al. (2015), Jabloun et al. (2015), Huang et al. (2017), Li et al. (2018) and many more have reported the leaching losses of nutrients by utilization of chemical or commercial fertilizers for different crops. The details of those studies have been given in Table 2.2.

2.1.2 Agricultural runoff and surface water pollution

The run off from the agricultural fields during heavy rain or flood contain chemicals (nitrate, ammonium, phosphate, calcium, magnesium, heavy metals, radionuclides etc.) used in the agriculture for intensifying the productivity (Gu et al. 2015). The main pollutants present in surface run off are N and P that are essential macro nutrients. The run off reaches to the nearby water bodies like ponds, lakes, rivers etc. and promote the growth of aquatic weeds and macrophytes etc. causing eutrophication (Vinod et al. 2015; Dari et al. 2017; Hua et al. 2017). Such pollution deteriorates the quality of aquatic systems and harms the lives of fishes and other aquatic creatures (Palanivelu et al. 2005). Previous studies of Zhao et al. (2014), Zhou and Bahl (2014), Jabloun et al. (2015), Huang et al. (2017), Li et al. (2018) and others have reported run off losses of different nutrients by application of chemical or commercial fertilizers for various crops. The details of those studies have been given in Table 2.2.

Table 2.2 Leaching and runoff losses of nutrients from the agricultural fields on application of commercial chemical fertilizers

Crops	Fertilizers	Leaching and run off losses of nutrients	References
Oryza sativa	165 kg N ha^{-1}	Nitrogen losses by NH_4^+ volatization	Li et al. (2018)
Zea mays	38-60 kg N ha^{-1}	NO_3^- leaching	Huang et al. (2017)
Oryza sativa	202-257 kg ha^{-1}	Nitrate leeching	Huang et al. (2015)
Triticum aestivum	25-135 kg N ha^{-1}	NO_3-N	Jabloun et al. (2015)
Triticum aestivum	120-150 kg N ha^{-1}	Nitrogen	Lehtonen and Rankinen (2015)
Triticum aestivum	180 kg N ha^{-1}	N leaching	Li-min et al. (2015)
Oryza sativa L.	Ammonium sulfate 300 kg N ha^{-1}	NH_4^+, NO_3^- leaching	Zhao et al. (2014)
Zea mays L.	20-178 kg N ha^{-1}	NO_3^- leaching	Zhou and Bahl (2014)
Zea mays L.	Urea 250 kg N ha^{-1}	NO_3^- leaching	Sanz-Cobena et al. (2012)
Zea mays L.	309-642 kg N ha^{-1}	NO_3^- leaching	Perego et al. (2012)

2.1.3 Volatilization, emission and air pollution

The chemical fertilizers contain volatile compounds containing alcoholic, ketonic, aldehydic groups that are emitted after their application in the agricultural fields (Insam and Seewald 2010). Volatilization of nitrogen in the form of ammonium & nitrous oxide (N_2O) and emission of different oxides of nitrogen and sulfur (NO_x and SO_x) cause air pollution (Cevallos et al. 2015; Fracetto et al. 2017). Emission of these chemicals especially NO_x and SO_x occur directly from agricultural fields or indirectly when N compounds and predominantly leached nitrate (NO_3^-) and volatilized ammonia (NH_3), are consequently converted into nitrous oxide (N_2O). Although, N fertilization is a necessary substrate for microbial nitrification and denitrification processes, excess usage of N generally lead to greater cumulative N_2O emissions (Zhou et al. 2014). According to Aguilera et al. (2013) the emissions from agricultural practices contain about 60% of worldwide anthropogenic N_2O emissions. Further, Oertel et al. (2016) reported that use of chemical N fertilizers like urea and

ammonium sulphate cause higher emission of N_2O under aerobic and saturated conditions of soil. Whereas, Syakila and Kroeze (2011) reported that about 23-31 % of all global N_2O emissions of about4.3-5.8 Tg N_2O-N yr^{-1} was caused by application synthetic N fertilizers in agricultural practices and improper manure management. The details of emission losses have been illustrated in Table 2.3-

Table 2.3 Emission losses from the agricultural fields on application of conventional chemical fertilizers

Fertilizers used	Emission Losses	Emission rate	References
Urea 120 kg N ha^{-1}	N_2O emission	231 kg CO_2 eq. ha^{-1}	Bhatia et al. (2010)
101-170 kg N ha^{-1}	N_2O emission	63-137 mg N m^{-2}	Chirinda et al. (2010)
120 kg N ha^{-1} Urea	N_2O	2.91 kg-N ha^{-1}	Behnke et al. (2011)
170 kg N ha^{-1} of ammonium nitrate and urea	N_2O emission	267- 810 g N ha^{-1}	Gu et al. (2011)
135 kg N ha^{-1} Urea	N_2O	2.5 kg-N ha^{-1}	Hoben et al. (2011)
Urea, diammonium phosphate and potassium sulphate (390-60-30 kg N-P-K kg ha^{-1})	N_2O and NO emission	1.5-1.6 and 1.9 (N_2O) and 0.8 and 1.8 (NO) kg N ha^{-1} for wheat and maize, respectively	Liu et al. (2011)
N- fixation- N besides dry atmospheric deposition (6-7 kg^{-1} ha^{-1} yr^{-1}, Liquid organic fertilizers (84 kg N ha^{-1})	N_2O emission	1-2% of total N output	Nylinder et al. (2011)
>80 N_2O %, >95 % NO and ~84% NH_3	N_2O, NO and NH_3	412 kg ha^{-1} N: P_2O_5: K_2O¼ 24%: 12%: 6%, 150 kg ha^{-1} Urea	Zhang et al. (2011)
4.0 and 3.0 kg ha^{-1} yr^{-1}	N_2O and NO	270 kg N ha^{-1}, 105 kg P_2O_5 ha^{-1}& 60 kg K_2O ha^{-1} (NPK)	Cui et al. (2012)
150 kg urea N ha^{-1}	CO_2-C, N_2O-N and CH_4-C	321, 0.258 and 0.096 µg m^{-2} ha^{-1}	Ruíz-Valdiviezo et al. (2013)
Organic and synthetic fertilizers	N_2O and CH_4	1.15 and 347.60 kg ha^{-1}	Aguilera et al. (2015)
Urea fertilizer	Greenhouse gas emission	2,155,691 tonnes CO_2-eq (cereals) and 200,137 tonnes CO_2-eq (legumes and oilseeds)	Tongwane et al. (2016)

2.1.4 Greenhouse gases and global warming

Unmindful and intensified usage of chemicals e.g. fertilizers, pesticides, herbicides, insecticides in agriculture sector has led severe environmental problem of greenhouse gases (GHGs) emission and global warming (Denman et al.2007; Wijesinghe et al. 2011; Zhou et al. 2014; Bin-feng et al. 2016). Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) are the most important GHGs that are totally associated modern agricultural practices (Snyder et al. 2009). According to FAO (2014) about 17 Mio. km^2 or 12.6 % of worldwide land surfaces are covered with arable lands under everlasting crops. Deforestation for the purpose of agriculture and application of chemical fertilizers causes global warming by direct and indirect GHG emissions (Oertel et al. 2016). Green houses gases (CO_2, CO, CH_4, NO_x, and SO_x) emitted by these chemicals may increase the ambient temperature which leads to global warming (Savci 2012; Huang et al. 2015; Bin-feng et al. 2016). Although global warming is a natural process and essential for sustaining life on the earth but in last two decades vehicular and industrial emission is increases many fold significantly increases the greenhouse effect (Fracetto et al. 2017). According to Charles et al. (2017) N_2O is a greenhouse gas with a global warming potential of 298 times that of CO_2 and nitric oxides (NO) that contribute significantly to the depletion of ozone (O_3) in the stratosphere, which poses a threat to human life, since it contributes approximately 6% in current greenhouse effect (Ravishankara et al. 2009; Senbayram et al. 2012). Bin-feng et al. (2016) have observed 78% increase in global warming potential of CH_4 and N_2O gases due to application of nitrogen (N) fertilizer in rice-paddy crops in China.

2.1.5 Food chain contamination and bioaccumulation

The inorganic and organic chemicals present in agro-chemicals have the potential to get accumulated in soil and plant system. Plants absorb these chemicals through the soil which may enter the food chain through bioaccumulation (Wijesinghe et al. 2011; Savci 2012). These contaminants get absorbed into the fatty tissues of living organisms and causes adverse health effects (Barkat 2011).

Moreover, crops grown on over fertilized or nourished soil are more prone to outbreak of insects and diseases. In this regard, the main objective is to minimize the

application of chemicals in the agricultural practices and develop such an alternative that not only provide high yielding but also cost effective that sustain the quality of soil. The use of organic compost, green manures and microbial fertilizers considered as an effective alternatives to conventional organic fertilizers and has been successfully applied by several researchers in the past (Kumar et al. 2014; Kantachote et al. 2016; Mukhtar et al. 2017; Mensah et al. 2018; Yao et al. 2018).

2.2 Biofertilizers

Maintaining food security for ever growing population in an ecofriendly and cost effective way has generated a great deal of interest in the use of bio fertilizers in agricultural practices over the last decades (Al-Tawei et al. 2010; Mukhtar et al. 2017). In the past, origin of biofertilizers was initiated with the discovery of "Nitragin" by Nobble & Hiltner in 1895, which was a laboratory culture of *Rhizobium*, trailed by the discovery of *Azotobacter*, cyanobacteria and some other microorganisms (Chatterjee et al. 2017; Singh et al. 2019). Biofertilizers comprise of different microorganisms like genera of bacteria, fungi, blue green algae (cyanobacteria), as well as their metabolites to be used as fertilizers (Mensah et al. 2018). These may be defined as artificially manufactured cultures of soil microbes or soil inoculants to increase richness, fertility and efficiency of soil and plants to enhance productivity (Singh et al. 2019). When bio fertilizers applied to the soil, they colonize in the rhizosphere and stimulate growth by accelerating the availability of essential nutrients to the host plant (Chatterjee et al. 2017). These biological agents also improve the physicochemical and biological characteristics of soil and also ensure the availability of nutrients to the plants (Tejada et al. 2016). The fertility of soil largely depends on the interactions of the microbial communities in the soil system around the rhizosphere (Li et al. 2016; Meena et al. 2017). These microbes actively contribute toward nutrient uptake, solubilization, mobilization and mineralization that help in up-surging plant growth and suppression of diseases (Abbasi et al. 2011; Sharma et al. 2012; Nath et al. 2017). Plant growth promotion (PGP) activity, nutrient solubilization, siderophore production, immune intonation, signal transduction and pest control are the most common processes oriented by microbial communities present in the soil (Chatterjee et al. 2017; Meena et al. 2017; Saritha et al. 2019). These processes are responsible for enhancing the growth and

productivity of crops. Depending upon these qualities, microbial biofertilizers have been used for enhancing the productivity of various economically important major food crops like wheat, rice, barley, maize, potato etc. (Mukhtar et al. 2016; Kumar and Singh 2018; Munda et al. 2018; Yao et al. 2018). In addition, the application of slow-release or controlled fertilizers or biofertilizers reduces N_2O emissions having potential to enhance the levels of secondary pollutants in atmosphere which ultimately increases global warming (Oertel et al. 2016). Thus, application of biofertilizers gives multiple benefits as it improves physicochemical properties of soil, inhibits environmental pollution, enhance productivity in an ecofriendly, cost effective and sustainable manner (Kowalska et al. 2014). Further, as biofertilizers contain large variety of microorganisms, so can be broadly categorized into following three kinds-

2.2.1 Plant growth promoting rhizobacteria (PGPR)

Firstly, Kloepper and Schroth (1978) have given the term 'rhizobacteria' to those soil bacteria that competitively colonized in plant roots and promoted growth and development of host plants with inhibition of plant diseases. Later in 1980, Kloepper and Schroth used the term plant growth-promoting rhizobacteria (PGPR) for them (Kloepper et al. 1980). Now, the perception of PGPR has restricted to different bacterial species that fulfill at least two of the three criteria of violent colonization, plant growth promotion and bio-control (Bhattacharyya and Jha 2012). Depending upon existence and their relationship with host plants PGPRs may be either rhizospheric or endophytic. The rhizospheric PRPRs colonize in the intercellular spaces of roots of plants whereas; endophytic PGPRs colonize in the apoplastic spaces inside host plants (Bhattacharyya and Jha 2012). PGPR contain nitrogen fixing and symbiotic bacteria viz. *Azospirillum*, *Azotobacter*, *Mycobacterium*, *Bacillus*, *Azobacter*, *Serratia*, *Xanthomonas*, *Proteus*, *Pseudomonas*, *Clostridium* etc. (Abbasi et al. 2011; Cortivo et al. 2017). These microbes fix atmospheric nitrogen into the organic forms and make it available to the plants. These bacteria used to colonize in the rhizospheric region and help in the plant growth promotion by various activities e.g. nitrogen fixation, siderophore production, phosphate solubilization, IAA production, enhancement in resistance towards biotic and abiotic stresses , 1-Aminocyclopropane-1-carbxylate deaminase (ACC), quorum sensing (QS) signal interference, suppression diseases etc. (Elekhtyar 2015; Cortivo et al. 2017; Kumar et

al. 2018). The benefits and reasons for applying PGPR as biofertilizers have been presented in Fig. 2.1.

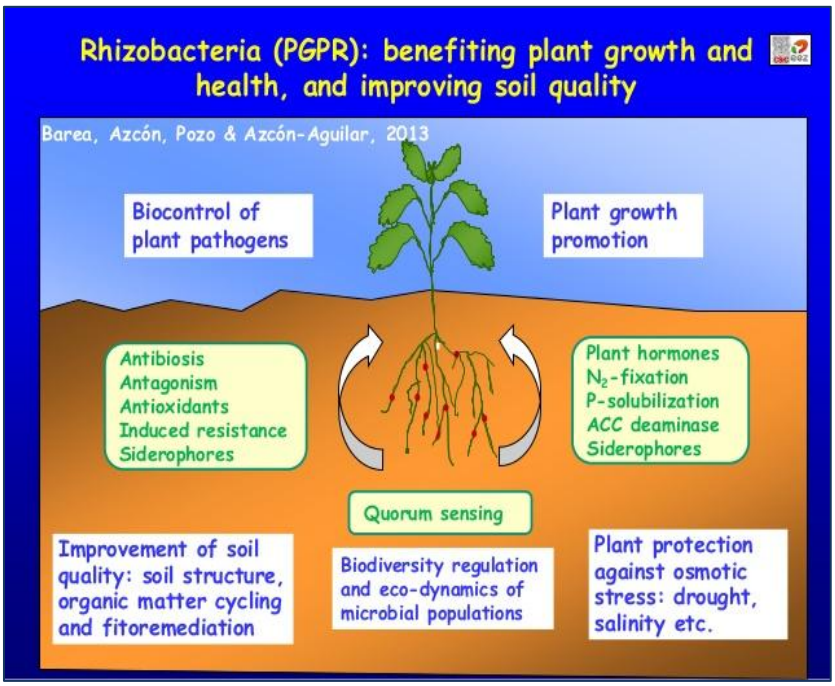

Fig 2.1 Benefits of plant growth promoting rhizobacteria (PGPR) (Source: Barea et al. 2013)

2.2.2 Plant growth promoting fungi (PGPF)

These comprise of various fungi e.g. different species of *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, etc. These fungi establish symbiotic relationships with the plants roots in the form of mycorrhiza and aid in the absorption of essential nutrients from soil and provide it to plants which stimulate growth and development of plants (Hossain et al. 2017; Mensah et al. 2018).

2.2.3 Cynobacteria and other algae

Cyanobacteria are also known as 'blue green algae (BGA)' and examples contain various species of *Oscillatoria*, *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira* etc. These cyanobacteria also help in nitrogen fixation and forms symbiotic association with aquatic fern *Azolla* that helps in enrichment of soil with nutrients. Apart from BGA, red and brown algae have also been used as probable biofertilizers (Chatterjee et al. 2017). These have the potential to enhance soil porosity by their filamentous structure and production of adhesives, secretion of phyto-hormones (auxin, gibberellin), vitamins, and amino acids (Rodriguez et al. 2006; Sahu et al. 2012). Further, these

increases soil nutrients contents by their death and decomposition and also prevents invasion of weeds and pathogens. All these activities help in the enhancement of growth and development of plants and ultimately the productivity (Chatterjee et al. 2017).

2.3 Mechanisms of plant growth promotion by biofertilizers

Plant roots secrete various exudates that contain larger amounts of carbohydrates, lipids, and amino acids which attract rhizospheric microbes to colonize the plant roots (Haldar and Sengupta). These microbes exhibits various mechanisms that directly or indirectly helps in the promotion or enhancement in growth and development of crop plants and end up with increase in the crop productivity (Glick 1995). Direct mechanisms are those that are associated with synthesis of substances by the microbes or help in uptake of nutrients (Elekhtyar 2015). These include phosphate solubilization, nitrogen fixation, production of siderophore, HCN, ammonia, vitamins, and phytohormones (Abbasi et al. 2011; Song and Liu 2015; Cortivo et al. 2017; Mukhtar et al. 2017). Whereas indirect mechanisms are those that do not directly related with improvement of growth but synthesize various inorganic and organic compounds by various mechanisms like secretion of antibiotics, cell wall damaging enzymes etc. (Bhattacharyya and Jha 2012 et al. 2017; Florencio et al. 2012). The outline of these mechanisms has been presented in Fig. 2.3 and described below-

2.3.1 Biological Nitrogen Fixation

Nitrogen is seventh most abundant element on the earth and become a limiting factor due to losses from agricultural fields as run off and leaching (Elekhtyar 2015). N is the constituent of chlorophyll and protein and thus, indispensible for photosynthesis as well as reproduction, growth and development plants (Saritha and Tollamadugu 2019). It is normally found to be present in non-soluble inorganic forms. Microbes present in the biofertilizers convert gaseous N into soluble forms and make it available to the plants in the organic forms especially in the form of nitrates (NO_3^-) and ammonium (NH_4^+). This conversion of atmospheric nitrogen in ammonium and nitrates in termed as 'nitrogen fixation'. Different species of *Azotobacter* spp., *Rhizobium* spp., *Azospirillum* spp., *Acetobacter* spp. *Herbspirillum* spp., *Anabaena* spp., *Nostoc* spp., *Plectonema* spp., *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* spp., *Aspergillus* spp., *Fusarium* spp., *Trichoderma* spp. etc. are profoundly used microbes in the biofertilizers that are renowned for their potential to fix atmospheric

and N and participate in the maintaining the N cycle on the earth (Egamberdieva et al. 2010; Ahmad et al. 2013;Cortivo et al. 2017; Meena et al. 2017). The fixation of atmospheric N may be symbiotic or non-symbiotic depending upon the strain of the bacteria (Bhattacharyya and Jha 2012). Inagaki et al. (2015) reported that seed inoculation with *Herbaspirillum seropedicae* boosted nitrogen concentration in leaves of maize due to upsurge in biological nitrogen fixation by these diazotrophic bacteria. A list of various biofertilizers containing different species of PGPR and PGPF has been given in Table 2.4.

Table 2.4 Different species of PGPR with their capability to fix atmospheric nitrogen in various economically important crops

PGPR	Relationship to host	Host crops	References
Rhizobium galegae and *Pseudomonas trivialis*	Endophytic & symbiotic	Fodder galega	Egamberdieva et al. (2010)
Rhizobium leguminosarium	Endophytic & symbiotic	Faba bean, chickpea and lupine	Shaban, and El-Bramawy (2011)
Rhizobium and *Pseudomonas*	Endophytic & symbiotic	Mung bean	Ahmad et al. (2013)
Cynobacteria (*Anabaena* spp.)	Free living & Symbiotic	Wheat	Swarnalakshmi et al. (2013)
Pseudomonas fluorescence	Endophytic & symbiotic	Rice	Elekhtyar (2015)
Acinetobacter spp. and *Bacillus* spp.	Endophytic & symbiotic	Medicinal plant *Phyllanthus amarus*	Joe et al. (2016)
Rhodopseudomonas palustris	Free living & non-symbiotic	Rice	Kantachote et al. (2016)
Azospirillum spp., *Azorhizobium* spp. and *Azoarcus* spp.	Free living & non-symbiotic and endophytic & symbiotic (*Azorhizobium*)	Wheat	Cortivo et al. (2017)
Pseudomonas spp., *Bacillus* spp. and *Acinetobacter* spp.	Free living and endophytic & Symbiotic and non-symbiotic	Mung	Kumari et al. (2018)
Azolla spp.	Free living & Symbiotic	Rice	Yao et al. (2018)

2.3.2 Phosphorus Solubilization

Phosphorus (P) is the second most vital macronutrient after nitrogen and sometimes become limiting feature due to unavailability, leaching and run off losses (Hoyos-Carvajal et al. 2009; Panda et al. 2013). P has significant importance in growth and development of plants as it is required in various pathways of photosynthesis, storage and transfer of energy, phyto-respiration etc. in the living plant cells (Mukhtar et al. 2017). Many soils are found to be deficit of P although they may have high amount of total P as reserve. This happens due to unavailability of soluble form of P i.e. phosphate as plants may take up P in organic soluble forms (Hong-yuan et al. 2015). Biofertilizers used to help in the conversion of inorganic P in organic forms by acidification, exudation of organic acids, chelation and exchange reactions and make it available to plants for its easy take up to improve plant growth and development (Richardson et al. 2009; Zhu et al. 2012; Mahanta et al. 2014; Meena et al. 2017). Different species of *Azospirillum, Azotobacter, Bacillus, Enterobacter, Burkholderia, Micrococcus, Microbacterium, Pseudomonas, Rhizobium, Serratia* etc. have been described to solubilize P significantly (Bhattacharyya and Jha 2012; Panda et al. 2013; Solangi et al. 2016). Jain et al. (2012) reported that application of *Aspergillus awamori* S29 significantly improved growth of mungbean, total P in soil and plant biomass. Inagaki et al. (2015) found that application of *Azospirillum brasilense* strain AbV5; and *Herbaspirillum seropedicae* strain SMR1 increased P solubilization and enhanced the growth of maize plants. Likewise, Hong-yuan et al. (2015) reported that *Aspergillus niger* 1107 worked as an efficient P solubilizing fungus (PSF) and enhanced growth of the Chinese cabbage plants by increasing available P content in soil. In another study Joe et al. (2016) applied *Acinetobacter* spp. and *Bacillus* spp. isolated from roots of *Phyllanthus amarus,* a medicinal plant. Both strains displayed salt tolerance and phosphate solubilizing characteristics and increased growth by higher vigor index, increase in percentage of germination, phenolic content and anti-oxidative activities as compared to uninoculated control. Likewise, Ng et al. (2015) have reported P solubilization by *Trichoderma* sp. that controlled bakanae pathogen (*Fusarium fujikuro*i) in rice. Mukhtar et al. (2017) have also found P solubilization as main mechanism involved in growth and promotion of wheat plants from bioinoculants of P solubilizing biofertilizers containing species of *Bacillus, Enterobacter* and *Virgibacillus*. The P solubilization by various ways and movement of P in the soil has been shown in Fig. 2.2.

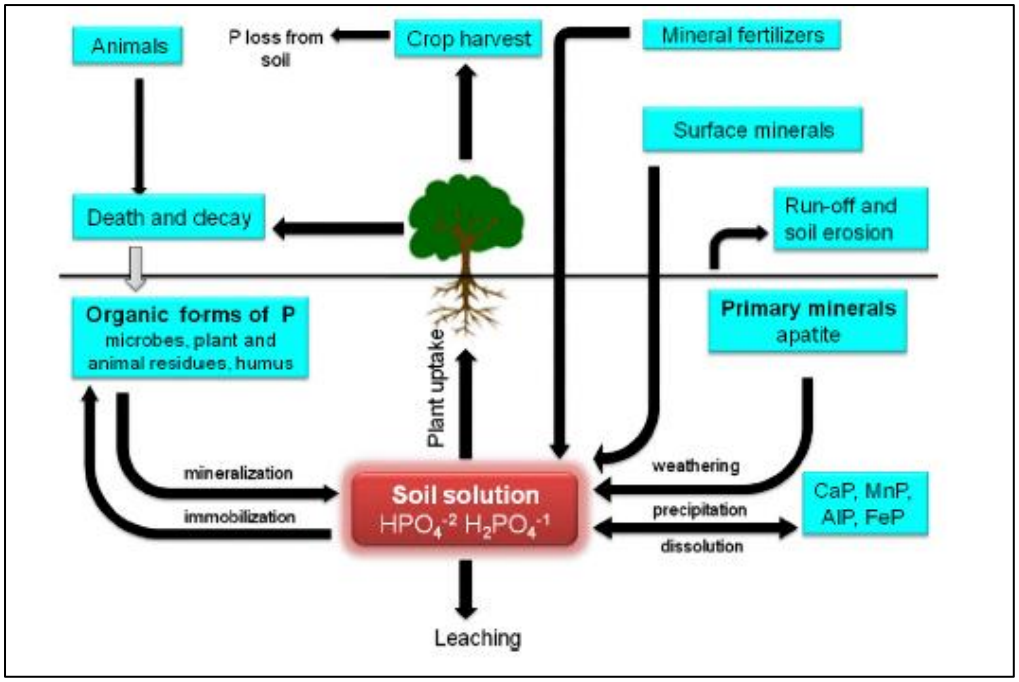

Fig. 2.2 Mechanism of phosphorus solubilization and its movement in the soil (Source: Ahemad and Kibret 2014)

2.3.3 Siderophore production

These are low molecular weight compounds which are produced under iron limiting circumstances, chelate ferric ion (Fe^{3+}) and transport it into microbial cells (Gupta and Gopal 2008). The term 'Siderophore' was firstly coined in 1973 and defined as low molecular weight molecules that have the tendency to bind ferric iron with an enormously high affinity (Bindu and Nagendra 2016). These are classified and signified by presence of ligands that chelate ferric iron and include catecholates, hydroxamates, and carboxylates (Louden et al. 2011). Iron (Fe) is an essential micronutrient and required as by plants a cofactor for proteins for their different physicochemical and metabolic activities including photosynthesis and respiration (Meena et al. 2017). Although Fe are naturally found to be present in soil at larger amounts as 4^{th} most abundant element on the earth, but are usually unavailable to plants or microbes for their uptake due to their insoluble complex forms (Ma et al., 2016). Siderophores produced by biofertilizers make it available to plants by converting into soluble and chelating form by available Fe complexes (Bhattacharyya and Jha 2012; Meena et al. 2017). Siderophores also inhibits pests and weeds by depriving these with iron nutrient and thus, help in improving growth and

development of plants and crop yield (Elekhtyar 2015). The chemical structure of siderophores retain electron rich or donor atoms like oxygen or nitrogen which have the ability to bind with metal cations (Ghavami et al. 2017). Previous studies of Bindu and Nagendra (2016); Ghavami et al. (2017), Venkat et al. (2017) etc. have showed prominent role of different siderophore producing microbial strains in growth promotion and biocontrol activity. Likewise, Bindu and Nagendra (2016) reported siderophore production by *Pseudomonas aeruginosa* isolated from paddy fields. Ghavami et al. (2017) found that *Micrococcus yunnanensis* YIM 65004 (T) and *Stenotrophomonas chelatiphaga* LPM-5 (T) isolated from rhizosphere of maize and canola plants enhanced Fe content in the soil and promoted the growth of host plants. Venkat et al. (2017) reported that *Bacillus* spp. and *Enterobacter* spp. isolated from Fe enriched produced siderophores which may play important role in medical and industrial fields.

2.3.4 Phytohormones production

Phytohormones are naturally occurring substances inside plants which regulate growth and development of plants by various physiological and metabolic activities like cell division, stem elongation, root development, activation of bud and branches, encouraging or delaying in leaf senescence, chlorophyll production etc. (Wong et al. 2015; Ma et al. 2016; Meena et al. 2017). These also act as messengers to synchronize and regulate abiotic stress responses and plant-pathogen interactions (Tsukanova et al. 2017). These are mainly comprised of auxin (indole acetic acid), cytokinin, ethylene, gibberellins, and abscisic acid (ABA), whereas, some new phytohormones are also added in this category e.g. brassinosteroids, jasmonates, and strigolactones which are supposed to help plants to endure abiotic stress (Bolimov et al. 2014; Maheshwari et al. 2015; Tsukanova et al. 2017; Singh et al. 2019). Indole acetic acid (IAA) is part of auxin and main precursor of cell division and elongation that influence growth of stem (Hoyos-Carvajal et al. 2009; Tsukanova et al. 2017). Likewise, cytokinin also activates cell division and promote growth plants by governing biosynthesis and chloroplast biogenesis (Cortleven and Schmulling 2015; Wong et al. 2015). Ethylene is the first gaseous phytoharomone that regulates the growth and senescence of plants and also helps in tolerating stress conditions (Nazar et al. 2014; Song and Liu 2015). However, excess of ethylene may cause harmful effect on the health of plants as root

curling and shortening. ACC deaminase activities of PGPRs help plant to fight with such problems and boost plant growth under environmental stresses (Cortivo et al. 2017; Elekhtyar 2015; Meena et al. 2017). Further, ABA improves plant defense mechanism and fights against different pathogens, regulates opening and closing of stomata and helps in survival of plants under biotic and/or abiotic stresses (Salomon et al. 2014; Sah et al., 2016). In addition to this, gibberellins are also an important plant growth stimulating hormones and are involved in developmental and physiological processes like seed germination, seedling emergence, floral initiation and fruit growth (Maheshwari et al. 2015). Microbes like *Pseudomonas, Rhizobacteria, Trichoderma, Azobacter, Bacillus* etc. present in different biofertilizers secrete these phytohormones and help in the growth and development of plants (Bhattacharyya and Jha 2012; Ng et al. 2015). Hayat et al. (2010) enlisted different strain of *Azospirillu*m, *Azobacter*, *Bacillus*, *Kluyvera*, *Paenibacillus*, *Pseudomonas*, *Rhizobacteria*, and *Rhizobium* potentially produced enormous amount of auxin. Salomon et al. (2014) observed that strains of *Bacillus licheniformis* Rt4M10 and *Pseudomonas fluorescens* Rt6M10 isolated from rhizosphere of *Vitis vinifera* profoundly synthesized ABA and reduced water stress on the host plants. Further, these microbes also produced IAA and gibberellin acids that also aid in the tolerance of plants for water stress conditions. Belimov et al. (2014) have also reported production of ABA by PGPR in rice fields. Kang et al. (2014) have reported secretion of gibberellic acid by *Pseudomonas putida* that helped soybean plants to cope up with saline stress and also improved plant growth. Maheshwari et al. (2015) have also described the potential of these phytohormones to inhibit the invasion of different pathogens including bacteria, virues, fungi and nematodes etc. The beneficial PGPRs are potential producer of these phytohormones that strengthened the induced systemic resistance (ISR) and systemic acquired resistance (SAR) that fight against the pathogenic and make the plants' immune system stronger (Glick et al. 1999; Abbasi et al. 2011).

2.3.5 Antibiotic and enzyme production

Catalases, ascorbate peroxidases (APX), peroxiredoxins (PRX), glutathione peroxidases (GPX), glutathione S-transferases (GST), aminocyclopropane-1-carboxylate (ACC)-deaminase etc. are the enzymes that actively participate in

metabolic and antagonistic activities of plants and microbes (Shaharoona et al. 2008; Bhattacharyya and Jha 2012). Biofertilizers containing PGPR and PGPF produce these enzymes and antibodies that regulate growth and development, as well as inhibit pathogenic activities (Cortivo et al. 2017; Singh et al. 2019). Various microbes present in biofertilizers like *Pseudomonas* spp. *Bacilllus* spp. *Streptomyces* spp. etc. have the tendency to produce 2,4-diacetylphloroglucinol (DAPG), β-1-3-glucanase, antibiotic samphisin, HCN, phenazine, tropolone, tensin, pyrrolnitrin, cyclic lipopeptides etc. (Bhattacharyya and Jha 2012; David et al. 2018; Singh et al. 2019). These substances have antibiotic, antibacterial, antifungal, antiviral, antioxidant and antitumor properties. These efficiently compete with pathogens and protect host plants from their harmful effects and helps in proper growth and development of plants. In a study, Ghodsalavi et al. (2013) reported production of protease and lipase enzymes from different PGPR species e.g. *Pseudomonas, Bacilus, Xanthomonas,* and *Agrobacterium* isolated from *Valeriana officinalis*.

2.3.6 Ammonium and hydrogen cyanide production

Production of ammonia (NH_3) and hydrogen cyanide (HCN) is also important plant growth promoting activities that help in the inhibition of plant pathogens (Mukhtar et al. 2017; Singh et al. 2019). HCN is a volatile, secondary metabolite that subdues development of pathogens by inhibiting their growth (Meena et al. 2017). HCN helps in chelating metal ions and phosphate solubilization. The production of NH_3 and HCN is supposed to be free from the boundations of genera and species (Rijavec and Lapanje 2016). Both of these two helps in accessibility of nitrogen to their host plant and improvement of growth parameters and thus boost productivity of *Zea mays* (Marques et al. 2010). HCN produced by strains of *Pseudomonas*, *Rhizobium*, *Bacillus*, *Alcaligenes*, and *Aeromonas* enhance efficiency of antifungal activity of these bacteria (Bahadur et al. 2017; Meena et al. 2017). Ng et al. (2015) have reported production of HCN by *Trichderma* sp. that helped in the inhibition of bakanae pathogen (*Fusarium fujikuroi*) in rice.

2.3.7 Zinc solubilization

Zinc (Zn) is a micronutrient, required by plants for regulating production of several proteins and have both catalytic and structural role in plants metabolism in very low

concentration as 5-100 mg kg^{-1} (Rana et al. 2012; Meena et al. 2017). It is consumed predominantly as a divalent cation (Zn^{2+}) by plants, but in high pH soils it as a monovalent cation $ZnOH^{+}$ (Meena et al. 2017). Microbes present in biofertilizers have been reported to solubilize Zn present in soil and activate it for different metabolic and catabolic processes. *Trichoderma* spp., *Anabaena* spp., *Calothrix* spp., *Anabaena* spp. etc. have significantly solubilize Zn and helped in the growth promotion and productivity of different plants including wheat (Rana et al. 2012; de Santiago et al. 2011; Mukhtar et al. 2017).

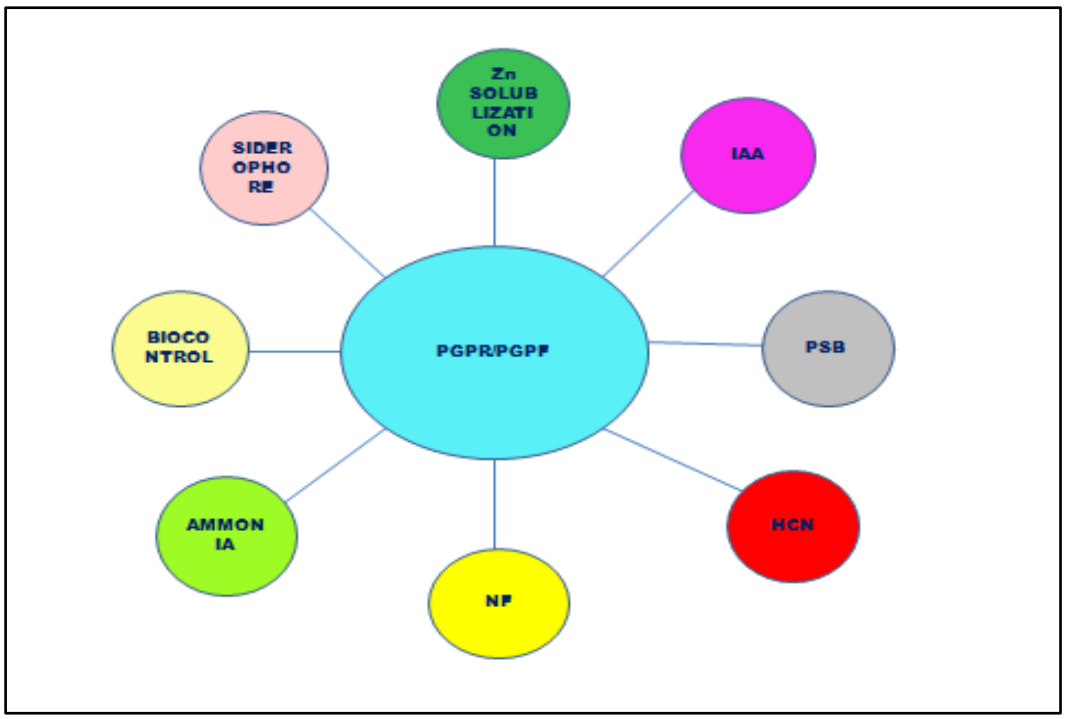

Fig. 2.3 Important mechanisms of biofertilizers to enhance growth and productivity of crops

2.4 Application of PGPR and PGPF in agriculture

Plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF) are important part of biofertilizers. These two have a massive range of usage in agricultural field but the chief purpose of their utilization is to enhance productivity by promoting growth of plants and inhibiting pathogens. Thus, the main applications of these two fertilizers are in the form of growth promoter and bio-control agent.

2.4.1 Growth promoter

The main purpose of the PGPR and PGPF application in agriculture practice is to improve the growth and development of plants that improve production without harming the environment (Kumar et al. 2018). Thus, these two main constituents of biofertilizers should work as growth promoter. The main mechanisms of plant growth promotion are the production of phytohormones like IAA, ABA, cytokinin and ethylene that helps in the cell division, cell elongation and expansion, seedling emergence, development of flowers and fruits, somatic embryogenesis and many more by PGPR and PGPF (Maheshwari et al. 2015; Meena et al. 2017). Whereas, these microbes participate in mineral solubilization, N fixation, siderophore, HCN and ammonium production, vitamins like niacin, thiamine, riboflavin, biotin and pantothenic acid which in turn aid in growth and development of plants (Cortivo et al. 2017; Elekhtyar 2015; Singh et al. 2019). Kuan et al. (2016) have reported that a group of PGPR species (*Klebsiella* spp., *Klebsiella pneumonia*, *Bacillus pumilus Acinetobacter* spp. and *B. subtilis*) have increased the productivity of maize by various mechanism including enhanced N fixation, phosphate solubilization, and IAA production. Nunkaew et al. (2014) have reported that some biofertilizers secrete some compounds like 5-aminolevulinic acid (ALA) that help in reduction of environmental stresses and encourage growth promotion of rice. It has been found that usage of some *Trichoderma* spp. rhizosphere-competent strains as biofertilizers have increased because of their positive effects on plants growth promotion and nutrient uptake, increase in rate of seed germination, and activiation of plant defense against environmental stresses (Sharma et al. 2012; Kowalska et al. 2014). Likewise, previous studies of different researchers like Murali et al. (2012), Ahmad et al. (2013), Akter et al. (2013), Yadav et al. (2013), Hosseini et al. (2014), Grobelak et al. (2015), Mensah et al. (2018) and many more have showed that PGPR and PGPF worked potentially as growth promoter and have fruitfully improved the productivity or yield of the crops. The details of these studies have been illustrated in Table 2.5-

Table 2.5 Effect of biofertilizers (PGPR and PGPF) on growth parameters of different crops

Biofertilizers	Plant	Remarks	References
	PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)		
Bacillus amyloliquefaciens, *B. pumilus*, *Glomus intraradices*	Tomato (*Solanum* lycopersicum, formerly (*Lycopersicon esculentum*)	Improved plant growth, yield, and nutrient uptake	Adesemoye et al. (2009)
PGPR isolates	Wheat (*Triticum aesiivum*)	Increased growth parameters, 1000-grain weight and grain yield. Uptake of N and P K also increased.	Abbasi et al. (2011)
Azospirillium lipoferum, Pseudomonas fluorescens and *P. putida*	Maize	Increased root biomass, biomass and productivity.	Adjanohoun et al. (2011)
Providencia spp., *Alcaligenes* spp., *Anabaena oscillarioides* and *A. torulosa*	Wheat *(T. aestivum)*	Growth parameters *viz*. enhancement in growth parameters of wheat.	Manjunath et al. (2011)
P. jessenii, and *P. synxantha*	Wheat (*T.* aestivum), rice (*Oriza sativa*) and black gram (*Vigna mungo*)	Increased grain yield, raw protein and mineral nutrient concentration of wheat grains (P, K, Cu, Fe, Zn, Mn). Further, soil quality was also improved indicated by increased soil enzyme activities.	Mäder et al. (2011)
Bacillus spp., *Providencia* spp. and *Brevundimonas diminuta*	Wheat (*T. aestivum*)	Enhanced growth parameters and productivity.	Rana et al. (2011)
B. polymyxa, *Azotobacter chroococcum* and *A. barasilense*	Wheat (*T. aesiivum*)	Enhancement in all growth parameters with higher wheat yield.	Eleiwa et al. (2012)
Rhizobium spp. and *Pseudomonas* spp.	Mung bean (*Vigna radiata*)	Improved plant height, growth, nodulation, dry matter, pods	Ahmad et al. (2013)

Biofertilizers	Plant	Remarks	References
		fresh weight, total grain weight, grain yield, proline content and K^+/Na^+ ratio and yield under salt-affected field conditions.	
B. cereus	*Vigna radiata* (Green gram), *Cyamopsis tetragonoloba* (Cluster bean) and *Brassica nigra* (Black mustard).	Increased root and shoot length, germination percentage and vigour index of seeds.	RS et al. (2013)
A. torulosa, *Azotobacter* spp., *Mesorhizobium* spp., *Serratia* spp. and *Pseudomonas* spp.	Wheat (*T. aesiivum*)	Enhancements in nitrogen fixing potential or ARA (Acetylene Reduction Activity) as well as soil biological and chemical properties were also increased.	Swarnalakshmi et al. (2013)
Rhizobium bacteria, *Azotobacter* spp., *Azospirillum*, *Pseudomonas* spp., and *Azospirillum* spp.	Mung bean (*Vigna radiate*)	Growth promotion and increased grain yield.	Hosseini et al. (2014)
B. megaterium, A. chlorophenolicus and Enterobacter sp.	Wheat (*T. aestivum*)	Height, yield, test weight as well as micronutrient content (Fe, Cu, Mn and Zn) were improved.	Kumar et al. (2014)
P. aeruginosa, *P. putida*, and *P. fluorescens*	Rice (*Oryza sativa*)	Treatment combinations increased grain yield, plant growth, nutrient contents in grain and straw of rice	Lavakush et al. (2014)
B. amyloliquefaciens, Bradyrhizobium japonicum	Soybean (*Glycine max*)	Enhanced the ability to colonize plant roots and increase the number of nodules.	Masciarelli et al. (2014)
Mesorhizobium, *A. chroococcum*, *P. aeruginosa* and *T. harzianum*	Chickpea (*Cicer arietinum*)	Enhanced growth, yield and phytopathogen growth inhibition.	Verma et al. (2014)
Ginger rhizobacteria *Burkholderia cepacia*, *B. amyloliquefaciens*, *Serratia*	Ginger (*Zingiber officinale Rosc*.)	Higher sprouting and lower disease incidence and greater rhizome yield wereobserved by B. *amyloliquefaciens* and *S.*	Dinesh et al. (2015)

Biofertilizers	Plant	Remarks	References
marcescens, *P. aeruginosa*		*marcescens*.	
Rhizobiaceae bacteria	Rape (*Brassica napus*) and fescue (*Festuca ovinia*)	Improved seed germination and plant growth.	Grobelak et al. (2015)
Sphingomonas spp., *Bacillus* spp. and *Methylobacterium* spp.	Tomato (*Solanum lycopersicum*)	Increased shoot and root biomass and chlorophyll contents.	Khan et al. (2016)
Azospirillum spp., *Azoarcus* spp. and *Azorhizobium* spp.	Wheat (*T. aestivum*)	Improvement in root growth, enhanced plant resistance to different stresses, and reduction in nitrogen losses.	Cortivo et al. (2017)
B. endophyticus, *B. sphaericus*, *Enterobacter aerogenes*, *B.* safensis, *B. megaterium*, Virgibacillus spp.	Wheat (*T. aestivum*)	Increased growth parameters and yield.	Mukhtar et al. (2017)
Flavobacterium johnsoniae, *P. putida*, *Achromobacter xylosoxidans* and *A. chroococcum*	Wheat (*T. aestivum*)	Enhanced bacterial growth and seed germination under salt stress by extracts from *Opuntia ficus-indica*, *Ulva lactuca* and *Enteromorpha intestinalis* which increased seed germination.	Rai et al. (2017)
	PLANT GROWTH PROMOTING FUNGUS (PGPF)		
T. harzianum and *T. atroviride*	Pea (Pisum sativum), tomato (*Lycopersicum esculentum*) and canola (*Brassica napus*)	Elevated Plant growth	Vinale et al. (2008)
T. virens and *T. atroviride*	Arabidopsis (*Arabidopsis thaliana*)	Increased biomass production and stimulated lateral root development which promoted plant growth	Contreras-Cornejo et al. (2009)
Trichoderma spp.	Bean (*Phaseolus vulgaris*)	Improvement in the growth of bean seedlings	Hoyos-Carvajal et al. (2009)

Biofertilizers	Plant	Remarks	References
T. harzianum	Chickpea (*Cicer arietinum*) .	Enhanced growth, yield and phyto-pathogen growth inhibition	Verma et al. (2014)
T. spp.	Bean (*Vicia fabae*), chickpea (*Cicer arietinum*) and lupine (*Lupines terms*)	Enhanced yield components and growth of all the plants	Shaban and El-Bramawy (2011)
T. harzianum	Tomato (*Lycopersicon esculentum* Mill.)	Improvement in yield and quality by increased total soluble solids, ascorbic acid, β-carotene, phosphorus, manganese content etc.	Molla et al. (2012)
Trichoderma spp.	Pearl millet	Highest disease protection.	Murali et al. (2012)
T. harzianum	Wheat (*T. aestivum*)	Significant increase in yield of wheat.	Sharma et al. (2012)
Trichoderma spp.	Cucumber (*Cucumis sativus*)	Increased seed germination, and improved growth and related parameters.	Akter et al. (2013)
T. harzianum Rifai	*Eruca sativa*	Improved plant growth.	Al-Rajhi (2013)
T. viride	Cabbage and red beet.	Enhanced growth by 27% at red beet and 29% at cabbage.	Topolovec-Pintarić et al. (2013)
T. asperellum	Chickpea (*Cicer arietinum*) and Rajma (*Phaseolus vulgaris*)	Enhanced seed germination and plant growth for both plants.	Yadav et al. (2013)
T. asperellum	Lemon balm *(Melissa officinalis)*	Growth promotion with an increase of dried mass of herb.	Kowalska et al. (2014)
Pleurotus tuber-regium, *Lentinus squarrosulus* and *Ganoderma* sp.	Wheat (*T. aestivum*) and tomato (*Lycopersicum esculentum*)	The growth parameters of both plants were increased by chitinase and esterage activities, siderophore production and phosphate solubilization by fungal strains.	Mensah et al. (2018)

2.4.2 Bio-control

Biofertilizers contain living microorganisms and biodegradable materials that are widely used to amplify the quality of soil and helps in prevention of pathogens by working as bio-control agent (Singh et al. 2019). Invasion of plant pathogens including various bacteria, viruses and other nematodes etc. are the main reason for reduction in the productivity and quality of crops. The usage of chemical pesticides to get rid of pathogen is not an intellectual option as these not only pollute the environment, contaminate food chain but are also very expensive (Ali et al. 2016). Thus, use of biological control over these is an efficient, cost effective and eco-friendly approach. PGPR and PGPF secrete various organic and inorganic substances, antibodies etc. that reduce the attack of pathogens and helps in the growth and development of plants (Tanaka et al. 2014). Further, PGPR and PGPF produce several enzymes and antibodies that hydrolyze cellulose, hemicelluloses, chitin, and proteins present in the cells of pathogens (Kumar et al. 2017). The production of chitinases enzymes by PGPFs helps in lysing of hyphal cell walls of fungal pathogens and protect host plants, whereas, esterases enzymes vitiate cutin and suberin in plant cuticles (Meena et al. 2017; Mensah et al. 2018). Production of siderophores by PGPRs and PGPFs generate competition for intake iron among plants, microbes and pathogens that inhibit pathogens (Hoyos-Carvajal et al. 2009; Angel et al. 2016). In addition, these also compete with pathogens for their nutrition and spacious requirements and inhibit their multiplication in the rhizosphere. *Serratia plymuthica* C48, *Serratia marcescens*, *Paenibacillus* spp., *Streptomyces* spp. and *Pseudomonas stutzeri* produce chitinase enzyme that degrade mycelia of fungal pathogens (Singh et al. 2019). β-1,3-glucanase produced by *Streptomyces*, *Paenibacillus*, and *Bacillus* spp. degrade fungal cell wall. Usage of various species of *Trichoderma* spp. for their antagonist nature toward different pathogens has increased in past few years. According to Wijesinghe et al. (2011) *Trichoderma* sp. produces antifungal metabolites and creates competition for space and nutrients that help in successful inhibition of *T. asperellum* over black rot disease of pineapple caused by a fungus *Thielaviopsis paradoxa*. Further, other biofertilizers frequently used contain numerous species of *Pseudomonas*, *Bacillus*, and mycorrhiza that have the ability to pursue ISR and SAR mechanisms and detoxify phytopathogen that help in enhancing plant defense against various pathogens (David et al. 2018). PGPR and PGPF helps in the

production of phytohormones like IAA, cytokinin, ethylene, gibberellins, ABA, salicylic acid, jasmonates, ethylene etc. which play important role in defense mechanisms of plants as these provide strength to the ISR and SAR of complex regulatory networks (David et al. 2015). Zhang et al. (2016) have found that *Pseudomonas fluorescens* FD6 possessed many advantageous qualities which are involved in bio-control of fungal pathogens *Botrytis cinerea* and *Monilinia fructicola*. Previous studies of Cordo et al. (2007), Sahebani and Hadavi (2008), Akter et al. (2013); Foroutan (2013); Koalwska et al. (2014); Zhang et al. (2014); Ng et al. (2015) etc. have reported successful application of PGPR and PGPF to work as bio-control agents. The details of these studies have been presented in Table 2.3.

Table 2.6 Previous studies on the application of biofertilizers (PGPR and PGPF) as bio-control agents

Biofertilizers (PGPR and PGPF)	Disease	Agent	Host Plant	Remarks	References
Trichoderma spp.	Leaf blotch	*Septoria tritici*	Wheat (*Triticum aestivum*)	No change in plant stem diameter or dry weight; and biochemical systemic induced response was stimulated to resist the disease.	Cordo et al. (2007)
Trichoderma harzianum	Root-knot nematode	*Meloidogyne javanica*	Tomato (*Lycopersicon sculentum*)	Reduction in the population of nematode and disease severity.	Sahebani and Hadavi (2008)
T. harzianum and *T. atroviride*	Fungal Pathogens	*Rhizoctonia solani*, *Pythium ultimum* and *Gaeumannomyces graminis* var. *tritici*	Pea (Pisum sativum), tomato (*Lycopersicum esculentum*) and canola (*Brassica napus*)	Reduction of disease symptoms	Vinale et al. (2008)
T. virens, T. koningii, T. koningiopsis, T. brevicompactum and *T. viridecens*	Wheat take-all disease	*Gaeumannomyces graminis* var *tritici*	Wheat (*T. aestivum*)	Reduced the mycelial growth and disease severity; and increased dry weight of shoots and dry weight of roots	Zafari et al. (2008)
Trichoderma spp.	Vascular wilt	*Verticillium* sp.	Tomato	Reduction in severity of disease, increased height and root shoot fresh weight.	Jabnoun-Khiaredinne et al. (2009)
T. harzianum and *T. koningii*	Leaf blotch	*Septoria tritici*	Wheat (*T. aestivum*)	Decrease in the disease, Reduced the incidence value and the severity value.	Perelló et al. (2009)

Biofertilizers (PGPR and PGPF)	Disease	Agent	Host Plant	Remarks	References
T. harzianum	Fungal Pathogens	*Fusarium oxysporum* and *Rhizoctonia solani*	Chickpea (*Cicer arietinum*)	Enhanced growth, yield and phyto-pathogen growth inhibition.	Verma et al. (2014)
T. viride and *B. megaterium*	Sheath spots disease	*Rhizoctonia oryzae*	Rice (*Oriza sativa*)	Inhibited growth of pathogen and showed growth promotion with increased grain and stem dry matter, panicles and harvest index in comparison to control plants.	Al-Taweil et al. (2010)
T. hazianum and *T. viride*	Soil born plant pathogens	Sclerotium rolfsii, Rhizoctonia solani and Sclerotinia sclerotiorum	Chilli	Inhibition of mycelial growth, reduction in pigmentation and plant growth promotion.	Joshi et al. (2010)
T. harzianum	Seed and seedling pathogen	*Pythium ultimu*	Jubilee tomato	Positive effect on seed germination and provided protection against oxidative damage.	Mastouri et al. (2010)
T. harzianum	Phyto-pathogenic fungus	*Fusarium oxysporum*	-	genes implicated in the mycoparasitism and increased bio-control.	López-Mondéjar et al. (2011)
Trichoderma spp.	Damping off and root rot	*Rhizoctonia solani* and *Sclerotium rolfsii*	Bean (*Vicia fabae*), chickpea (*Cicer arietinum*) and lupine *Lupines terms*	Significant bio-control of the diseases in all the plants.	Shaban and El-Bramawy (2011)
T. atroviride and T.	Foliar	*Botrytis cinerea*	Tomato	Induction of systemic resistance	Tucci et al.

Biofertilizers (PGPR and PGPF)	Disease	Agent	Host Plant	Remarks	References
harzianum	pathogen			mechanism which reduced the disease severity.	(2011)
T. asperellum	Black rot	*Thielaviopsis paradoxa*	Pine apple	All treated fruits were free of disease at the end of the incubation period. No change in pH, total soluble solids and titratable acidity between treated and control.	Wijesinghe et al. (2011)
Trichoderma spp.	Take-all Pathogen of Wheat	*(Gaeumannomyces graminis* var. *tritici, Ggt*)	Cucumber (*Cucumis sativus*)	Disease inhibition.	Akter et al. (2013)
T. harzianum and *T. viride*	Root rot	*Fusarium graminearum*	Wheat (*T. aestivum*)	Reduction in incidence and disease severity; and, Increased grain weight.	Foroutan (2013)
T. harzianum	Seed born disease	*Bipolaris sorokiniana*, *Fusarium graminearum* and *Aspergillus flavus*	Wheat (*T. aestivum*)	Reduced radial growth of pathogens, increased growth and grain yield.	Hasan et al. (2012)
T. harzianum	-	*Fusarium solani* f.sp. *pisi*	Pea seeds	Reduced the Fsp population, and colonization of roots.	Kim and Knudsen (2013)
T. harzianum	Foot and root rots	*Fusarium oxysporum* f. sp. *lycopersic*i	Tomato	Reduction of the disease; and, Increase the emergence and growth.	Marzano et al. (2013)
T. asperellum	Fungal Pathogens	*Septoria melissae*	Lemon balm (*Melissa officinalis*)	Stimulated growth and an increase in dried mass of herb.	Koalwska et al. (2014)

Biofertilizers (PGPR and PGPF)	Disease	Agent	Host Plant	Remarks	References
Mesorhizobium spp. *A. chroococcum*, *P. aeruginosa* and *T. harzianum*	Root rot and wilt diseases	*Fusarium oxysporum* and *Rhizoctonia solani*	Chickpea (*Cicer arietinum*)	*Pseudomonas* and *Trichoderma* improved growth attributes, yield and inhibited phyto-pathogen growth in comparison to control.	Verma et al. (2014)
T. longibrachiatum	Cereal cyst nematode	*Heterodera avenae*	Wheat (*Triticum aestivum*)	Increased chitinase activity and bio-control.	Zhang et al. (2014)
Trichoderma spp.	Bakanae disease	*Fusarium fujikuroi*	Rice (*Oryza sativa*)	About 45 % Inhibition of Radial Growth against *F. fujikuroi*. Disease incidence and severity were also significantly reduced.	Ng et al. (2015)
Pseudomonas spp., *Bacillus* spp., *Acinetobacter* spp.	Root rot	*Rhizoctonia solani*	Mung bean (*Vigna radiate*)	*Pseudomonas* showed most potent antifungal activity against *R. solani* followed by other two.	Kumari et al. (2018)
Trichoderma spp.	Black pepper and ginger pathogens	*Fusarium oxysporum*, *Rhizoctonia solani* and *Phytophthora capsici*	Black pepper (*Piper nigrum*) and ginger (*Zingiber officinale* Rosc.)	The isolates showed maximum bio-control property against plant all pathogens.	Das et al. (2019)

2.5 Application of PRPRs and PGPFs for enhancing the productivity of wheat (*Triticum aestivum* L.)

Wheat (*Triticum aestivium*) is the most common and widely cultivated food crop all over the globe and considered to be the basis of food security (Mukhtar et al. 2017). Nitrogen and phosphorus based chemical fertilizers have been profoundly used worldwide to intensify the productivity of this major food crop without concerning its side effects on the health of the environment (Munda et al. 2018). Some farmers apply ca. 500-600 kg N $ha^{-1}yr^{-1}$as an N fertilizer in order to achieve high yields of maize and wheat on a crop rotation system (Jia et al. 2014). According to Lenka et al. (2013) about more than or equal to172 kg N ha^{-1} of nitrate-N accumulation was observed in 90 cm soil depth during wheat growing season in seven wheat cultivating regions of North China Plain. According to Cui et al. (2010) this amount was so huge that 55% farmers will not require applying N fertilizers before sowing of wheat. Some parts of such a huge amount of chemical fertilizers may be lost in the environment causing environmental pollution. So, it is the necessity of the present to adopt such a measure that not only increase the productivity of major food crops like wheat but also provide a safe and sustainable future to the generations to come. The application of biofertilizers in the place of chemicals is a greener and cost effective measure to take upon. It has been reported that bacterial population associated with roots of wheat work as an enhancer of yield and shows antagonists activities towards oil borne phytopathogens that increases the productivity of the crop (Mukhtar et al. 2017). A large number of PGPRs and PGPFs including isolates from the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Paenibacillus*, *Trichoderma*, *Mesorhizobium*, *Providencia*, *Alcaligenes*, *Anabaena* etc. have been obtained from the rhizosphere of various crops, including wheat (Manjunath et al. 2011; Tahir et al. 2015; Cortivo et al. 2017; Mukhtar et al. 2017; Rai et al. 2017). These microbes have increased the productivity of wheat by inducing various mechanisms like enhanced nutrient uptake, mineral solubilization, production of enzymes and phytohormones, by strengthening SAR and ISR mechanism to fight against pathogens etc. Further, previous studies of Abbasi et al. (2011), Manjunath et al. (2011) Rana et al. (2011), Eleiwa et al. (2012), Swarnalakshmi et al. (2013), Kumar et al. (2014), Zhang et al. (2014) Cortivo et al. (2017), Kumar and Singh (2015); Mukhtar et al. (2017), Rai et al. (2017), Kumar and Singh (2018); Kumar et al. (2018); Mensah et al. (2018) etc.

have successfully utilized various genera of PGPR and PGPFs for enhancing the growth and productivity of wheat crop. The details of these studies have been presented in Table 2.2 and 2.3.

2.6 Limitations in the application of biofertilizers

Although application of biofertilizers provide eco-friendly and cost effective approach for sustainable agriculture but there lie some limitations also. The constrains that limit the usage of biofertilizers are-

1. Unavailability of suitable resources and strains at local level.
2. Absence of infrastructures, space for establishment of laboratory, cold storage equipment for inoculum and production with proper storage conditions of biofertilizers.
3. Financial crisis related to purchase, transportation and storage of biofertilizers for underprivileged farmers.
4. The lack of research and development for quality assurance and limited production to fulfill the demand of biofertilizers.
5. Shortage of skilled personnel to guide regarding the amount, suitable environmental factors (soil and climatic factors), time duration and season of applying biofertilizers to different crops.
6. Application of biofertilizers do not give immediate effect and takes time to visualize the results in comparison to chemical fertilizers that detract farmer's will to use it in their fields.
7. Avoidance of quality control measures due to unawareness of farmers towards the benefits of technology.
8. This technique requires different methods of inoculation that creates problems towards adoption of technology for local farmers.

These constrains should be overcome to successfully use biofertilizers for enhancing the productivity of major food crops like wheat and rice. The success and commercialization of biofertilizers are governed by the connections between

the scientific organizations and industries (Bhattacharyya and Jha 2012). Farmer oriented policies should be developed by the governments to attack farmers to encourage the usage of biofertilizers in the agriculture. Financial support and awareness should also be generated by state and central governments to safeguard the environment from environmental pollution caused by agrochemicals (Saritha and Tollamadugu 2019). Further, a regulatory and monitoring organization should be formed with main focus on research and development of cost efficient and ecofriendly biofertilizers with easy marketing strategies that favors wellbeing of farmers (Kumar and Singh 2015). Thus, biofertilizers can fulfill diverse advantageous interactions in plants leading to auspicious elucidations for sustainable and environment-friendly agriculture.

Chapter 3

Materials and Methods

3 Material and Methods

3.1 Collection of soil samples

Soil samples were collected from rhizosphere of wheat crops. Healthy wheat plants were uprooted randomly and soil from rhizosphere region (about of 4 inch deep) were collected from about 25 places around Periurban region of Lucknow Uttar Pradesh India. The root system was plowed and the rhizospheric soil samples were collected, mixed and kept in an icebox in sterilized polyethylene bags and maintained in refrigerator till further study.

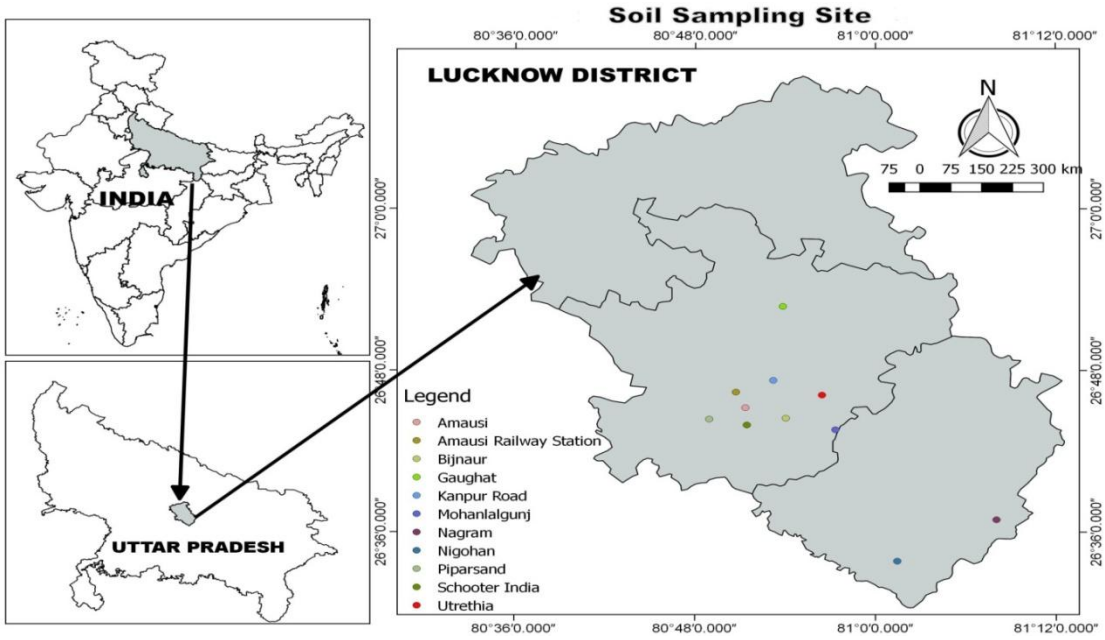

Fig. 3.1 Soil sampling sites

3.2 Isolation of plant growth promoting microorganisms

Isolation of bacteria and fungi from the rhizospheric soil samples was carried out using serial dilution method (Aneja 2005). The diluted samples (0.1ml) were aseptically introduced into a sterile Petri plate containing sterile nutrient medium and assayed for growth of the bacterial and fungal colonies. The plates were incubated at 27±2 ºC for 2 days (for bacterial colonies) and 3-5 days (for fungal colonies). After growth, purification of the colonies was done on the same solid medium by placing a small portion of colonies by streaking method. All cultures were sub cultured periodically to preserve the viability and purity of cultures. The media used for the

isolation of bacteria was nutrient agar (NA) and different for fungi which were Potato dextrose agar (PDA) purchased from Hi-Media Lab, Pvt. Ltd.

3.3 Characterization and identification

The isolated bacteria were identified morphologically on the basis of shape of bacteria as well as the size, shape and colour of the colon using Bergey's manual (Garrity et al. 2005). Identification of fungal colonies was done by Manual of fungi imperfecti by Barnett and Hunter (1998).

3.3.1 Morphological characterization

Colony morphology of the isolates was determined by observing their growth on solid media plates and observed the growth pattern, colour and shape of their colonies. Cell morphology of the bacterial isolates was determined by Gram staining following Gram (1884). Clean slides were used in the method with smear of freshly prepared culture of each bacterial isolates. Bacterial smears were inundated with crystal violet stain for 60 seconds and rinsed afterward with distilled water. Then few drop of Gram's iodine was added on the smear for 60 seconds which was washed with 95% alcohol (drop by drop) until the purple dye completed removal of purple dye from the smear. It was once again washed gradually with distilled water. At last, the smears were flooded with a counter stain safranin for 30 seconds and washed with distilled water and waited for drying of the slides. Now the slides were observed under by phase contrast microscope at 100X for determining the shape and Gram's nature of the bacterial isolates.

3.3.2 Cell morphology of fungi

The fungal isolates were stained by lactophenol cotton blue and observed under phase contrast microscope at 40X by following the methods of Aneja (2005).

3.4 Physiological characterizations

3.4.1 Growth of PGPMs at different salt concentrations

Salt tolerance of isolates was determined on (for bacteria) at various concentrations of NaCl (1 to 10%) which was used for growth of PGPMs (Chaiharn and Lumyong

2009) The plates were incubated at 28±1 °C for 3 to 5 days in saline environment in media. Growth of isolates was recorded by the colony appearance.

3.4.2 Growth of PGPMs at different temperature

The effect of temperature on growth of isolates was observed on the same media used for culture of PGPMs. The respective media plates were inoculated and kept for 3 to 5 days at various temperatures ranging from 5 to 40 °C. After incubation growth of the isolates at each temperature was observed by colonies appearance on the respective temperature (Chaiharn and Lumyong 2009)

3.5 Biochemical test for primary identification of bacteria

Biochemical tests of bacterial isolates were done to identify the primary characteristics up to genus level. The details of main tests performed have been given below-

3.5.1 Nitrate reductase activity

This test was performed on tryptone yeast extract (TYE) medium containing 0.1 % KNO_3 (Campbell 1999). Bacterial isolates were inoculated and incubated for 4 days at 28±2ºC. After incubation few drops of sulphanilic acid (8 g l^{-1}) in 5M acetic acid) and α-naphthylamine (5 g l^{-1} in 5M acetic acid) was added in each tubes including control. Change in colour of solution after incubation was observed to determine the occurrence of the activity qualitatively.

3.5.2 Urease test

Yeast Mannitol Agar (YEMA) media was prepared in which 2% (w/v) urea and 0.012% (w/v) phenol red was added. Slants of urea containing medium was prepared for each bacterial culture including control (No culture) and incubated at 28ºC for 48 hrs. Change of media colour from light yellow to red was observed which was indicator of urease production (Lindström and Lehtomäki 1988).

3.5.3 Gelatinase test

Gelatinase media was prepared and poured in separate test tubes for each isolates including one control (uninoculated) and after inoculation it was incubated for 3-5

days at 28ºC. After incubation test tubes were placed in refrigerator at 4ºC for 15 minutes and liquidification of media was observed. Partial or total liquid state of gelatin confirmed the positive test (Champman 1952).

3.5.4 Citrate utilization test

In this test slant of Simmon's citrate agar media was prepared in test tube and inoculated with bacterial culture by streaking on it (Simmon 1926). All tubes including one uninoculated control was incubated at 28ºC for 24 h and the colour change from green to blue was observed.

3.5.5 Amylase test

Starch agar media was prepared and spot inoculated with bacterial culture and incubated at 28ºC for 48 h (Lennette et al. 1985). After incubation plates were flooded with Gram's iodine solution for 4-5 minute. After removing the extra iodine solution, plates were observed for the clear zone around colonies.

3.5.6 Lipase test

Lipase test was done on Tween 80 agar media (Samad et al. 1989). Media plates were prepared, inoculated and incubated for 2-3 days at 28ºC. After incubation opaque zone formation was observed around the bacterial colonies on transparent media.

3.5.7 Protease test

For this test skim milk agar media was prepared, inoculated and incubated for 48 h at 28ºC. After incubation clear zone formation was observed around the bacterial colonies (Kasana et al. 2011).

3.5.8 Cellulase test

Czapek's mineral salt agar media was prepared, inoculated and incubated for 2-3 days 28ºC. After the growth of the colonies, plates were flooded with dye (congo red) for 15 minutes for staining and 1% NaCl for destaining for 15 minutes. After destaining hollow zone formation around the bacterial colonies was observed (Florencio et al. 2012).

3.5.9 Indole test

Tryptone broth was prepared and inoculated with loopfull of culture and incubated for 24 h at 28ºC. Then after incubation 1 ml of Kovac's reagent was added to each tube including control and the tubes were observed for the formation of "Red colour ring'' on the top of the broth (Cheesbrough 1985).

3.5.10 Hydrogen sulphide test

The Sulphide Indole Motility Media (SIM) agar was prepared in the test tubes and the cultures were stabbed into the tube. The tubes were incubated for 48 h at 28ºC. After incubation the black colour along the stabbing line stabbing was considered to be a positive result.

3.6 Plant growth promoting activities of isolates

After biochemical characterization of the bacterial and fungal isolates, their plant growth promoting potentials were also determined by performing various tests as described as following-

3.6.1 Nitrogen fixation

Nitrogen free Jensen's medium was used for this assay (Jensen 1942). This medium is recommended for detection and cultivation of nitrogen fixing bacteria. Solid agar media plate was prepared and inoculated with bacterial culture. After inoculation plates were incubated for 3-7 days at 28±2ºC and observed the growth of colonies.

3.6.2 Phosphate solubilisation

This assay was done on plates containing Pikovskaya's agar media. Solid agar media plates were prepared and spot inoculated with fresh bacterial culture (Pikovskaya 1948). After inoculation plates were incubated at 27±2ºC for 2-4 days and observed the halo zone formation around the bacterial colonies. Halo zone formation was used for the determination of phosphate solubilisation index (PSI) by applying the following formula (Edi-Premono et al. 1996).

$$PSI = \frac{\text{Colony diameter} + \text{halo zone diameter}}{\text{Colony diameter}}$$

In vitro phosphate solubilization by *Trichoderma* spp. strain was initially cultured on potato dextrose agar (PDA) culture medium at 27±2 °C for one week. Subsequent colonies about 5.0 mm in diameter discs containing mycelium and conidia were placed in a 500 ml Erlenmeyer flask containing 100 ml of modified NBRIP media (Nautyal 1999). The flasks were incubated at 27±2 °C for seven days at 180 rpm for a week. The evaluation was conducted at seven days. The colorimetric method of Murphy and Riley (1962) was used to determine the soluble Phosphate concentration.

3.6.3 Hydrogen cyanide (HCN) production

HCN production ability of isolates was determined according to Bakker and Schippers (1987). For this test HCN induction solid agar media plates were prepared by adding 4.4 g/l glycine in Luria Bertani (LB) agar both culture were spread on separate plate. One uninoculated plate was used as control. Filter paper (Whatman No. 1) dipped in picric acid solution (2% sodium carbonate mixed with 0.5% picric acid solution) was placed in the lid of petri plates. All the plates were sealed with parafilm and incubated at 27±2 ºC for 4 days. After incubation colour change of filter paper from yellow to brown was observed against control plate.

3.6.4 Siderophore production

The detection of siderophore production ability of both isolates was done by universal chrome-azurol sulfonate (CAS) assay (Schywn and Neilands 1987). Firstly, glasswares were washed with 3M hydrochloric acid (HCl) and subsequently washed in deionized water to remove iron (Cabaj and Kosakowska 2007).Siderophore production was estimated qualitatively. CAS reagent was prepared as described by Schwyn and Neilands (1987). Briefly, 121 mg CAS dye was dissolved in 100 ml distilled water and 20 ml of 1 mM ferric chloride ($FeCl_3 6H_2O$) solution prepared in 10 mM HCl. This solution was added to 20 ml hexadecyl trimethyl ammonium bromide (HDTMA) solution (729 mg HDTMA in 400 ml distilled water) with continuous stirring. This is called as CAS-HDTMA solution which was sterilized before further use.

3.6.5 Indole Acetic Acid (IAA) production

The bacterial and fungal cultures were grown on minimal broth with 0.1% tryptophan for 3–7 days (Brick et al. 1991). After isolates growth the broth was centrifuged at (10,000 rpm for 30 min) and culture supernatant was used for IAA estimation. About 1 ml supernatant of each broth was mixed with 4 ml of Salkowski reagent (1.0 ml of 0.5 M ferric chloride- $FeCl_3$, 50 ml of distilled water and 30 ml of concentrated sulphuric acid-H_2SO_4) and incubated for 30 min. The development of pink colour indicated IAA production. (Optical density) OD of each sample including control was taken at 530 nm.

3.6.6 Ammonia production

Production of ammonia was observed by following the methods of Cappuccino and Sherman (1992). Peptone water broth media was used in the experiments for analysis the amount of ammonia produced by bacterial and fungal isolates. The microbial isolates were inoculated in 10 ml of freshly cultured peptone water after incubating period of 3-7 days at temperature of 27±2 ºC. After that 0.5 ml Nessler's reagent was added to observe the development of yellow to brown colour which indicated positive test.

3.7 Biocompatibility test

3.7.1 Compatibility test among bacterial and fungal isolates

Compatibility among bacterial (*A. faecalis* and *M. phyllosphaerae*) and fungal (*T. virens*) isolates In vitro compatibility test using dual culture plate method according to Siddiqui and Shaukat (2003).

After streaking *A. faecalis* vs *T. virens and M. phyllosphaerae* vs *T. virens* fresh culture of *A. faecalis* and *M. phyllosphaerae* streaked one side of sterilized petri plate of PDA and other side of petri plate inoculated with 5mm block of *T. virens* plates were incubated at 25±2°C and to observed zone of inhibition but there is no any inhibition zone found which is indication of the compatibility with each other

3.7.2 Compatibility between *A. faecalis* and *M. phyllosphaerae*

The isolates were checked for their compatibility between each other by the method of Fukui et al. (1994). The bacterial strains were streaked vertically and horizontally to each other on sterilized NA media plates. The plates were incubated at (25±2°C) for observation of inhibition zone. No observation of inhibition zone indicated the compatibility with each other bacterial strains

3.8 Molecular characteristics of the isolates

3.8.1 Bacterial isolates

Universal primer was used for sequencing of bacterial isolate by 16S rRNA method. Briefly, 5ul of genomic DNA was extracted and amplified by forward 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-CGGTTACCTTGTTACGACTT-3'). At the time of sequencing, PCR product primers were used as 785F (GGATTAGATACCCTGGTA) and 907R (CCGTCAATTCMTTTRAGTTT) s per method described by Saitou and Nei (1987).

PCR conditions

Reaction Mixture (50 µl)		**Cycling Conditions**		
Template DNA	100 ng	Initial Denaturation	2 minutes at 95°C	
Forward Primer	0.3 µM	Denaturation	30 seconds at 95°C	35 Cycles
Reverse Primer	0.3 µM	Anealing	30 seconds at 52°C	
Master Mix	25 µl	Extension	2 minutes at 72°C	
Nuclease Free Water	Volume makeup 50 µl	Final Extension	15 minutes at 72°C	

Primer details for PCR

No.	**Oligo Name**	**Sequence (5`→ 3`)**	**Tm (°C)**	**GC- Content**
1	27F	AGAGTTTGATCMTGGCTCAG	56.3	47.5%
2	1492R	CGGTTACCTTGTTACGACTT	55.3	45%

Primer Details (For Sequencing)

No.	Oligo Name	Sequence (5`→ 3`)	Tm (°C)	GC- Content
1	785F	GGATTAGATACCCTGGTA	56.3	47.5%
2	907R	CCGTCAATTCMTTTRAGTTT	55.3	45%

3.8.2 Fungal isolates

DNA isolation

DNA was isolated using standard protocols optimized at our research and development facility and details are shown below-

PCR Conditions

Reaction Mixture (50 µl)		**Cycling Conditions**		
Template DNA	100 ng	Initial Denaturation	2 minutes at 95°C	
ITS 1	0.3 µM	Denaturation	30 seconds at 95°C	40 Cycles
ITS 4	0.3 µM	Annealing	30 seconds at 54°C	
Master Mix	25 µl	Extension	1 minutes at 72°C	
Nuclease Free Water	Volume makeup 50 µl	Final Extension	15 minutes at 72°C	

Primer details

No.	Oligo Name	Sequence (5`→ 3`)	Tm (°C)
1	ITS 1	´TCCGTAGGTGAACCTTGCGG	61.0

All sequencing process was performed in Aakaar Biotechnologies Pvt. Ltd., Lucknow, India. The obtained data of base pair sequence was analysed through BLAST method available in Gene bank (www.ncbi.nlm.nih.gov) for the identification of bacteria and fungi for the final sequence was submitted in NCBI for obtaining accession number.

3.1 The detail layout of experimental design for pot and field experiments with different treatments

Treatments	Details layout of treatments
Con	Control
Commer.	Commercial biofertilizer market based (recommended dose)
U+D	Urea and di-ammonium phosphate (recommended dose)
$S5_1$	*Microbacterium phyllosphaerae* (1x 10^8)
SDA_3	*Alcaligenes faecal* (1x 10^8)
T_2	*Trichoderma virens* (2x10^8)
S51+SDA_3	*Microbacterium phyllosphaerae + Alcaligenes faecalis*
$S5_1$+T_2	*Microbacterium phyllosphaerae + Trichoderma virens*
SDA_3+T_2	*Alcaligenes faecalis+ Trichoderma virens*
$S5_1$+SDA_3+T_2	*Microbacterium phyllosphaerae +Alcaligenes faecalis+ Trichoderma virens*

3.10 Preparation of bacterial and fungal culture

Mass culture of bacterial cell was prepared by fresh culture.It was inoculated into Erlenmeyer flask (1 L) containing 250 ml nutrient broth (NB) incubated 25±2 °c for 1-2 days on orbital shaker prepared culture applied on wheat seeds. Spore suspension of *Trichoderma* spp. was prepared by seven days-old culture of *Trichoderma* spp. It was inoculated into Erlenmeyer flask (1 L) containing 250 ml potato dextrose broth (PDB) and incubated at 28±2°C for 5-6 days on orbital shaker. After incubation, prepared suspension of conidia was utilized for seed priming.

3.11 Seed inoculation

Seeds of wheat (*Triticum aestivum* L.), variety PBW-343, were purchased from local market of Lucknow, Uttar Pradesh, India. The seeds were surface sterilised by 0.1% $HgCl_2$ for 2 min and rinsed properly for 4-5 times with sterilised water. These sterilised seeds were inoculated with isolates for seed priming as per Vidhyasekaran and Muthamilan (1995) whereas un-inoculated seeds were used as a control.

3.12 Pot experimental plan

The experimental site selected within the BBA University, Lucknow, was located at (26°46'5.77"N and 80°55'38.92"E). Fifteen seeds of wheat (*T. aestivum*) were sown in 12 inch diameter earthen pots filled with 8 kg soil and placed under the net house as presented in Fig. 3.2. Irrigation was given equally to each pot. The randomly sampled wheat plants were uprooted at interval of 40, 80 and 120 days and were analysed for growth parameters, for example, root length, shoot length, fresh weight and dry weight as well as yield parameters such as grain weight per plant and length of spikelet.

Fig. 3.2 Experimental set-up of wheat in earthen pots within the net house

3.13 Designs for field experiment

The experiment was conducted in the natural field conditions in Research Field Station, Babasaheb Bhimrao Ambedkar University, Lucknow, India over four subsequent years (2004-15, 2015-16, 2016-17 and 2017-18) with cropping of wheat (*Triticum aestivum* L. cv. PBW-343). The experimental plots were arranged in 4 m^2 (2x2 m) area of random block design (RBD) as presented in Fig. 3.3.Three replicates were maintained for each treatment and samples for studies were taken in replicates (n=12).

Fig. 3.3 Field experimental site with random block design

3.14 Measurement of growth and productivity of Wheat after inoculation of PGPMs

3.14.1 Root Length and shoot length

Root length and shoot lengths were measured by erecting the plant parts using meter scale.

3.14.2 Leaf number and number of roots

Number of root hairs and leaves were counted by manual counting of each sample.

3.14.3 Fresh weight

The fresh weight of plant parts was recorded using single pan electric balance. The plant parts were washed with distilled water after removing impurities kept within two layers of filter paper before weighing**.**

3.14.4 Dry weight

Plant samples were oven dried at 70°C till constant weight was gained and afterwards dry weight was measured

3.14.5 Weight of grain

Grain weight per plant was recorded by electronic weighing balance and length of spikelets at the time of harvesting was also measured using meter scale

3.15 Analysis of physicochemical parameters of soil

Soils were sampled from the pots. The top 0-12 cm soil from between the plant roots was collected and then analysed. The main parameters of soil testing have been given below-

3.15.1 pH (pouvoir hydrogen or hydrogen power)- Electronic pH meter (Model: Elico PE 138) was used to determine pH of soil.

Procedure

- 4 g of soil samples were taken in replicate and mixed with 20 ml of distilled water on orbital shaker up to 30 minutes after that pH was noted.

3.15.2 Electrical conductivity- The conductance generated by various ions present in soil samples was analysed by electronic conductivity meter (Model: Elico PE 138).

Procedure

- 20 g of soil was shaken intermittently with 40 ml of distilled water in a 250 ml conical flask for 1 hr and allowed to stand.
- The conductivity of the supernatant liquid was measured with the help of conductivity meter.

3.15.3 Organic carbon and organic matter - Organic carbon was estimated by Walkley and black method (1934) by rapid dichromate oxidation technique.

Chemicals and reagents

- Potassium dichromate, 1N: Dissolved exactly 49.04 g of AR (Analytical grade) $K_2Cr_2O_7$ (dried at 105^oC) in1Lof distilled water.
- Ferrous ammonium sulphate 0.5N (approx.): Dissolved 196.0 g of the hydrated crystalline $FeSO_4$ $(NH_4)_2.6H_2O$ per litre containing 20 ml of conc.H_2SO_4.
- Diphenylamine indicator: Dissolved 0.5 g $(C_6H_5)_2NH$ in a mixture of 20 ml of water and 100 ml of conc. H_2SO_4.
- Conc. Sulphuric acid, H_2SO_4 (Sp. Gravity 1.84)
- Ortho-phosphoric acid (85%) and or sodium fluoride.

Procedure

- Standardisation of ferrous ammonium sulphate solution- Took 10 ml of 1N Potassium dichromate solution in a 250 ml of conical flask. Then very carefully added 150 ml of conc. H_2SO_4 which generated heat. Swirled the mixture and allow it to cool. Afterwards, added 200 ml of distilled water, 10 ml of ortho-phosphoric acid and 1ml of indicator solution with thorough mixing. Finally, added ammonium ferrous sulphate from the burette, swirl the flask until the colour changed from blue to green. These data were also used as the blank reading.
- **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. Then 10ml of potassium dichromate was

added in the conical flask and the flask was swirled gently to disperse the soil in the dichromate solution. Following that 20 ml of conc. H_2SO_4 was carefully added from a measuring cylinder. Swirled it two to three times. The flask was allowed to stand for 30 minutes. After that, 200 ml of distilled water and 10 ml of ortho-phosphoric acid was added to get a sharper end point of the titration. 1ml of di-phenyl indicator was added and titrated with ferrous ammonium sulphate solution till the colour flashed from blue-violet to green. Simultaneously a blank was also run without soil.

- *Calculation*- Following formula was used to calculate organic carbon (%)-

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

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, and S= Weight of soil in g.

Whereas, organic matter (%) was calculated by following formula-

$$Organic\ matter\ \ \% \ = organic\ carbon\ \times 1.724$$

3.15.4 Nitrite estimation- 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 method of Stevens and Oaks (1973) was used.

Chemicals and reagents

- $NaNO_2$ stock solution- Dissolved 0.001 g of $NaNO_2$ in 100 ml distilled water.
- 1 % sulphailamide (LR) solution (4-amono benzene sulphonamide, ($C_6H_8N_2O_2S$)- Dissolved 1 gm sulphanilamide in 100 ml 1N HCl.
- 0.01% NED (N-1-napthyl ethyldiamine dihydrochloride (GR) $C_{12}H_{16}C_{l2}N_2$ Dissolved 0.01 g of NED in 100 ml distilled water.

Procedure

- Standards of known concentrations i.e. 0.05, 0.10, 0.15, 0.20 and 0.25 mg L^{-1} were prepared with sodium nitrite for calculation of k factor (Fig. 3.4).

- 1.0 g of sample was crushed with 6 ml of distilled water which was centrifuged at 5000 rpm for 10 minutes.
- Took 0.1 ml of supernatant and added 1.0 ml of 1 % sulphanilamide and 1.0 ml of 0.02 % NED.
- After 10 minutes, with appearance of pink colour, optical density (OD) was taken on spectrophotometer at 540 nm.

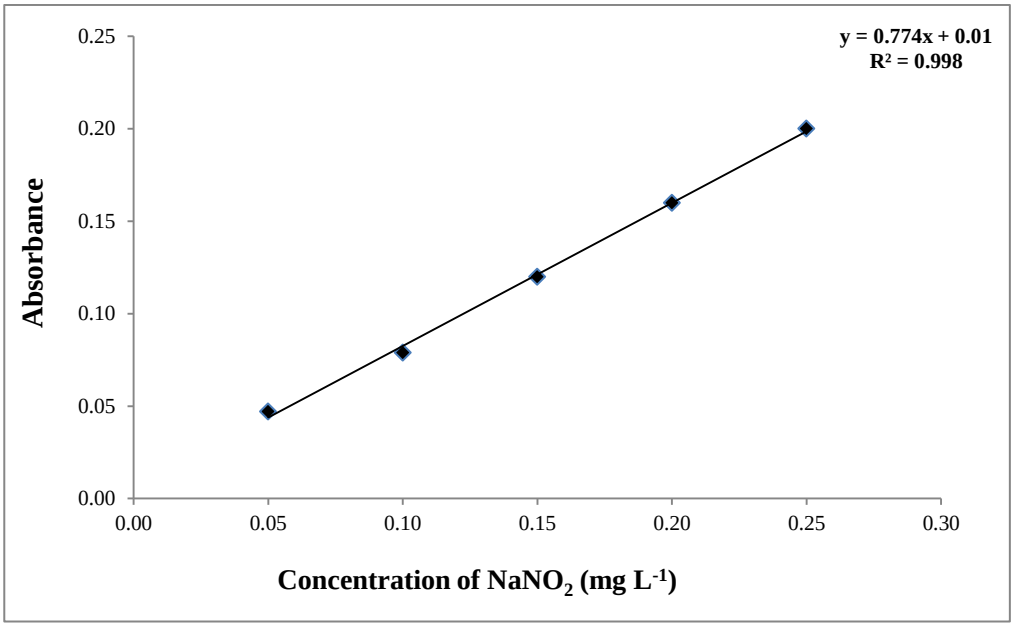

Fig. 3.4 Standards curve of nitrite prepared form known concentrations of $NaNO_2$

3.15.5 Nitrate estimation- Cataldo method (Cataldo et al. 1975) was used for nitrate estimation in the soil samples.

Chemicals and reagents

- Stock solution of KNO_3- Dissolved 0.1g of KNO_3 in 100 ml distilled water.
- 5 % salicylic acid- Dissolved 5.0 g of salicylic acid in 100 ml of conc. H_2SO_4.
- 2N NaOH- 20.0 g of NaOH was dissolved in 250 ml of distilled water.

Procedure

- Standards of known concentrations i.e. 5, 10, 15 and 20 mg L^{-1} were prepared with potassium nitrate for calculation of k factor (Fig. 3.5).
- 0.1 ml of distilled water , 0.4 ml of 5 % salicylic acid and 9.5 ml 2N NaOH was taken in a test tube.

- 1.0 g soil sample was crushed with 6 ml of distilled water and centrifuged at 5000 rpm for 10 minutes.
- 0.1 ml of supernatant was taken into a test tube and 0.4 ml of 5 % salicylic acid 9.5 ml 2N NaOH was added into it. After 20 minutes orange-yellowish colour appeared.
- Then, OD was taken on spectrophotometer at 410 nm.

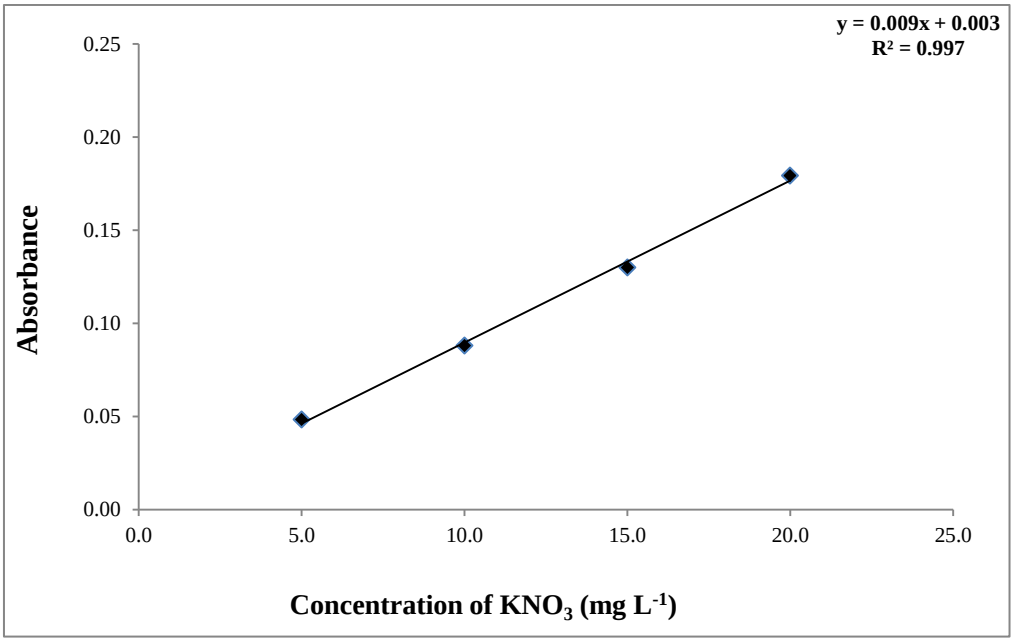

Fig. 3.5 Standards curve of nitrate prepared form known concentrations of KNO_3

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

Chemicals and reagents

- Ammonium molybdate **(**$(NH_4)6Mo_7O_{24}$)- 1.25l g of ammonium molybdate was dissolved in 50 ml of conc. H_2SO_4.
- Stannous chloride ($SnCl_2$) - Dissolve 0.61 g in 25 ml of glycerol.

Procedure

- Standards of known concentrations i.e. 5, 10, 15 and 20 mg L^{-1} were prepared with phosphorus penta oxide for calculation of k factor (Fig. 3.6).
- Crushed 2.0 g of soil sample in 12 ml distilled water and centrifuge it to obtain the supernatant.

- 10 ml of supernatant was taken in a test tube and added 0.4 ml of ammonium molybdate and 1 drop of stannous chloride.
- After that OD was taken on spectrophotometer at 680 nm.

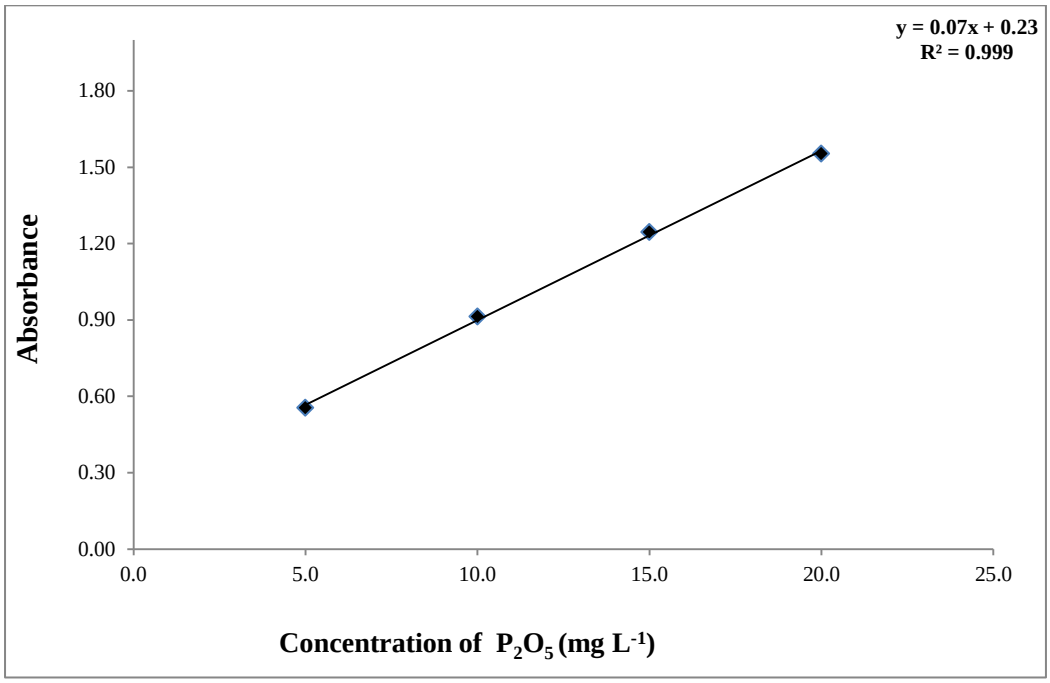

Fig. 3.6 Standards curve of phosphate prepared form known concentrations of P_2O_5

3.15.7 Ammonium estimation- Ammonium was estimated by Weatherburn (1967) method using Nesseler's reagent method.

Chemicals and reagents

- Nesseler's Reagent

Procedure

- Standards of known concentrations i.e. 2, 4, 6, 8, and 10 mg L^{-1} were prepared with ammonium sulphate for calculation of k factor (Fig. 3.7).
- Crushed 1.0 g of soil sample in 6 ml of distilled water and centrifuged it to obtain supernatant.
- 2.5 ml of supernatant was taken in a test tube and added 1.5 ml of Nessler's reagent.
- After change in colour from red to brown OD was taken on spectrophotometer at 420 nm.

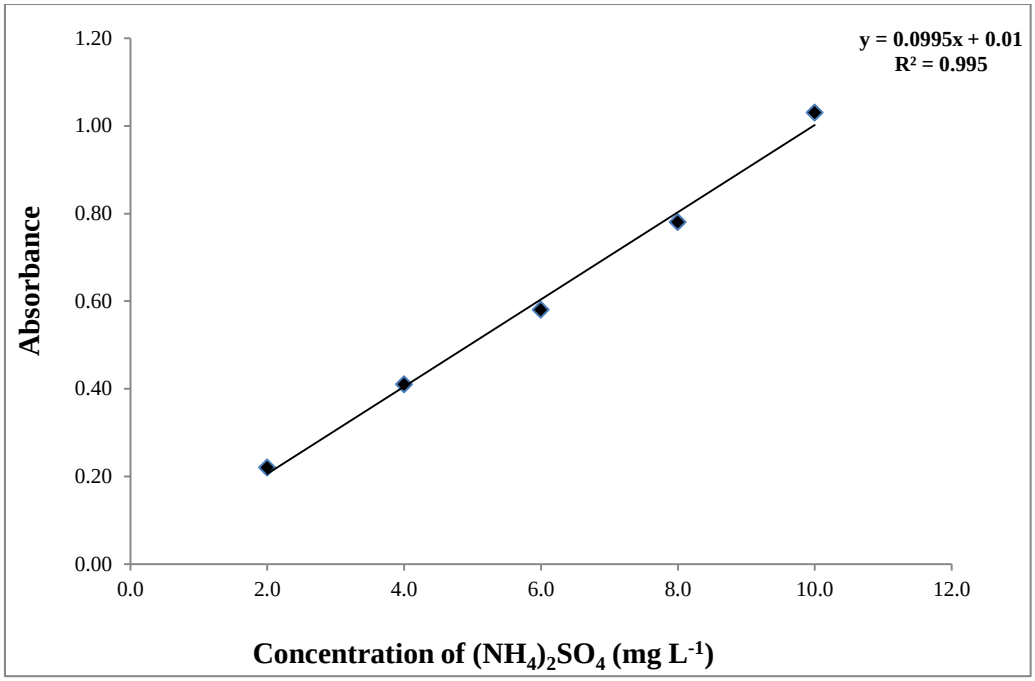

Fig. 3.7 Calibration curve for ammonium prepared from standard solution of $(NH_4)_2SO_4$

Calculation- Concentration of nitrite, nitrate, phosphate and ammonium was calculated by following formula-

$$\text{Concentration } \mu g\ ml-1 = \text{K factor} \times \text{Absorbance (OD)}$$

$$K\ factor = \frac{Absorbance\ (OD)}{Concentration}$$

3.15.8 Alkaline phosphatase activity- It was determined by following Tabatabai and Brenner (1969) method described as following-

- 1.0 gm 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 0.025mol L^{-1} p-nitrophenol phosphate and incubated for 1hr at 37ºC.
- After incubation, the mixture was mixed well with 1.0 ml of 0.5mol L^{-1} $CaCl_2$ and 4.0 ml of 0.5 mol L^{-1} NaOH and then filtered through a filter paper.
- The concentration (mg g^{-1} hr^{-1}) of product (p- nitrophenol) in filtrate was determined colorimetrically at 400nm. Activity was quantified by reference to a calibration curve construction using phenol standards under the same condition describe above.

3.15.9 Dehyrogenase activity- It determined by Casida et al (1964) method described as following-

- 2.0 g of soil was placed in 18mm×20mm glass tube and incubated with 2ml of 3% 2, 3, 5, tri-phenyl chloride (TTC) for 24h at 30°C.
- After incubation, 10 ml acetone was added, and the suspension was homogenized with intermittent agitation for 2hr (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 2ml buffer, instead of TTC, was used as control.
- Dehyrogenase activity was expressed in µg TPF/24hr.

3.16 Statistical analysis

The data were analyzed statistically by one way analysis of variance (SPSS, 20 Statistical Package and MS office 2010) using Duncan's Multiple Range Test (DMRT) and t test to determine the significance of difference among treatments at probability (p) 0.05.

3.17 Cost benefits analysis

The cost of wheat cultivation for all treatment was analyzed by manual calculation based on the price of commercial and chemical fertilizers available in the market. The cost-benefit analysis was also done by comparing the rates of market based fertilizers with formulated biofertilizers. The costs of manufacturing in the formulated fertilizers were based upon tentative price of manufacturing and its packaging. The details of cost benefit analysis have been described in detail in result section.

Chapter 4

Results

4. Results

4.1 Evaluation of plant growth promoting activities of the different isolates of *Trichoderma* and PGPR and its similar commercially available products in pot conditions.

4.1.1 Isolation of plant growth promoting rhizobacteria (PGPR)

Thirty three soil samples were collected from rhizosphere of wheat (*Triticum aestivum* L.) crop of wheat fields around Lucknow (Uttar Pradesh), India. Twenty three bacteria were isolated as described in the section 3.2 of material and methods from the soil samples. Out of twenty three bacterial samples two were selected for further studies based on best plant growth promoting activities (PGPAs).

4.1.2 Morphological characterization of PGPRs

All bacterial colonies appeared on Nutrient Agar Media were whitish, yellow or light green in color, smooth or rough in texture. According to Gram staining, strains S_1, S_6, SS_4, $S5_1$ and M_3 were Gram positive and all other strains were Gram negative. The growth of bacterial colonies was also determined visually. Out of 23 bacteria isolated, nine fast growing colonies were designated as S_3, S_6, S_7, $SS3_1$, SDA_1, SDA_2, SDA_3, M_3 and M_4, whereas two moderately growing colonies were named as $SS4_2$ and $S8_Y$ and twelve slow growing as S_1, S_2, S_5, S_9, SS_1, SS_4,$SS3_2$, $S5_1$, $S5_2$, $S8_W$, M_1 and M_2. Among these two (Fig. 4.1) were selected for further studies on growth and yield of wheat based on best PGPAs. All of the morphological and phenotypic characteristics of different bacterial isolates have been summarized in Table 4.1.

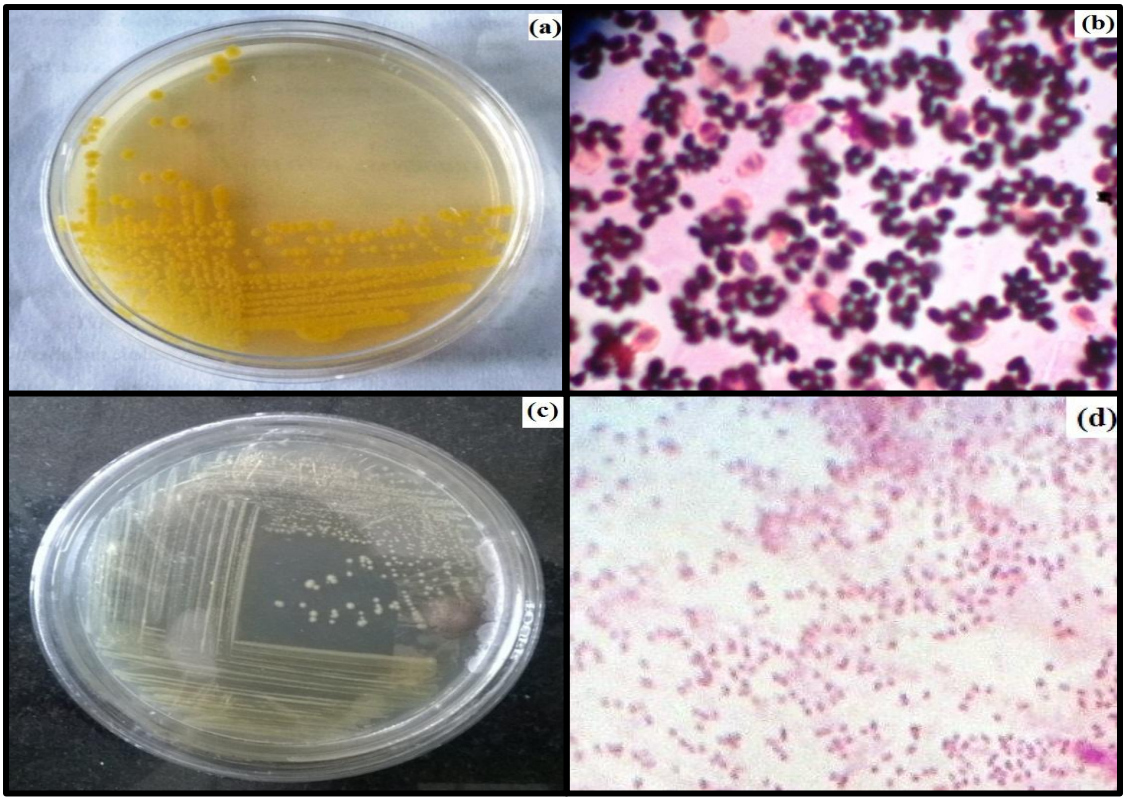

Fig. 4.1 Pure culture and microscopic image of isolate *Microbacterium phyllosphaerae* (a and b) and *Alcaligenes faecalis* (c and d)

Table 4.1 Phenotypic characteristics of different bacterial isolates

Isolates	Colony color	Growth rate	Morphology	Shape	Gram staining
S_1	White	Slow	Smooth	Rod	Positive
S_2	White	Slow	Smooth	Rod	Negative
S_3	Whitish	Fast	Rough	Rod	Negative
S_5	Green	Slow	Smooth	Rod	Negative
S_6	Light yellow	Fast	Smooth	Rod	Positive
S_7	Whitish	Fast	Rough	Rod	Negative
S_9	Whitish	slow	Rough	Rod	Negative
SS_1	White	Slow	Smooth	Short rod	Negative
SS_4	white	slow	Rough	Rod	Positive
$SS4_2$	white	Moderate	Smooth	Rod	Negative
$SS3_1$	white	Fast	Smooth	Rod	Negative
$SS3_2$	Whitish	Slow	Rough	Rod	Negative
$S5_1$	yellow	Slow	Smooth	Short rod	Positive

$S5_2$	White	Slow	Smooth	Rod	Negative
$S8_Y$	Pale yellow	moderate	Smooth	Rod	Negative
$S8_W$	White	Slow	Rough	Rod	Negative
SDA_1	Whitish	Fast	Smooth	Rod	Negative
SDA_2	White	Fast	Smooth	Rod	Negative
SDA_3	Whitish	Fast	Rough	Rod	Negative
M_1	White	Slow	Smooth	Rod	Negative
M_2	Whitish	Slow	Rough	Rod	Negative
M_3	White	Fast	Smooth	Rod	Positive
M_4	Light green	Fast	Smooth	Rod	Negative

4.1.3 Biochemical characterization of microbial isolates from rhizospheric soil

All of the bacterial isolates showed positive test for indole except for S_5, SS_4, M_1 and M_3 (Fig. 4.2a and Table 4.2) and for nitrate except S_5, S_2, $SS3_1$, $S8_Y$ and M_1 showed positive test nitrate (Fig. 4.2b and Table 4.2). The amylase test was also recorded positively in the bacterial isolates S_2, S_3, S_5, S_7, S_9, SS_4, $S8_W$, M_1, M_3 and M_4 whereas, other did not show it (Fig. 4.2c and Table 4.2). Among twenty three bacterial isolates only three namely $S5_1$, M_1 and M_3 showed positive citrate test and remaining strains showed negative results (Fig. 4.2d and Table 4.2). Likewise, only seven bacterial isolates S_1, S_5, SS_1, $S5_1$, $S8_W$, M_2 and M_4 showed positive test for H_2S production and remaining were negative (Fig. 4.2e and Table 4.2). All bacterial isolates except S_5, SS_4, $SS3_1$, M_2 and M_3 showed positive lipase test (Fig. 4.2f and Table 4.2). The protease test was positive only for twelve bacterial isolates namely S_2, S_5, S_7, S_9, SS_1, $SS3_2$, $S5_1$, $S8_Y$, SDA_1, SDA_2, SDA_3 and M_4 (Fig. 4.2g and Table 4.2) Whereas all bacterial isolates showed positive results for cellulase test (Fig. 4.2 h and Table 4.2). In contrast to these tests, none of the bacterial isolate showed positive test for urease and gelatin (Table 4.2).

Table 4.2 Biochemical characterizations of isolates

	Biochemical tests									
Strain	**Urease**	**Cellulase**	**H_2S**	**Lipase**	**Gelatin**	**Amylase**	**Citrate**	**Nitrate**	**Indole**	**Protease**
S_1	-	+	+	+	-	-	-	+	+	-
S_2	-	+	-	+	-	+	-	-	+	+
S_3	-	+	-	+	-	+	-	+	+	-
S_5	-	+	+	-	-	+	-	-	-	+
S_6	-	+	-	+	-	-	-	+	+	-
S_7	-	+	-	+	-	+	-	+	+	+
S_9	-	+	-	+	-	+	-	+	+	+
SS_1	-	+	+	+	-	-	-	+	+	+
SS_4	-	+	-	-	-	+	-	++	-	-
$SS4_2$	-	+	-	+	-	-	-	+	+	-
$SS3_1$	-	+	-	-	-	-	-	-	+	-
$SS3_2$	-	+	-	+	-	-	-	+	+	+
$S5_1$	-	+	+	+	-	-	+	++	+	+
$S5_2$	-	+	-	+	-	-	-	+	+	-
$S8_Y$	-	+	-	+	-	-	-	-	+	+
$S8_W$	-	+	+	+	-	+	-	+	+	-
SDA_1	-	+	-	+	-	-	-	+	+	+
SDA_2	-	+	-	+	-	-	-	+	+	+
SDA_3	-	+	-	+	-	-	-	+	+	+
M_1	-	+	-	+	-	+	+	-	-	-
M_2	-	+	+	-	-	-	-	+	+	-
M_3	-	+	-	-	-	+	+	+	-	-
M_4	-	+	+	+	-	+	-	+	+	+

(-) = negative for the test, positive (+) = positive for the test, (++) = more positive for different tests

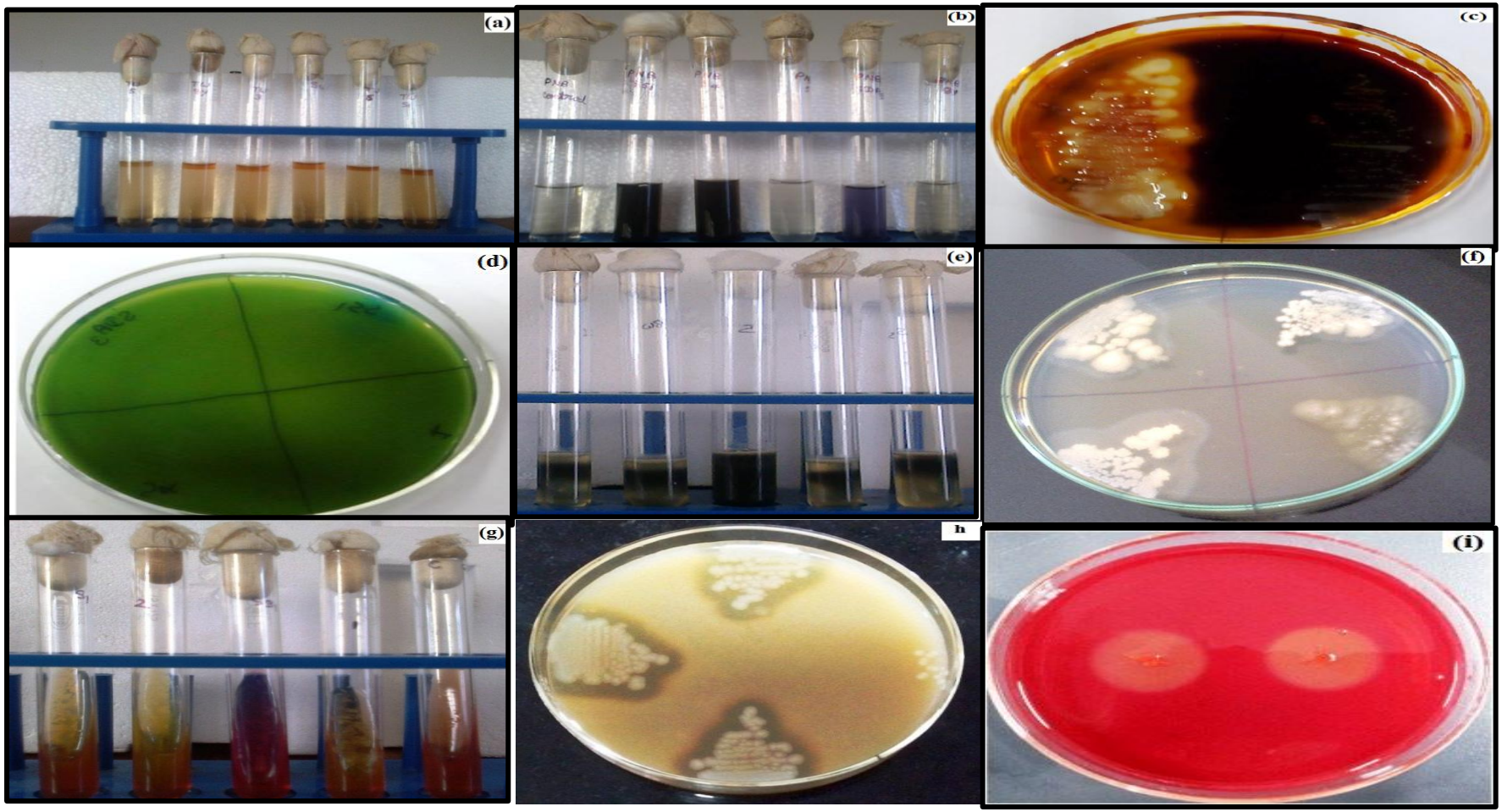

Fig. 4.2 Results of biochemical tests; Indole test (a), Nitrate test (b), Amylase test of isolates (c), Citrate test (d), H_2S test (e) Lipase test (f), Urease test (g), Protease test (h) and Cellulase test (i)

4.1.4 Isolation of *Trichoderma* spp.

Similar to PGPR two *Trichoderma* spp. were isolated from crop of wheat fields around Lucknow (Uttar Pradesh), India. The details of the process have been described in material and method section 3.2. Out of two *Trichoderma* species one was selected for further studies on the basis of PGPAs.

4.1.5 Morphological characterization of *Trichoderma* spp.

The *Trichoderma* colonies were green in color with concentric ring like structures. Out of two *Trichoderma* isolated one was fast growing on Potato Dextrose Agar (termed as T_1) and other was slow growing (T_2). Between these two *Trichoderma* species one (Fig. 4.3) was selected for further studies for enhancing growth and yield of wheat on the basis of its PGPAs. All of the morphological and phenotypic characteristics of different fungal isolates have been summarized in Table 4.3.

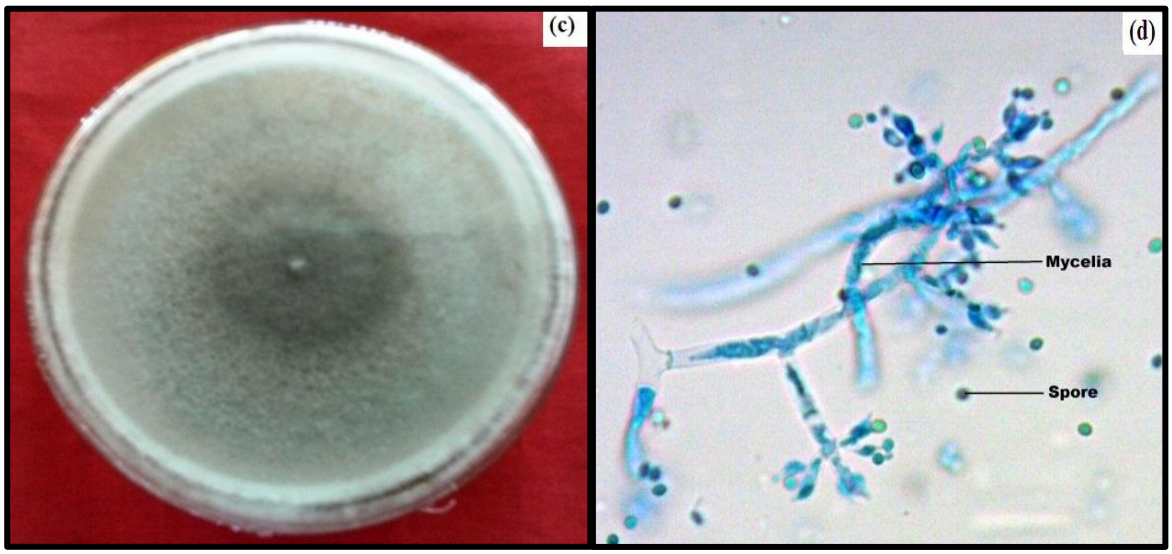

Fig. 4.3 Pure culture (a) and microscopic images of isolate *Trichoderma virens* (b)

Table 4.3 Phenotypic characteristics of fungal isolates

Isolates	Colony color	Growth rate	Morphologically	Shape
T_1	Green	Slow	Cotton like and powdery	Finger like hyphae
T_2	Green	Fast	Cotton like and powdery	Finger like hyphae

4.1.6 Plant growth promoting characteristics of the isolated soil microbes

4.1.6.1 Phosphate solublization

Out of twenty three isolated strains 76% isolates were able to solubilize phosphate on Pikovskaya agar media (Table 4.4). Among phosphate solubilizing bacteria one strain named SDA_3 showed maximum phosphate solubilized zone i.e. 2.0 psi by making clear zone and $SS3_2$ showed smallest clear zone 1.0 psi. (Fig 4.4 a and h).

4.1.6.2 Hydrogen cyanide production (HCN) production

None of the isolates showed positive result for HCN production as per observation of color change from yellow to orange in filter papers soaked in picric acid (Fig 4.4 b and Table 4.4).

4.1.6.3 Indole acetic acid (IAA) production

IAA production ability was checked in all isolates on the basis of morphologically color change as well as spectrophotometric measurement of the color. The SDA_3 (100.32 µg ml^{-1}) showed maximum IAA production and S_3 (61.69 µg ml^{-1}) showed minimum IAA production in in vitro conditions in labeled strain of isolated bacteria (Fig 4.4 c and Table 4.4).

4.1.6.4 Ammonium production

Among all isolates 76% isolates showed positive result from ammonium production which is indicator of their ability to fix nitrogen. The bacterial strain labeled $S5_1$ produced maximum ammonium and S_1 produced minimum which is evident by yellow or brown color appearance after addition of Nessler's reagent in their solution (Fig 4.4 d and Table 4.4).

4.1.6.5 Siderophore production

Siderophore production was shown by 56% bacterial isolates, forming orange colored zone around the colony is qualitative analysis of siderophore production on CAS agar plates. Bacterial strain $S5_1$ showed highest siderophore production ability with larger orange zone on CAS plate (Fig 4.4 e and f and Table 4.4).

4.1.6.6 Nitrogen fixation

Among all isolates only 56 % isolates showed positive results for nitrogen fixation which was observed by their growth on nitrogen free media plate (Fig 4.4 g and Table 4.4).

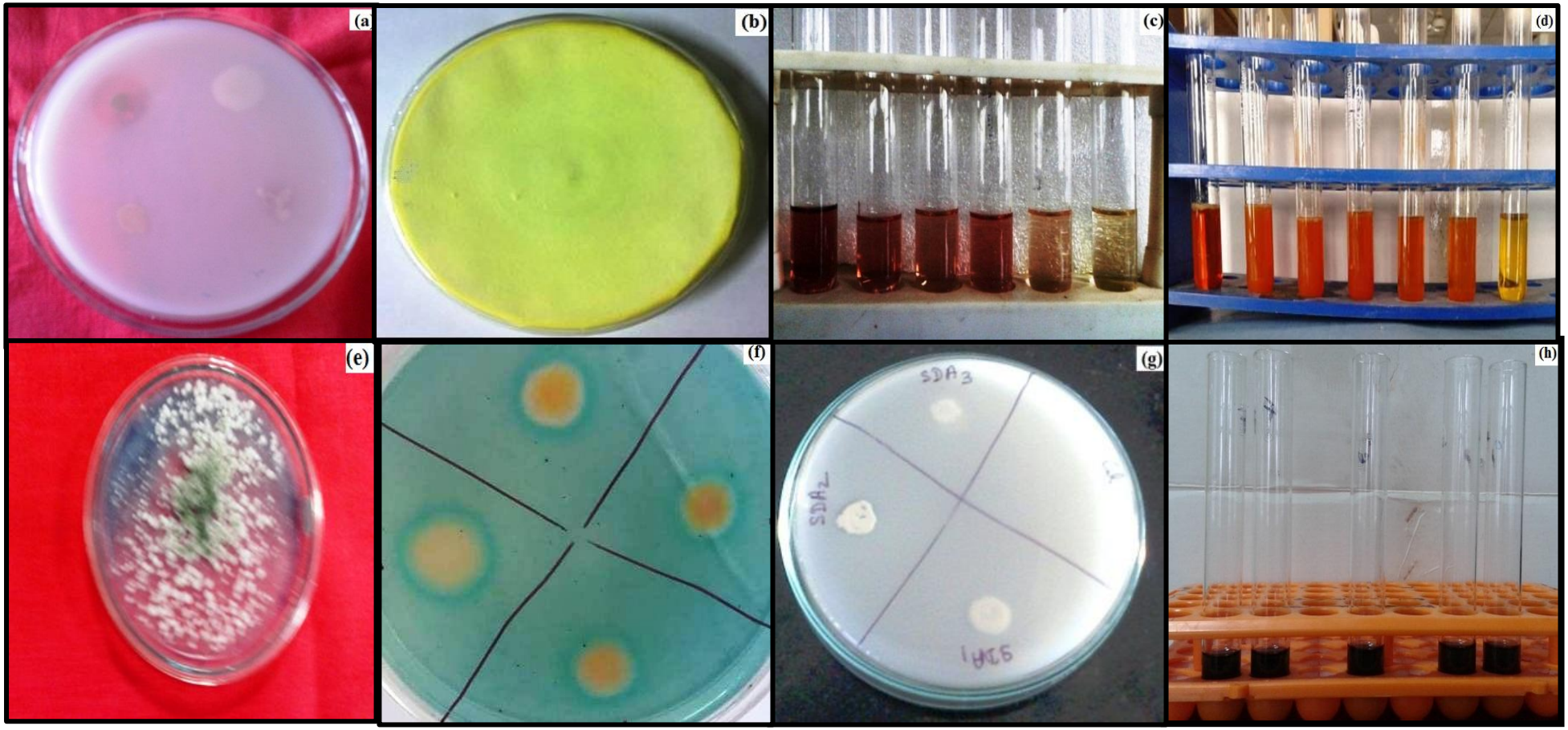

Fig. 4.4 Plates showing some PGP activities detectable visually; a) Phosphate solubilization by bacterial isolates, h) Phosphate solubilization by fungal isolate, b) HCN test (no change in color) for microbial isolates, c) Production of IAA by microbial isolates, d) Ammonia production by microbial strains, e) Siderophore production by fungal isolates, f) Siderophore production by bacterial isolates and g) Nitrogen fixation by microbial isolates

Table 4.4 Plant growth promoting properties of rhizospheric bacteria and fungi for the study (quantitative and qualitative)

Strains	Nitrogen Fixation	Phosphate Solubilization (Psi)	Ammonia Production	Siderophore production	HCN Production	IAA Production ($\mu g\ ml^{-1}$)
S_1	-	+(1.3)	+	-	-	-
S_2	+	-	+	+	-	-
S_3	+	+(1.2)	+	-	-	+(61.69)
S_5	-	+(1.5)	+	-	-	+(72.84)
S_6	+	+(2.7)	+	-	-	-
S_7	-	-	+	+	-	-
S_9	+	+(1.6)	+	+	-	+(74.74)
SS_1	-	+(1.4)	+	-	-	-
SS_4	-	-	+	+	-	-
$SS4_2$	-	-	-	-	-	+(78.69)
$SS3_1$	+	+(1.2)	-	-	-	-
$SS3_2$	+	+(1.0)	+	+	-	+(72.92)
$S5_1$	+	+(1.5)	++	++	-	++(99.84)
$S5_2$	-	+(1.2)	-	+	-	+(75.37)
$S8_Y$	+	+(1.1)	-	+	-	-
$S8_W$	+	-	-	+	-	-
SDA_1	+	+(1.5)	+	-	-	-
SDA_2	+	+(1.2)	+	-	-	+(75.30)
SDA_3	+	++(2.0)	+	+	-	++(100.32)
M_1	-	-	+	+	-	+(79.21)
M_2	+	+(1.3)	+	-	-	+(77.59)
M_3	-	+(1.2)	++	-	-	-
M_4	+	+(1.2)	-	+	-	-
			***Trichoderma* spp.**			
T_1	ND	+(157 $\mu g\ ml^{-1}$)	+	+	-	++(95.19)
T_2	ND	++(227 $\mu g\ ml^{-1}$)	+	+	-	++(99.78)

*(-) = negative for the test; Positive (+) = positive for the test, (++) = highly positive for the test and ND = Not detected

4.1.7 Compatibility test among selected microbial isolates (*Alcaligenes faecalis, Microbacterium phyllosphaerae* and *Trichoderma virens*)

The compatibility among the selected three isolates *T. virens, A. faecalis and M. phyllosphaerae* was checked and results showed absence of inhibition zone around the colonies which indicated that these were compatible with each other (Fig. 4.5).

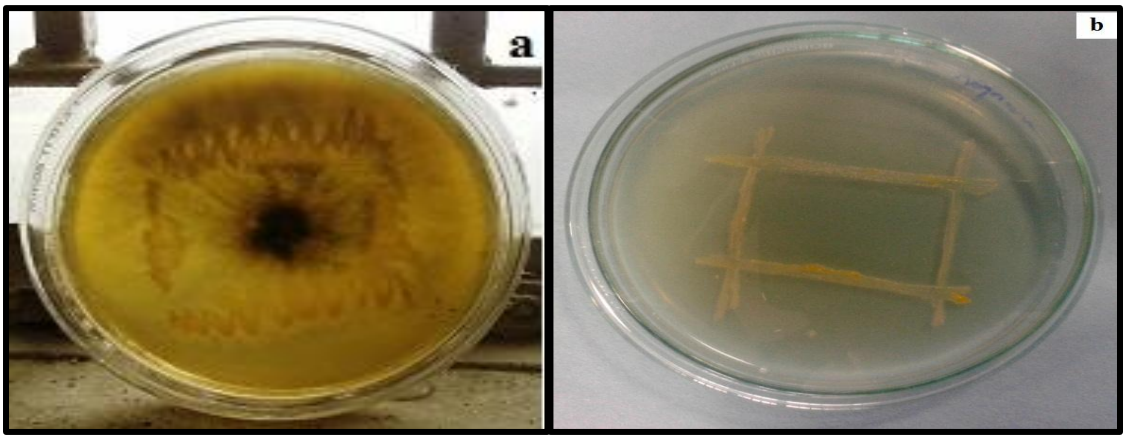

Fig. 4.5 Compatibility of *Trichoderma virens* with *Alcaligenes faecalis* and *Microbacterium phyllosphaerae* (a), Compatibility between (*A. faecalis* and *M. phyllosphaerae*) (b)

4.1.8 Genotypic characterization of microbial isolates

4.1.8.1 16S rRNA sequencing and phylogenetic analysis of bacterial isolates

After the morphological and biochemical characterization, bacterial isolates were further characterized by 16S rRNA gene sequencing (Fig. 4.6 and 4.7) isolates were characterized at genotypic level. On the basis of phylogenetic analysis these isolates were identified as strains of different genera belonging to *Microbacterium phyllosphaerae* ($S5_1$) and *Alcaligenes faecalis* (SDA_3). Further, the sequence data of both isolates were submitted to GenBank (NCBI) and allotted their respective accession numbers (Table 4.5).

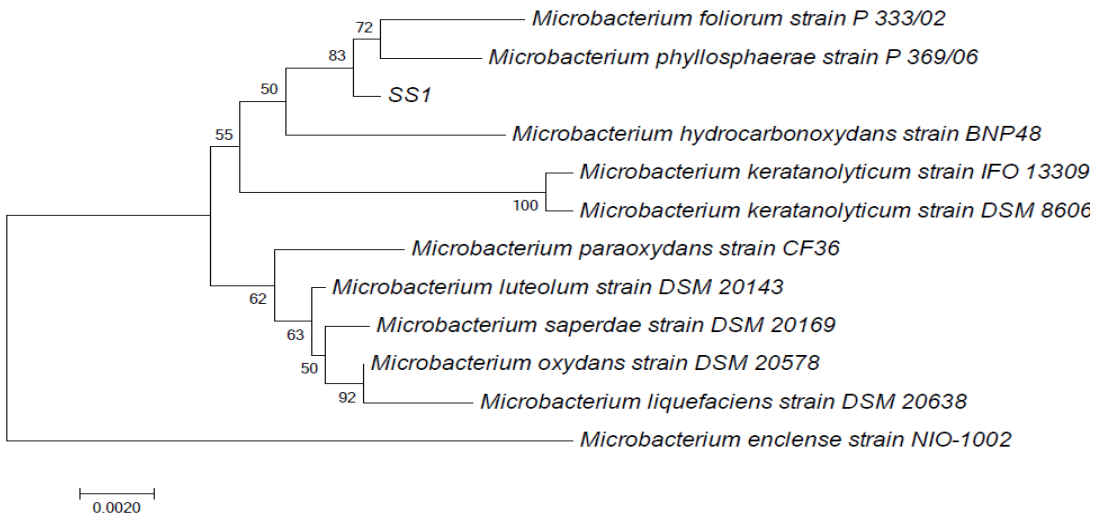

Fig. 4.6 Phylogenetic tree of bacterial isolate *Microbacterium phyllosphaerae*

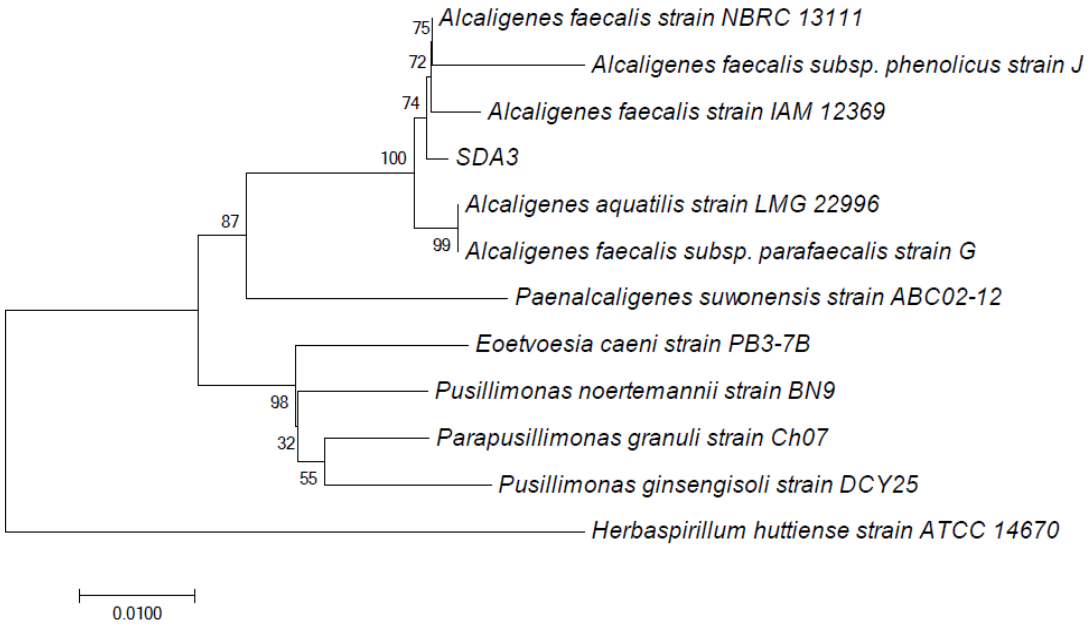

Fig. 4.7 Phylogenetic tree of bacterial isolate *Alcaligenes faecalis*

4.1.8.2 18S rRNA sequencing and phylogenetic analysis of fungal isolates

The selected fungal isolate was further characterized by 18S rRNA gene sequencing (Fig. 4.8) at its genotypic level. On the basis of phylogenetic analysis, this isolate belonged to *Trichoderma virens* (T_2). Further, the sequence data of fungal isolate was submitted to GenBank (NCBI) and allotted its respective accession numbers (Table 4.5).

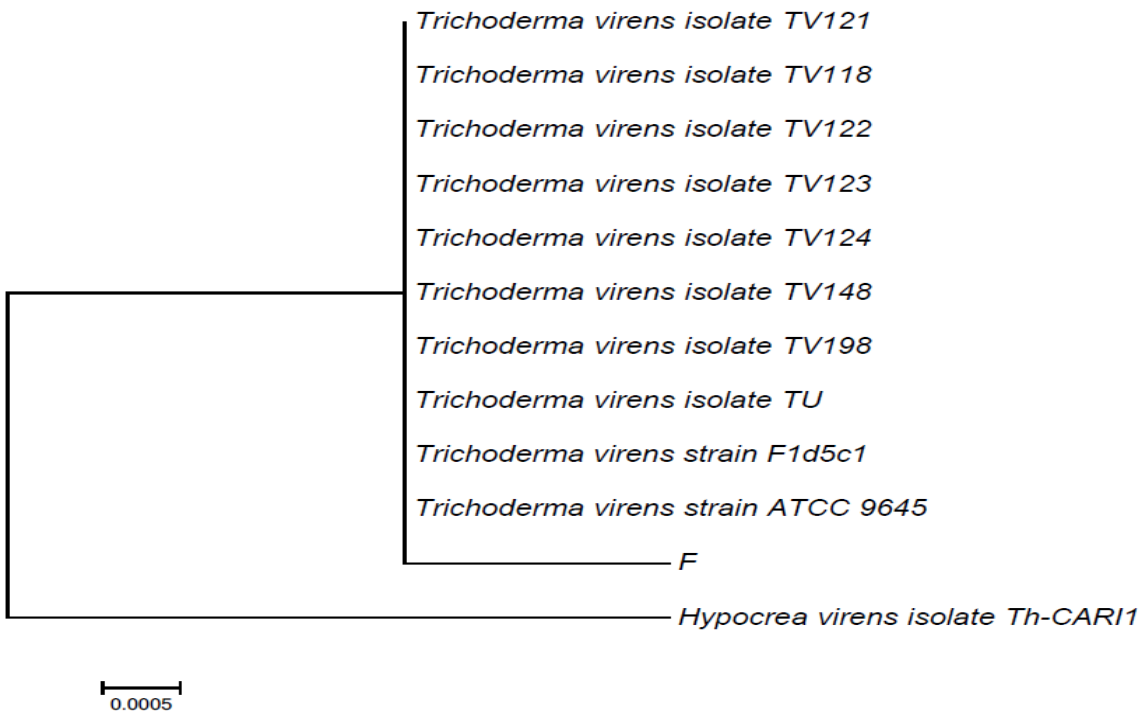

Fig. 4.8 Phylogenetic tree of fungal isolate *Trichoderma virens*

Table 4.5 Details of isolated strains from host plant wheat (*Triticum aestivum* L.) achieved after submission in National Center for Biotechnology Information (NCBI)

Isolates	Strain (Identified as)	Accession no.
$S5_1$	*Microbacterium phyllosphaerae*	**KY936459.1**
SDA_3	*Alcaligenes faecalis*	**KY936458.1**
T_2	*Trichoderma virens*	**KY962814.1**

4.2 Evaluation of plant growth promoting activities of the different isolates of *Trichoderma* and PGPR and its similar commercially available products in pot conditions.

To assess the plant growth promoting activities (PGPA) of selected isolates of *Trichoderma virens*, rhizobacteria *Alcaligenes faecalis* (SDA_3) and *Microbacterium phyllosphaerae* ($S5_1$) in comparison to the chemical fertilizers Urea and DAP were applied for 40, 80 and 120 days in pots placed in house during the cultivation of wheat (*Triticum aestivum* L.). The results were compared with control without any amendment. The growth parameters examined were root and shoot length, number of roots and leaves along with fresh and dry weights of wheat plants at different intervals.

4.2.1 Effects on root length of wheat

The root length of control plants were recorded to be 4.67, 8.33 and10.67 cm after 40, 80 and 120 days after sowing (DAS). The plants treated with commercial biofertilizers (Commer.) showed 29.43, 12.44 and 32.47 % increase root length in comparison to control plant after 40, 80 and 120 DAS. Whereas, treatment of selected microbial isolated *A. faecalis* (SDA_3), *M. phyllosphaerae* ($S5_1$) and *T. virens* (T_2) were further improved the root length by 8.21, 18.20 and 34.34 %, 22.36, 13.16 and 39.22 % and 19.14, 9.44 and 46.91 %, respectively after 40, 80 and 120 DAS, respectively.

Likewise, the consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* showed 37.14, 25.20 and 47.19 %, 58.57, 32.0 and 53.13 % and 67.86, 56.80 and 63.75 % increase in root length, respectively after 40, 80 and 120 DAS. The consortium of three microbes comprising of two rhizobacteria and one fungus i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* showed the highest increase in the root length in comparison to control as 75.0, 74.40 and 75.63 % after 40, 80 and 120 DAS. Further, chemical fertilizer urea and DAP (U+D) were also examined which showed 82.86, 98.0 and 97.50 % increase in the root length in comparison with control after 40, 80 and 120 DAS. The results showed that consortium of isolates exhibited higher potential of enhancing root length than individual microbial treatment.

The trend of enhancing root length at the time of harvesting was Control< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens* < consortium of *A. faecalis* and

M. phyllosphaerae< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* < Urea and DAP as shown in Fig. 4.9.

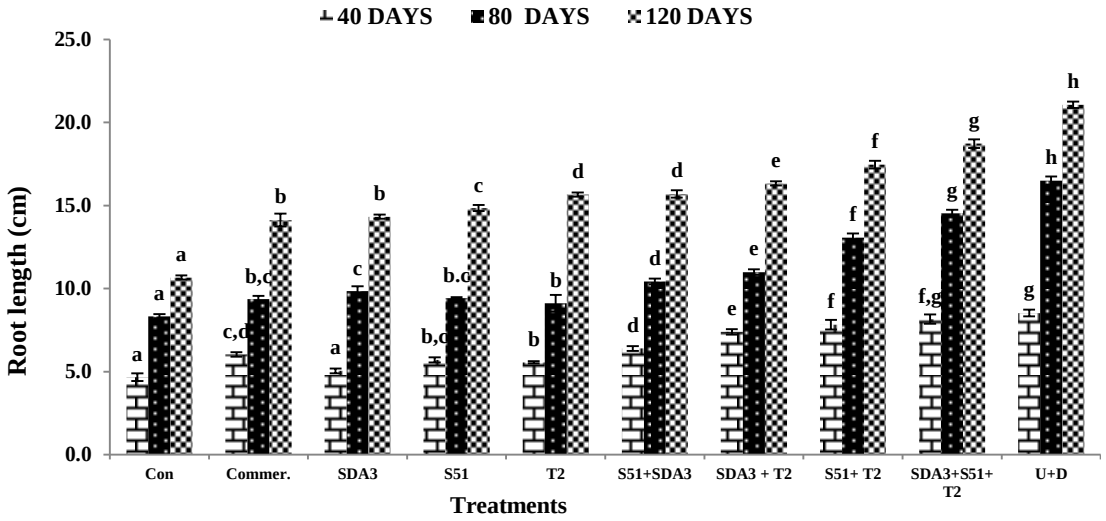

Fig. 4.9 Effect of different treatments on root length of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. The alphabets are significant differences between different treatments. [Where, Con= Control (without any treatment), Commer.= Commercial biofertilizer market based (recommended dose), SDA_3= *Alcaligenes faecalis*, $S5_1$= *Microbacterium phyllosphaerae*, T_2= *Trichoderma virens*, $S5_1+SDA_3$= Consortium of *Microbacterium phyllosphaerae* and *Alcaligenes faecalis*, SDA_3+T_2= Consortium of *Microbacterium phyllosphaerae* and *Trichoderma virens*, $S5_1+T_2$ = Consortium of *Alcaligenes faecalis* and *Trichoderma virens*, $SDA_3+S5_1+T_2$= Consortium of *Microbacterium phyllosphaerae*, *Alcaligenes faecalis* and *Trichoderma virens* and U+D= chemical fertilizer Urea and di-ammonium phosphate (recommended dose)].

4.2.2 Effect on shoot length of wheat

The average shoot length of control was recorded to be 20.27, 44.50 and 54.20 cm after 40, 80 and 120 DAS, respectively. Treatment of market based biofertilizer enhanced shoot length of wheat plants by 31.88, 15.96 and 26.26 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, treatment of individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased the shoot length by 30.76, 17.54 and 28.08 %, 34.54, 18.46 and 30.09 % and 37.34, 20.28 and 31.67 % after 40, 80 and 120 DAS, respectively.

Higher enhancements in the shoot length were observed by the treatment of consortium of two treatments i.e. consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* in comparison to control and individual treatments. It increased shoot length by 40.30, 29.59 and 37.52 %, 53.95, 31.39 and 39.11 % and 60.53, 35.81 and 41.24 %, after 40, 80 and 120 DAS, respectively. Further, highest increase in the sooth length of wheat plants were observed by the consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. The results showed 65.13, 40.45 and 43.02 % increase in the shoot length after 40, 80 and 120 DAS. Whereas, treatment of chemical fertilizer Urea and DAP enhanced shoot length by 69.41, 44.72 and 46.01 % after 40, 80 and 120 DAS.

Control< Commer.< *A. faecalis* < *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*< Urea and DAP as shown in Fig. 4.10.

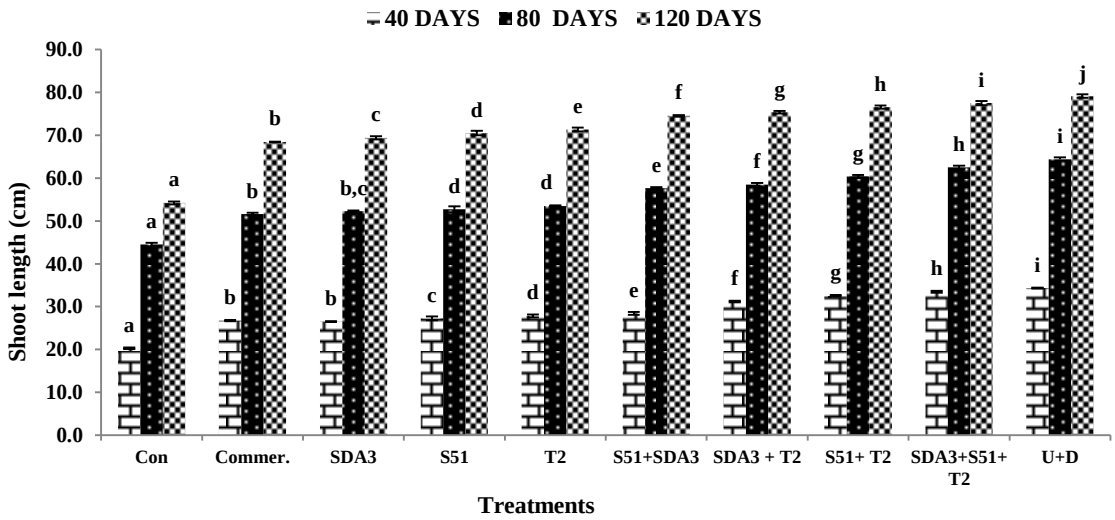

Fig. 4.10 Effect of different treatments on shoot length of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at p< 0.05 (remaining details are similar to Fig. 4.9)

4.2.3 Effect on number of roots of wheat

The numbers of roots of wheat plants were counted by manual counting. The average number of roots in control wheat plants was 5.33, 10.33 and 11.67 after 40, 80 and

120 DAS, the number of roots was increased by the application of commercial biofertilizers was 43.75, 9.68 and 2.86 % in comparison with control after 40, 80 and 120 DAS, respectively. Whereas, considerable higher increase in number of roots were shown by the treatment of individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens*. These treatments enhanced no. of roots by 62.50, 6.45 and 8.57 %, 75.0, 9.68 and 8.57 % and 81.25, 12.90 and 14.29 % in comparison with control after 40, 80 and 120 DAS, respectively.

Likewise, consortium of two isolates like *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* exhibited higher increase in number of roots in comparison with control as 125.0, 48.39 and 45.71 %, 143.75, 77.42 and 68.57 % and 150.0, 90.32 and 91.43 % after 40, 80 and 120 DAS, respectively. On the other hand, the highest increase was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* which improved number of roots by 156.25, 116.13 and 97.14 % in comparison with control after 40, 80 and 120 DAS, respectively. Whereas, treatment of Urea and DAP also enhanced root number by 175.0, 125.81 and 111.43 % in comparison to control after 40, 80 and 120 DAS, respectively. The trend of increase in no. of roots was also found to be same as root and shoot length at the time of harvesting as presented in Fig. 4.11.

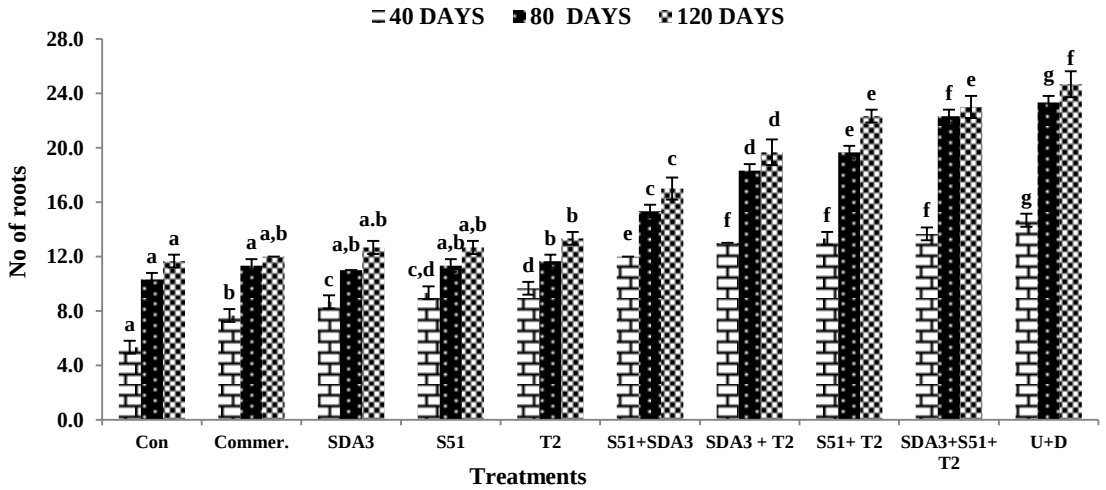

Fig. 4.11 Effect of different treatments on number of roots of wheat (T*riticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p < 0.05$ (remaining details are similar to Fig. 4.9)

4.2.4 Effect on number of leaves of wheat

The average number of leaves in wheat plants of control was recorded 4.67, 6.67 and 3.0 % after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers increased the number of leaves by 35.64, 54.95 and 111.0 % in comparison to control after 40, 80 and 120 DAS, respectively. Treatment of individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced number of leaves by 42.93, 60.05 and 122.33%, 42.93, 60.05 and 111.0 % and 50.0, 69.95 and 155.67 % after 40, 80 and 120 DAS, respectively.

Whereas, consortium of the two isolates i.e. consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* showed higher increase in number of leaves by 50.0, 80.0 and 155.56 %, 64.29, 95.0 and 155.56 % and 71.43, 100.0 and 177.78 % respectively, in comparison to control after 40, 80 and 120 DAS. Further, the highest increase was obtained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. This treatment enhanced the number of leaves by 85.71, 100.0 and177.78 % in comparison with control after 40, 80 and 120 DAS, respectively. Whereas, Urea and DAP showed 100.0, 115.0 and 200.0 % increase after 40, 80 and 120 DAS. The trend of increase in number of leaves were also same as root and shoot length as well as number of roots at harvesting time as represented in Fig. 4.12.

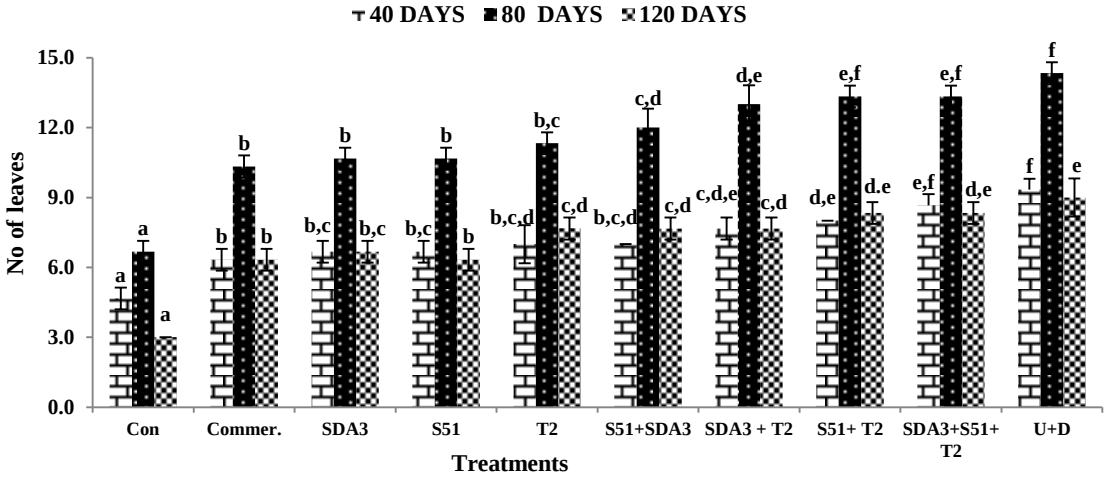

Fig. 4.12 Effect of different treatments on number of leaves of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p < 0.05$ (remaining details are similar to Fig. 4.9)

4.2.5 Effect on fresh weight of wheat

The fresh weight of wheat was recorded immediately after uprooting in grams. The average fresh weight of control wheat plants were 2.84, 6.75 and 2.98 g plant^{-1} after 40, 80 and 120 DAS, respectively. The commercial biofertilizers showed increase in the fresh weight in comparison to control plants by 92.60, 9.98 and170.66 % after 40, 80 and 120 DAS, respectively. Further, selected isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* also enhanced fresh weight by 99.53, 40.59 and 176.26 %, 79.55, 42.07 and 180.18 % and 128.79, 52.10 and 203.25 % in comparison to control after 40, 80 and 120 DAS, respectively.

Whereas, consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced fresh weights of wheat by 161.46, 67.21 and 221.61%, 242.77, 71.06 and 259.46 % and 248.18, 86.67 and 281.08 % in comparison to control after 40, 80 and 120 DAS, respectively. But, among all of treatments of biofertilizers the maximum increase in fresh weight was secured by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 273.33, 89.63 and 287.01 % in comparison to control after40, 80 and 120 DAS, respectively. Treatment of chemical fertilizer U+D also enhanced fresh weight by 283.43, 95.95 and 316.13 %, in comparison to control after 40, 80 and 120 DAS, respectively. The trend of increase in fresh weight were also same as length of root and shoot , number of roots and leaves at the time of harvesting as shown in Fig. 4.13.

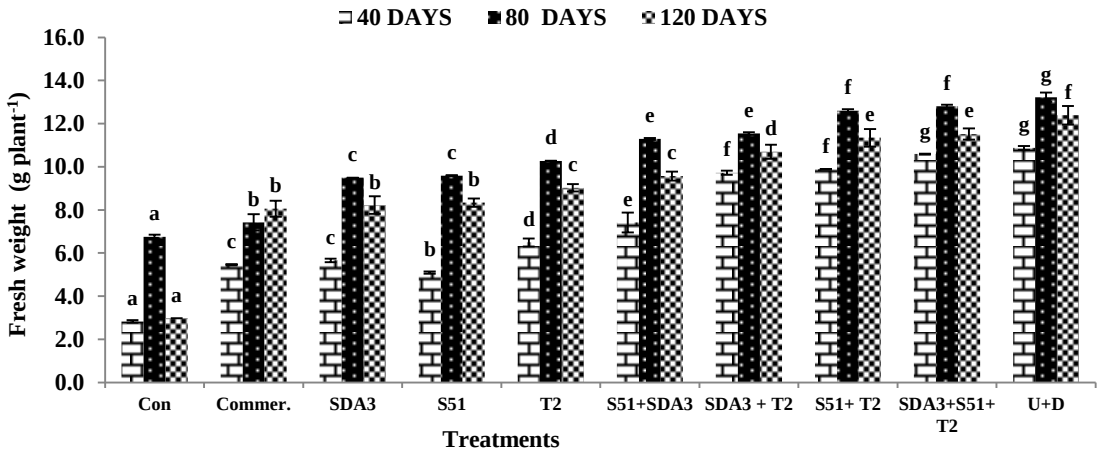

Fig. 4.13 Effect of different treatments on fresh weight of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$ (remaining details are similar to Fig. 4.9)

4.2.6 Effect on dry weight of wheat

The dry weight of a plant demonstrates the amount of protoplasm after complete dehydration. The dry weight of control plants was 0.13, 1.17 and 1.82 g plant^{-1} after 40, 80 and 120 DAS, respectively. The treatment of commercial biofertilizers was enhanced dry weight by 117.95, 34.38 and 32.54 % in comparison to control after 40, 80 and 120 DAS, respectively. Likewise, isolated rhizobacteria *A. faecalis, M. phyllosphaerae* and fungi *T. virens* significantly enhanced dry weight by 153.85, 19.89 and 42.60 %, 123.08, 7.39 and 44.97 % and 179.49, 29.83 and 55.76 % in comparison to control after 40, 80 and 120 DAS, respectively.

Whereas, consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* significantly enhanced dry weight in comparison to control by 207.69, 32.10 and 66.36 %, 253.85, 36.93 and 72.03 % and 317.95, 38.07 and 77.70 % after 40, 80 and 120 DAS, respectively. But, the maximum upsurge in dry weight was recorded by treatment of consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 371.79, 40.34 and 91.04 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, chemical fertilizer Urea and DPA also improved dry weight by 435.90, 47.16 and 97.62 % after 40, 80 and 120 DAS, respectively. The trend of increase in dry weight was also same as length of root and shoot, number of roots and leaves and fresh weight at the time of harvesting as shown in Fig. 4.14.

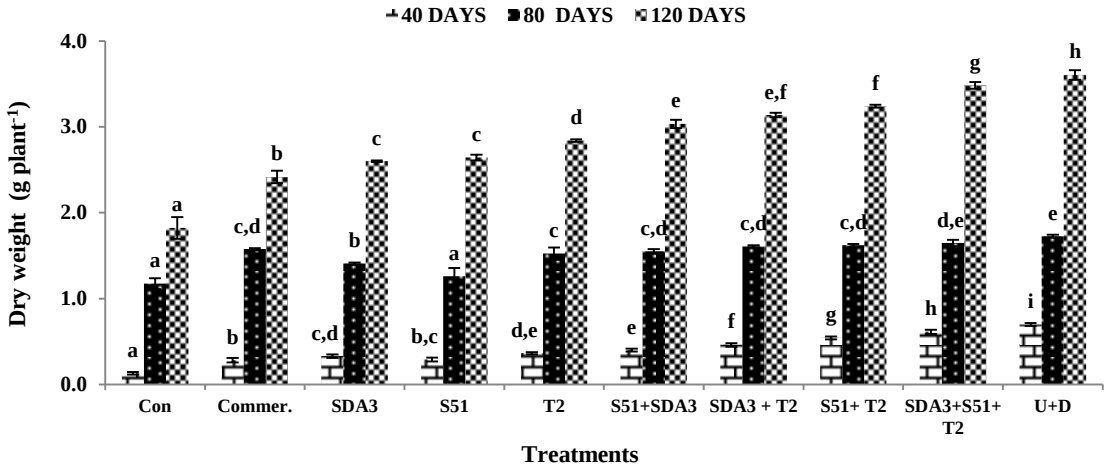

Fig. 4.14 Effect of different treatments on dry weight of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$ (remaining details are similar to Fig. 4.9)

4.3 Evaluation of best performing isolates in different combinations for growth and productivity of wheat in earthen pots.

4.3.1 Effects of best performing microbial isolates on wheat crop for growth and productivity in earthen pots

On the basis of optimization processes (as described in sec. 4.2), the best performing isolates were identified. The details have been presented in Table 4.6. Among three selected isolates, two were rhizobacteria namely *Alcaligenes faecalis* (SDA_3) and *Microbacterium phyllosphaerae* ($S5_1$) and one was fungus *Trichoderma virens* (T_2). The average root length of wheat plants without any treatment (control) was 10.67 cm which became 14.33, 14.85 and 15.67 cm after treatment of *A. faecalis*, *M. phyllosphaerae* and *T. virens*, respectively. Likewise shoot length also increased from 54.20 cm to 69.42, 70.51 and 71.37 cm, respectively. In the same manner, number of roots (from 11.67 to 12.67, 12.67 and 13.33) and leaves (from 3 to 6.67, 6.33 and 7.67) and fresh (2.98 g $plant^{-1}$ to 8.22, 8.34 and 9.03 g $plant^{-1}$) and dry weights (1.82 g $plant^{-1}$ to 2.60, 2.64 and 2.84 g $plant^{-1}$) also increased as given in Table 4.6.

The productivity of control plants was 0.79 g $plant^{-1}$ which was observed to increase by treatments of isolates *A. faecalis* (1.26 g $plant^{-1}$), *M. phyllosphaerae* (1.31 g $plant^{-1}$) and *T. virens* (1.32 g $plant^{-1}$). Although, individually all three isolates improved growth parameters at harvesting time as well as productivity in comparison to control, but the highest enhancements and productivity were recorded by the fungus *Trichoderma virens.* Thus, isolates of *Trichoderma virens* (T_2) showed highest increase in the growth parameters and productivity of wheat plants in comparison to *Alcaligenes faecalis* (SDA_3) and *Microbacterium phyllosphaerae* ($S5_1$) at the time of harvesting. Thus, it can be concluded that the best performing isolate was and *T. virens* followed by *M. phyllosphaerae* and *A. faecalis*. The effects of *A. faecalis*, *M. phyllosphaerae* and *T. virens* after 40 DAS (Fig. 4.15) and at harvesting time (Fig. 4.16) in comparison to control over growth and development of wheat plants in earthen pots have been demonstrated in Fig. 4.16 and 4.17.

Table 4.6 Comparison of effect of selected isolates on growth parameters of wheat (*Triticum aestivum* L.) at harvesting time in comparison to control (no fertilizer) in earthen pots

Growth parameters	Con	SDA_3	$S5_1$	T_2
Root length (cm)	10.67 ± 0.12^{a}	14.33 ± 0.12^{b}	14.85 ± 0.19^{c}	15.67 ± 0.12^{d}
Shoot length (cm)	54.20 ± 0.33^{a}	69.42 ± 0.39^{c}	70.51 ± 0.50^{d}	71.37 ± 0.45^{e}
No. of roots	11.67 ± 0.47^{a}	$12.67\pm0.47^{a,b}$	$12.67\pm0.47^{a,b}$	13.33 ± 0.47^{b}
No. of leaves	3.00 ± 0.0^{a}	$6.67\pm0.47^{b,c}$	6.33 ± 0.47^{b}	$7.67\pm0.47^{c,d}$
Fresh weight (g plant^{-1})	2.98 ± 0.01^{a}	8.22 ± 0.40^{b}	8.34 ± 0.19^{b}	9.03 ± 0.18^{c}
Dry weight (g plant^{-1})	1.82 ± 0.13^{a}	2.60 ± 0.01^{c}	2.64 ± 0.03^{c}	2.84 ± 0.02^{d}
Productivity (g plant^{-1})	0.79±0.01	1.26±0.01	1.31±0.01	1.32±0.0

*Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. [Where, Con= Control (without any treatment), SDA_3= *Alcaligenes faecalis*, $S5_1$= *Microbacterium phyllosphaerae* and T_2= *Trichoderma virens*)]

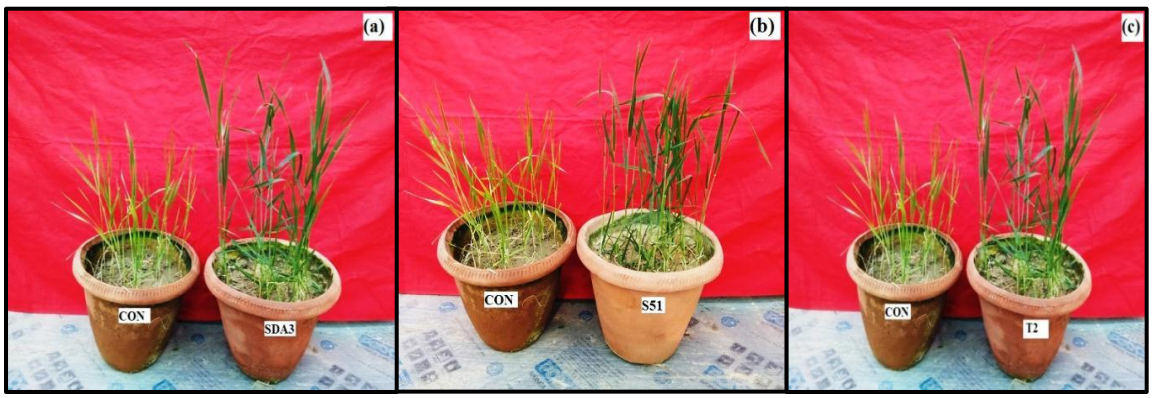

Fig. 4.15 Effect of best isolates SDA_3 (a), $S5_1$ (b) and T_2 (c) over growth and development of wheat (*Triticum aestivum* L.) after 40 DAS in comparison to control (CON). (All details are same as described in Fig. 4.9)

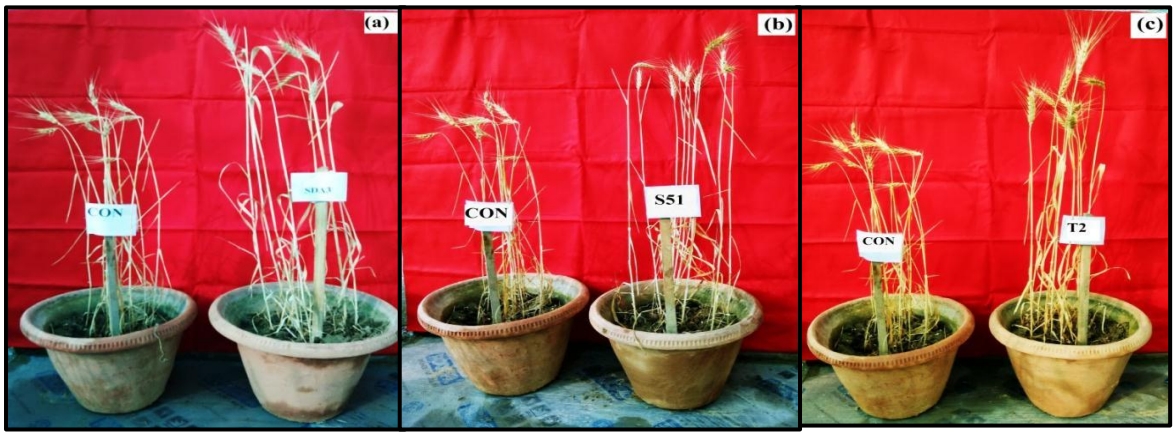

Fig. 4.16 Effect of SDA_3 (a), $S5_1$ (b) and T_2 (c) over growth and development of wheat (*Triticum aestivum* L.) at harvesting time in comparison to control (CON) (All details are same as described in Fig. 4.9)

4.3.2 Effect of combinations of best performing isolates on growth parameters and productivity of wheat in earthen pots

The best performing isolates were used in consortium to observe effects of various combinations over growth parameters of wheat plants. Four combinations were developed for this purpose, first combination included consortium of two of rhizobacteria *M. phyllosphaerae* and *A. faecalis* ($S5_1$+SDA_3), second was consortium of one rhizobacteria *M. phyllosphaerae* and one fungus *T. virens* ($S5_1$+$T_{2)}$, third was consortium of second rhizobacteria (*A. faecalis*) and fungus *T. virens* (SDA_3+T_2) and last included consortium of two rhizobacteria *A. faecalis* and *M. phyllosphaerae* and one fungus *T. virens* (SDA_3+$S5_1$+T_2).

The consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* increased root length of wheat plants as it was recorded to be 10.67 cm in control which increased and became 15.70, 16.33 and 17.47 cm, respectively. The highest growth was recorded from consortium of three isolate which included two rhizobacteria and one fungus i.e. *A. faecalis, M. phyllosphaerae* and *T. virens*. The root length enlarged from 10.67 to 18.73 cm by the effect of this consortium. Further, shoot length of control was 54.20 cm and treatment of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens*; and *A. faecalis, M. phyllosphaerae* and *T. virens* enhanced it to become 74.53, 75.40, 76.55 and 77.52 cm, respectively. Likewise, all other growth parameters (no. of roots and leaves and fresh and dry weight) were also increased in comparison to control as presented in Table 4.7 and Fig. 4.17. Thus, it may be concluded that in comparison to consortium of two isolates (*A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens*), consortium of three isolates (*A. faecalis, M. phyllosphaerae* and *T. virens*) significantly enhanced growth parameters of wheat plants in earthen pots.

The enhancements in growth parameters of wheat were recorded maximum by treatment of consortium of *A. faecalis, M. phyllosphaerae* and *T. virens* followed by consortium of *M. phyllosphaerae* and *T. virens*, *A. faecalis* and *T. virens* and *A. faecalis* and *M. phyllosphaerae* at harvesting time. The effects of these treatments over growth and development of wheat in earthen pots after 40 DAS (Fig. 4.18) and at

harvesting time (Fig. 4.19) in comparison to control have been represented in Fig 4.18 and 4.19.

The grain yield of control plants were recorded to be 0.79 g plant^{-1} which was enhanced by the effect of consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* and became 1.43, 1.61 and 1.70 g plant^{-1} respectively. The percentage increase was calculated to be 81.27, 104.08 and 115.92 % by *A. faecalis*, *M. phyllosphaerae* and *T. virens*, respectively in comparison to control. Further, the highest productivity was observed by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 1.84 g plant^{-1} i.e. about 133.66% of control. Whereas, treatment of commercial and chemical fertilizers Urea and DAP also enhanced productivity as recorded to be 1.11 (40.70%) and 1.92 (143.80%) g plant^{-1} (Fig. 4.19). The enhancements in productivity by commercial biofertilizer were low in comparison to collective effects of consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* and chemical biofertilizer. Further, chemical fertilizer U+D showed almost same productivity of 1.92 g plant^{-1} as that of *A. faecalis*, *M. phyllosphaerae* and *T. virens* i.e. 1.84 g plant^{-1}. Therefore, application of *A. faecalis*, *M. phyllosphaerae* and *T. virens* should be encouraged over chemical fertilizers as the productivities were nearly same.

Table 4.7 Effects of combinations of best performing isolates on growth parameters of wheat (*Triticum aestivum* L.) at harvesting time in comparison to control (no fertilizer)

Growth parameters	Con	$S5_1$+SDA_3	SDA_3+T_2	$S5_1$+T_2	SDA_3+$S5_1$+T_2
Root length (cm)	10.67±0.12^{a}	15.70±0.22^{d}	16.33±0.12^{e}	17.47±0.24^{f}	18.73±0.26^{g}
Shoot length (cm)	54.20±0.33^{a}	74.53±0.17^{f}	75.40±0.29^{g}	76.55±0.39^{h}	77.52±0.49^{i}
No. of roots	11.67±0.47^{a}	17.0±0.82^{c}	19.67±0.94^{d}	22.33±0.47^{e}	23.0±0.82^{e}
No. of leaves	3.00±0.0^{a}	7.67±0.47^{c,d}	7.67±0.47^{c,d}	8.33±0.47^{d,e}	8.33±0.47^{d,e}
Fresh wt. (g plant^{-1})	2.98±0.01^{a}	9.57±0.21^{c}	10.70±0.32^{d}	11.34±0.41^{e}	11.52±0.26^{e}
Dry wt. (g plant^{-1})	1.82±0.13^{a}	3.03±0.05^{e}	3.14±0.03^{e,f}	3.24±0.02^{f}	3.48±0.04^{g}
Yield (g plant^{-1})	0.79±0.01	1.43±0.02	1.61±0.01	1.70±0.02	1.84±0.02

*Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. The alphabets show statistically significant differences (Where, Con= Control, $S5_1$+SDA_3= Consortium of

Microbacterium phyllosphaerae and *Alcaligenes faecalis*, SDA_3+T_2= Consortium of *Alcaligenes faecalis* and *Trichoderma virens*, $S5_1+T_2$ = Consortium of *Microbacterium phyllosphaerae* and *Trichoderma virens* and $SDA_3+S5_1+T_2$= Consortium of *Alcaligenes faecalis, Microbacterium phyllosphaerae* and *Trichoderma virens*)

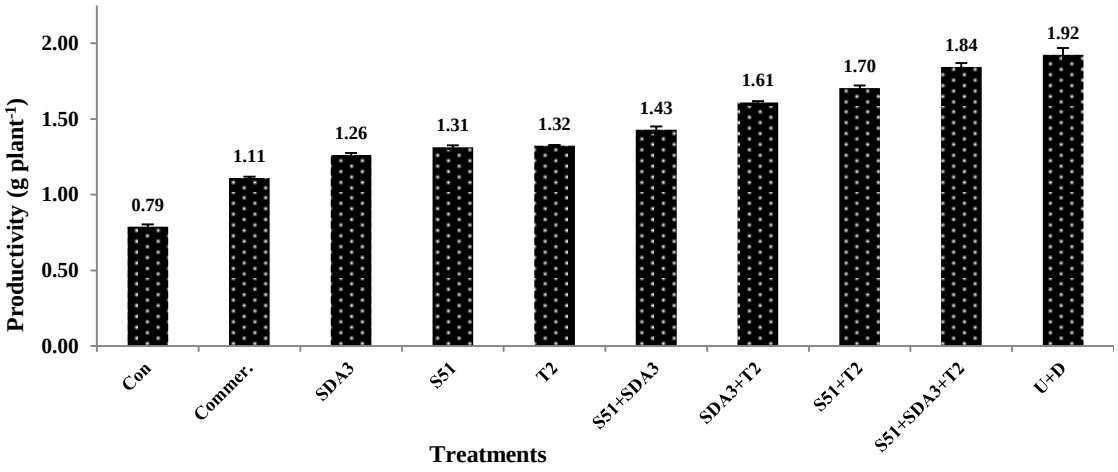

Fig. 4.17 Effect of different treatments on productivity of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$ (remaining details are similar to Fig. 4.9)

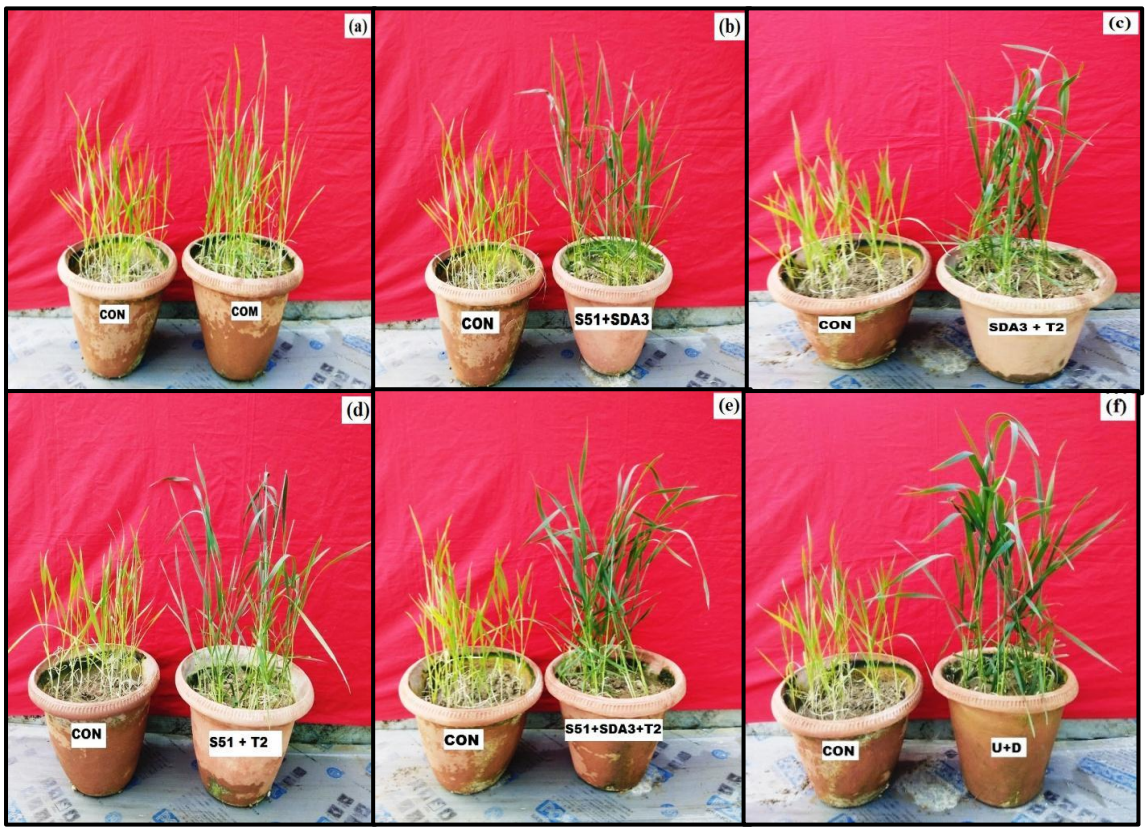

Fig. 4.18 Effect of Com (a), $S5_1$+SDA_3 (b), SDA_3+T_2 (c), $S5_1$+T_2 (d), SDA_3+$S5_1$+T_2 (e) and U+D (f) over growth and development of wheat (*Triticum aestivum* L.) after 40 DAS in comparison to control (CON). [Where, (without any treatment), CON= Control (without any treatment), Com= Commercial biofertilizer market based (recommended dose), $S5_1$+SDA_3= Consortium of *Microbacterium phyllosphaerae* and *Alcaligenes faecalis*, SDA_3+T_2= Consortium of *Microbacterium phyllosphaerae* and *Trichoderma virens*, $S5_1$+T_2 = Consortium of *Alcaligenes faecalis* and *Trichoderma virens*, SDA_3+$S5_1$+T_2= Consortium of *Microbacterium phyllosphaerae*, *Alcaligenes faecalis* and *Trichoderma virens* and U+D= Urea and di-ammonium phosphate (recommended dose)]

Fig. 4.19 Effect of Com (a), $S5_1$+SDA_3 (b), SDA_3+T_2 (c), $S5_1$+T_2 (d), SDA_3+$S5_1$+T_2 (e) and U+D (f) over growth and development of wheat (*Triticum aestivum* L.) at harvesting time in comparison to control (CON). (remaining details are similar to Fig. 4.9)

4.4 Studies on availability of nutrients in soil and its distribution in plant parts during application of *Trichoderma* and PGPR.

The nutrients available in cultivated soil have important significance in growth and development of wheat plants. Nitrate, nitrite, ammonium and phosphate are the very common and essential nutrients for plant's growth and development. Further, accumulation of these nutrients in plants also helps in development processes. The details of these studies have been given below-

4.4.1 Physicochemical properties of pots and experimental field soils

Physicochemical properties influence growth and development of the plants by providing them essential nutrients for their metabolic and catabolic activities. The main characteristics of soil include pH, electrical conductivity (EC), nitrate, nitrite, ammonium, phosphate, organic carbon, organic matter and potassium ion concentrations. Details of these physicochemical properties of soil at the time of wheat sowing have been given in Table 4.8. pH of the soil defines the nature of soil to whether it is acidic or basic. The growth of wheat plants is favored in slightly acidic to neutral condition i.e. at pH 6.0 to 7. It was recorded to be 6.5 for pot soil and 6.37 for field soil which might facilitate wheat growth and development. Whereas, EC of soil define its ability to conduct electricity which was observed to be 0.09 and 0.14 mS cm^{-1} for pot and field soil, respectively. Further, nitrogen and phosphorus content are vital for survival of all plants as these are essential micro nutrients (Solangi et al. 2016). The concentrations of nitrate, nitrite, ammonium and phosphate at sowing time were 0.13 and 0.58, 0.05 and 0.06, 0.17 and 0.21 and 0.02 and 0.06 mg g^{-1} in pot and field soils, respectively. Likewise, the amount of organic matter present the soil also provides nutrition to plants and associated beneficial microorganisms. Organic carbon in pot and field soils was recorded to be 0.46 and 0.69 %, respectively, whereas organic matter was 0.79 and 1.18 % in pot and field soils, respectively at the time of sowing. Potassium is also a micronutrient required for activation of different bio-chemical reactions. It was found to be 10.43 and 11.8 mg g^{-1} in pot and field soils, respectively at the time of sowing.

Table 4.8 Physicochemical properties of soil at sowing time

Physicochemical properties	Earthen Pot	Experimental field
pH	6.5±0.4	6.37±0.15
Electrical conductivity (mS cm^{-1})	0.09±0.0	0.14±0.01
Nitrate (mg g^{-1})	0.13±0.03	0.58±0.02
Nitrite (mg g^{-1})	0.05±0.0	0.06±0.0
Ammonium (mg g^{-1})	0.17±0.0	0.21±0.0
Phosphate (mg g^{-1})	0.02±0.0	0.02±0.0
Organic carbon (%)	0.46±0.05	0.69±0.09
Organic matter (%)	0.79±0.09	1.18±0.15
Potassium (mg g^{-1})	10.43±0.60	11.8±0.72

*Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D.

4.4.2 Alterations in physicochemical properties of soil of pot soils by application of isolated microorganisms as biofertilizers

Application of isolated microorganisms as biofertilizers altered the physicochemical properties of pot soils through degradation and solubilization mechanisms. The details of these alterations on different physicochemical properties have been given below-

4.4.2.1 Effect of biofertilizers containing isolated microorganisms on nitrate levels of pot soil

The nitrate contents in the soil of control were 2.59, 3.63 and 3.0 mg g^{-1} after 40, 80 and 120 DAS, respectively. The commercial biofertilizers increased level of nitrate in soil by 21.43, 16.33 and 14.81 % in comparison to control after 40, 80 and 120 DAS, respectively. After application of isolated microbes, individually increase at all levels were recorded. *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced the levels of nitrate by 97.14, 59.18 and 61.73, 130, 75.51 and 109.88 and 140.0, 79.59 and 103.70 %, respectively in comparison to control after 40, 80 and 120 DAS.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced nitrate content by 132.86, 141.84 and 181.48 %, 152.86, 235.71 and 220.99 % and 262.86, 274.49, and 319.75 %, respectively after 40, 80 and 120 DAS in comparison to control. But, the highest increase in nitrate content containing consortium of three isolates *A.*

faecalis, *M. phyllosphaerae* and *T. virens* was recorded as 412.86, 351.53 and 338.27 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, treatment of chemical fertilizer Urea and DAP also enhanced nitrogen content by 441.43, 61.22 and 38.27 % in comparison to control after 40, 80 and 120 DAS, respectively. The increase by chemical fertilizers in initial 40days were higher as 441.4% which decreased after 80 (61.22 %) and 120 (38.27 %) days in comparison to control. The trend of increase in nitrate content by examined biofertilizers and chemical fertilizer was Control< Urea and DAP < Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Thus, it may be concluded that the highest increase in the level of nitrate was obtained by the application of consortium of three isolates i.e. consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.20.

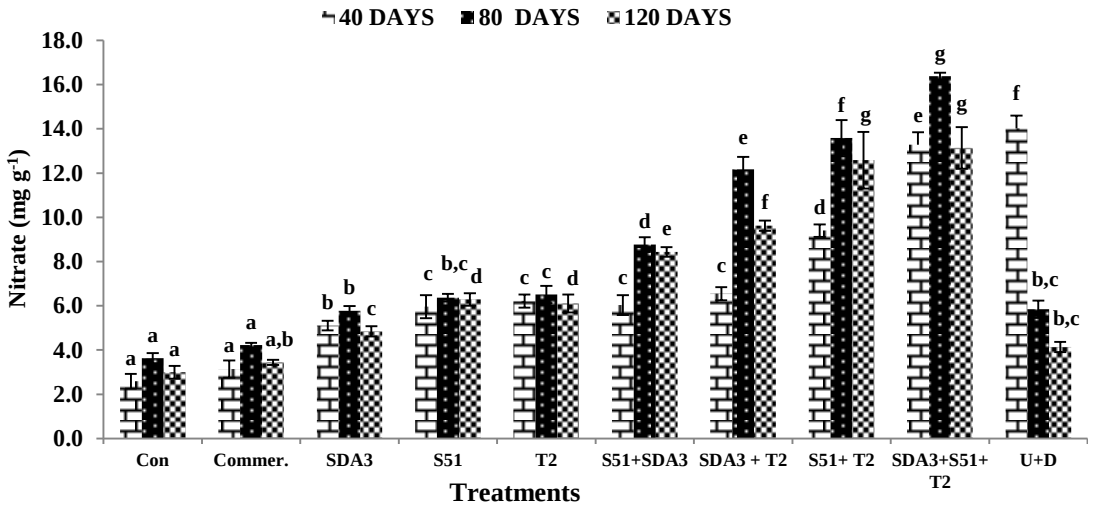

Fig. 4.20 Levels of nitrate (mg g^{-1}) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. The alphabets are significant differences between different treatments [Where, Con= Control (without any treatment), Commer.= Commercial biofertilizer market based (recommended dose), SDA_3= *A. faecalis*, $S5_1$= *M. phyllosphaerae*, T_2= *T. virens*, $S5_1$+SDA_3= Consortium of *M. phyllosphaerae* and *A. faecalis*, SDA_3+T_2= Consortium of *M. phyllosphaerae* and *T. virens*, $S5_1$+T_2= Consortium of *A. faecalis* and *T. virens*, SDA_3+$S5_1$+T_2= Consortium of *M. phyllosphaerae*, *A. faecalis* and *T. virens* and U+D= chemical fertilizer Urea and di-ammonium phosphate (recommended dose)].

4.4.2.2 Effect of biofertilizers containing isolated microorganisms on nitrite levels of pot soil

The concentrations of nitrite in pot soil of control were 0.076, 0.083 and 0.078 mg g^{-1} after 40, 80 and 120 DAS, respectively. Treatment of commercial biofertilizer (Commer.) enhanced nitrite content in pot soil by 19.78, 12.56 and 16.13 % in comparison to control after 40, 80 and 20 DAS, respectively. Whereas, application of isolated microbes i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly enhanced nitrite concentration in pot soil by 24.18, 16.58 and 1.61 %, 23.63, 21.91 and 23.12 % and 28.02, 25.63 and 29.03 % after 40, 80 and 120 DAS, respectively.

However, the levels of nitrite were increased more significantly with treatment of consortium of two isolates, *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 30.22, 28.64 and 35.48 %, 35.71, 31.66 and 37.10 % and 41.76, 52.26 and 44.09 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, highest increase in levels of nitrite was observed with the effect of consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 46.15, 69.35 and 76.88 %, respectively after 40, 80 and 120 DAS. The treatment of chemical fertilizers i.e. Urea and DAP also enhanced the level of nitrite in pot soil. In initial 40 days 75.33 % increase in levels of nitrite was observed in comparison to control which decreased to 15.58 % after 80 DAS and at harvesting time only 18.82 % higher nitrite remained in the soil. At last, the trend of increase in nitrite levels in pot soils was same as that of nitrate levels i.e. Control< Urea and DAP< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Thus, it may be concluded that the highest increase in the level of nitrite was gained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.21.

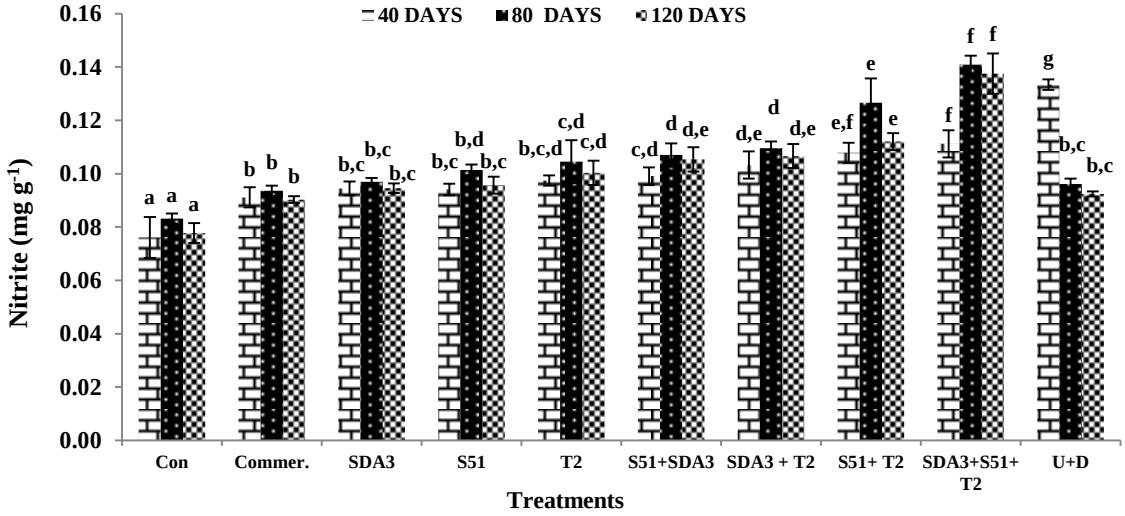

Fig. 4.21 Levels of nitrite (mg g^{-1}) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.3 Effect of biofertilizers containing isolated microorganisms on ammonium levels of pot soil

Ammonium contents in pot soil of control were 1.87, 2.07 and 1.998 mg g^{-1} after 40, 80 and 120 DAS, respectively. Commercial biofertilizer (Commer.) improved its concentration in pot soil by 40.97, 32.47 and 15.05 % in comparison to control after 40, 80 and 20 DAS, respectively. Further, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly enhanced ammonium levels in pot soil by 65.65, 96.91 and 83.81 %, 95.55, 107.0 and 103.39 % and 99.82, 291.60 and 282.36 % after 40, 80 and 120 DAS, respectively.

Whereas, levels of ammonium were increased more significantly with treatment of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 312.16, 348.63 and 356.14 %, 343.83, 540.16 and 532.62 % and 579.07, 656.30 and 652.55 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, highest increase in levels of ammonium was observed with consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 694.28, 744.91 and 727.15 %, respectively after 40, 80 and 120 DAS. Likewise, treatment of chemical fertilizers Urea and DAP also enhanced level of ammonium in pot soil by 731.84, 134.09 and 110.36 %, respectively after 40, 80 and 120 DAS. In addition to this, tendency of increase in

ammonium levels in pot soils was same as that of nitrate and nitrite levels and highest increase was gained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.22.

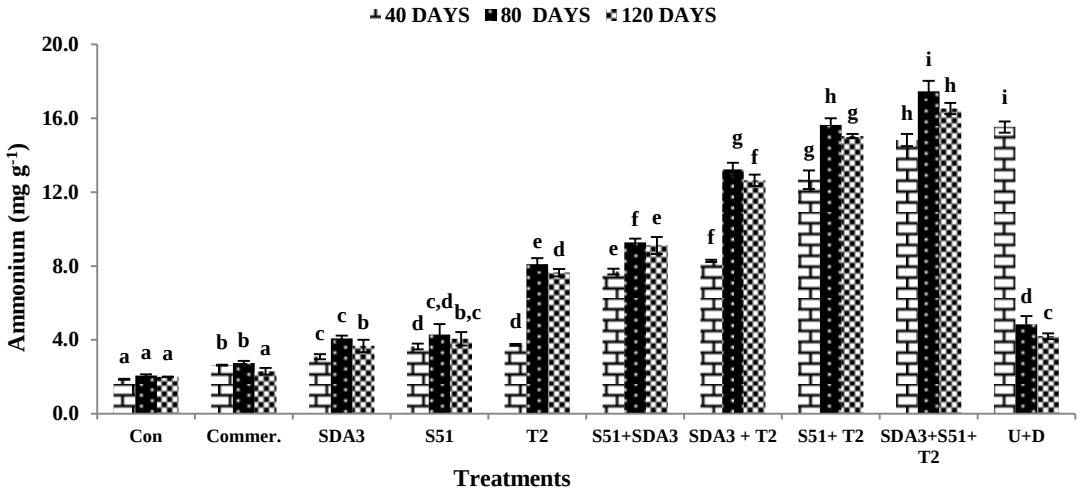

Fig. 4.22 Levels of ammonium (mg g^{-1}) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.4 Effect of biofertilizers containing isolated microorganisms on phosphate levels of pot soil

The concentration of phosphate in pot soil of control was 1.05, 1.27 and 1.19 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of market available biofertilizer (Commer.) enriched phosphate concentration in pot soil by 21.0, 9.41 and 14.51% in comparison to control after 40, 80 and 20 DAS, respectively. But, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved phosphate levels in pot soil by 25.62, 44.03 and 39.43 %, 67.62, 65.0 and 47.13% and 68.68, 70.03 and 61.99 %, respectively after 40, 80 and 120 DAS.

Whereas, more significant increase in levels of phosphate were observed with treatment of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 75.80, 125.29 and 100.32 %, 138.43, 149.82 and 134.35 % and 157.65, 220.59 and 222.08 % in comparison to control after 40, 80 and 120 DAS, respectively. However, highest increase in levels of phosphate was observed with consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 161.92, 252.94 and 236.85 %, respectively after 40,

80 and 120 DAS. Further, same tendency of increase in phosphate content was recorded as that of nitrate, nitrite and ammonium with treatment of chemical fertilizers i.e. Urea and DAP. It boosted level of phosphate in pot soil by 180.07, 28.82 and 11.04 %, respectively after 40, 80 and 120 DAS. The tends of increase in phosphate levels in pot soils was same as that of nitrate, nitrite and ammonium levels and highest increase was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time as shown in Fig. 4.23.

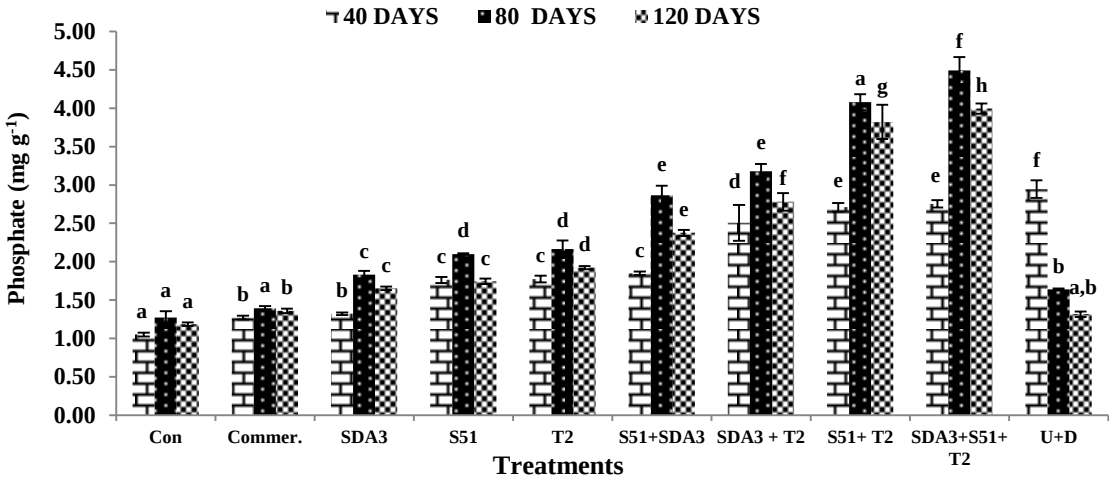

Fig. 4.23 Levels of phosphate (mg g^{-1}) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.5 Effect of biofertilizers containing isolated microorganisms on organic carbon levels of pot soil

The concentrations of organic carbon at seed sowing and harvesting time were 0.46 and 0.77 %, respectively in pot soil. Application of all treatments had same organic carbon content in pot soil, but harvesting time it changed according to effects of different treatments. Market based biofertilizer enhanced organic carbon by 18.04 % and chemical fertilizer (Urea and DAP) by 68.04 % in comparison to control. Whereas, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved organic carbon by 102.48, 118.52 and 149.41 % respectively in comparison to control.

Further, more significant increase in carbon content was recorded by the application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A.*

faecalis and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 175.31, 212.36 and 243.22 %, respectively. The highest increase in carbon content was recorded by application of consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 290.15 %. Unlike trends of increase in nitrate, nitrite, ammonium and phosphate levels, organic carbon was found to increase in a trend of Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis, M. phyllosphaerae* and *T. virens* at harvesting time. Thus, highest increase was recorded by collective effects of three isolates in the form of consortium (*A. faecalis, M. phyllosphaerae* and *T. virens*) as presented in Fig. 4.24.

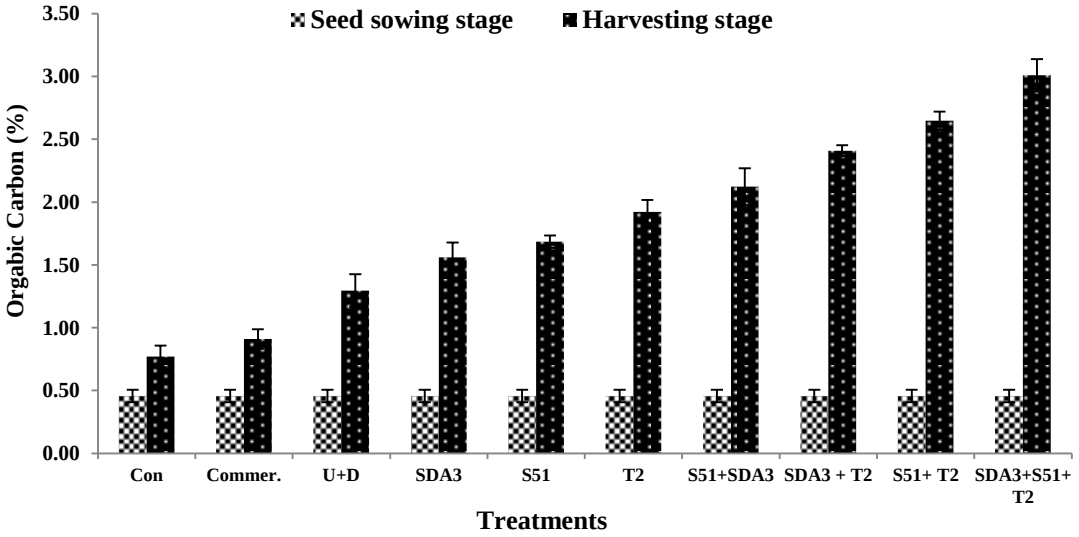

Fig. 4.24 Levels of organic carbon (%) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.6 Effect of biofertilizers containing isolated microorganisms on organic matter levels of pot soil

The concentrations of organic matter in pot soil at seed sowing and harvesting time were 0.79 and 1.33 %, respectively. Similar to organic carbon, all treatments had the same organic matter content at seed sowing time and changed at harvesting time accordingly. Commercial biofertilizer and chemical fertilizer (Urea and DAP) enhanced organic matter in pot soil by 18.04 and 68.04 %, respectively in comparison

to control. However, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved organic matter 102.48, 118.52 and 149.41 % respectively in comparison to control.

Moreover, more significant increase in carbon matter was found by consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* which recorded 175.31, 212.356 and 243.22 % increase, respectively. But, the highest increase in carbon matter was recorded by applying consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 290.15 %. Trends of increase in organic matter was found to be similar to organic matter as Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Thus, it may be concluded that just like organic carbon the highest increase in organic matter was recorded by consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* as shown in Fig. 4.25.

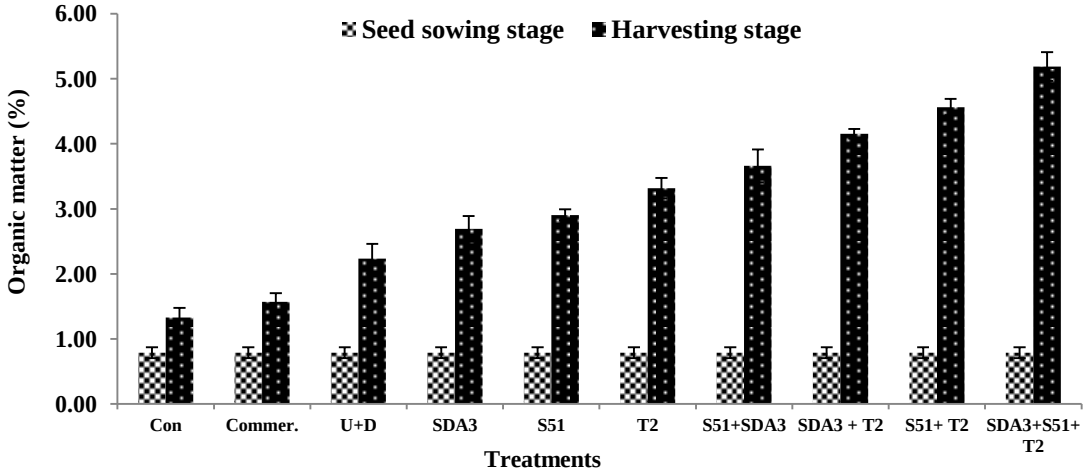

Fig. 4.25 Levels of organic matter (%) in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.7 Effect of biofertilizers containing isolated microorganisms on alkaline phosphatase activity in pot soil

Alkaline phosphatase activity (APA) is an enzymatic property of soil which helps in growth and development of plants. It was found to be 0.26 mg g^{-1} per hour at sowing time (zero day). In control soil it was slightly higher as 0.36 mg g^{-1} per hour at harvesting time. This showed that in control treatment APA was increased by 41.56 % at harvesting time. The application of market based biofertilizer (Commer.) increased its activity by 11.19 %. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved APA 22.84, 27.89 and 62.11% respectively in comparison to control.

Further, more significant increase in APA was observed by application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 64.50, 78.07 and 118.35%, respectively at harvesting time in comparison to control. Whereas, the highest increase of 204.40 % was recorded by application of consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Unlike to all other treatments of biofertilizer, decrease of 7.89 % in APA was observed by chemical fertilizer (Urea and DAP) in comparison to control. Unlike trends of increase in nitrate, nitrite, ammonium, phosphate levels, organic carbon and organic matter, APA was found to increase in a trend of Urea and DAP< Control< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Thus, highest increase in APA was recorded by collective effects of three isolates in the form of consortium (*A. faecalis*, *M. phyllosphaerae* and *T. virens*) as presented in Fig. 4.26.

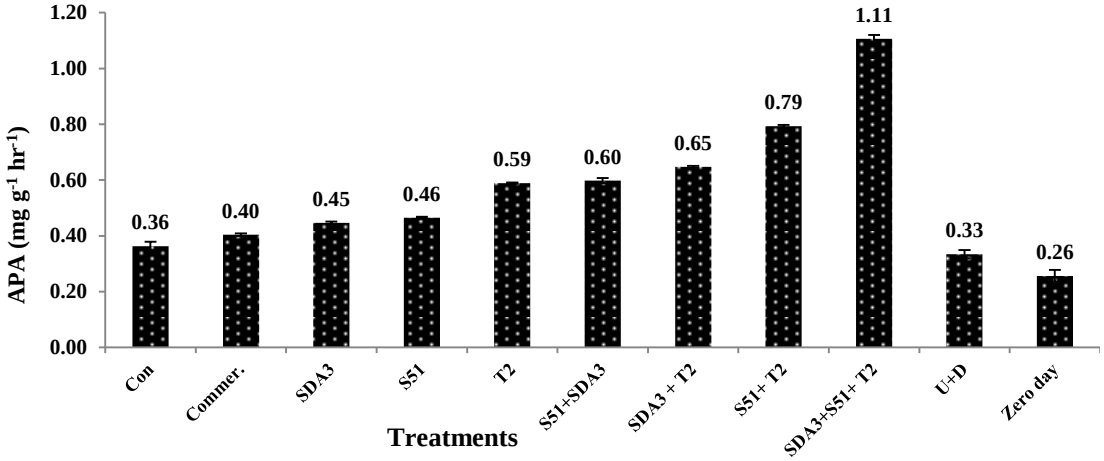

Fig. 4.26 Levels of alkaline phosphatase (mg g^{-1} hr^{-1}) activity in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.2.8 Effect of biofertilizers containing isolated microorganisms on dehydrogenase activity in pot soil

Dehydrogenase activity (DHA) is also an enzymatic property of soil like APA. DHA was reported to be 7.86 µg TPF per 24 hours at sowing time (zero day). At harvesting time, DHA in control soil was 10.71 µg TPF per 24 hours at harvesting time which was 36.38 % higher than sowing time. Market based biofertilizer (Commer.) merely improved its activity by 0.80 % in comparison to control. However, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* improved DHA by 3.32, 12.78 and 13.62 % respectively in comparison to control.

Moreover, significant increase in DHA was recorded by application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* which were 26.98, 31.44 and 56.87 %, respectively at harvesting time in comparison to control. But, the highest increase of 60.59 % was shown by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Similar to APA, decrease of 5.14 % in DHA was reported by chemical fertilizer (Urea and DAP) in comparison to control. The trend of increase in DHA was also same as that of APA. Lastly it may be concluded that the highest increase in DHA was recorded by consortium of three isolates (*A. faecalis*, *M. phyllosphaerae* and *T. virens*) as presented in Fig. 4.27.

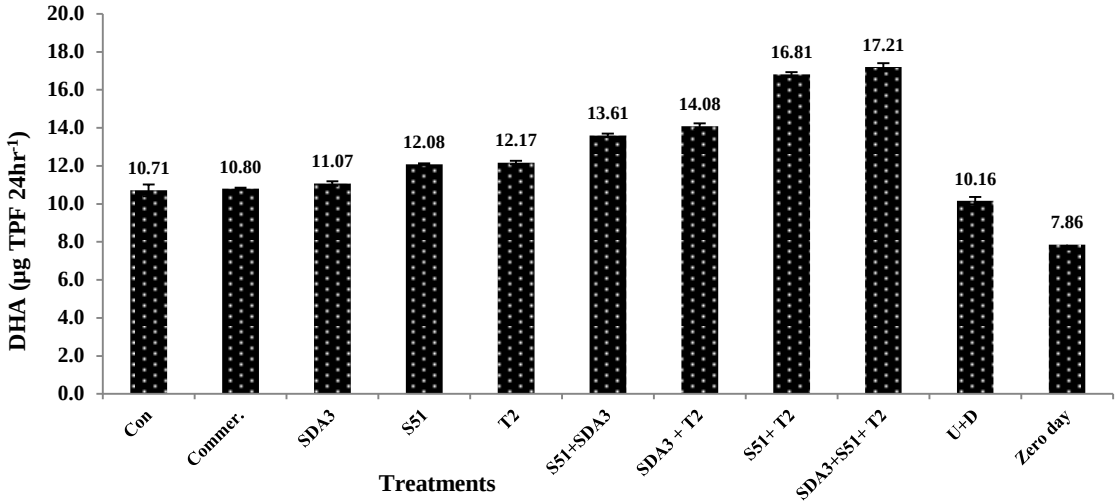

Fig. 4.27 Levels of dehydrogenase (µg TPF 24hr^{-1}) activity in soil of earthen pots of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.3 Accumulation of nutrients in wheat (*Triticum aestivum* L.) of pot experiments

Uptake and distribution of different nutrients (nitrate, nitrite, ammonium and phosphate) are important factor that signifies the applicability of these nutrients in growth and development of plants. Among the nutrients present in pot soil, how much of was used and accumulated in wheat plants with the effect of different treatments was studied and results have been given below-

4.4.3.1 Accumulation of nitrate in wheat plants from earthen pots

The concentration of nitrate in wheat plants of control was 22.01, 30.82 and 25.16 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers was increased uptake and accumulation of nitrate in plants by 20.29, 15.71 and 15.63 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, application of individual isolated microbes increased uptake and accumulation of nitrate as *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced it by 97.14, 61.22 and 62.25 %, 127.0, 76.22 and 110.0 % and 128.71, 76.73 and 104.13 %, respectively in comparison to control after 40, 80 and 120 DAS.

However, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* more significantly

increased nitrate uptake and accumulation by 137.43, 142.45 and 186.19 %, 153.14, 236.73 and 227.75 % and 266.71, 272.96 and 333.75 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, the highest increase in nitrate accumulation containing consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* was recorded as 427.14, 354.49 and 365.0 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, treatment of chemical fertilizer U+D also enhanced nitrate accumulation by 444.43, 56.63 and 40.0 % in comparison to control after 40, 80 and 120 DAS, respectively. It was observed that accumulation of nitrate by chemical fertilizers in initial 40days were higher which decreased after 80 and 120 DAS accordingly in comparison to control. The trend of increase in accumulation of nitrate Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of nitrate was obtained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.28.

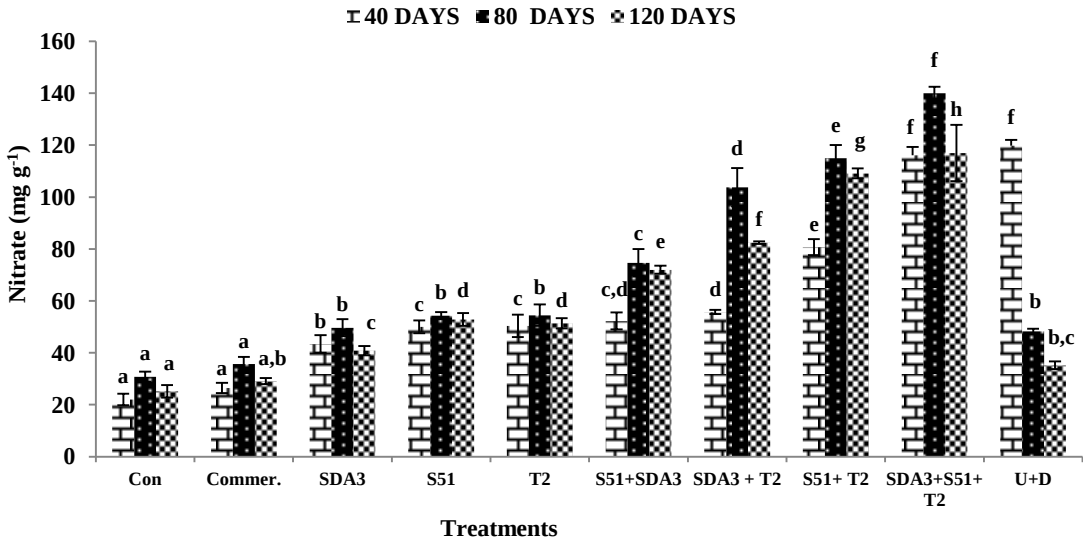

Fig. 4.28 Accumulation of nitrate (mg g^{-1}) in wheat (*Triticum aestivum* L.) plants from earthen pots applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.3.2 Accumulation of nitrite in wheat plants from earthen pots

Accumulation of nitrite in wheat plants of control was 0.42 , 0.46 and 0.43 mg g^{-1} after 40, 80 and 120 DAS, respectively. Commercial biofertilizers increased accumulation of nitrite in plants by 19.78, 12.56 and 16.13 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased accumulation of nitrite by 24.18, 16.58 and 21.61 %, 23.63, 21.91 and 23.12 % and 28.02, 25.63 and 29.03 %, respectively, after 40, 80 and 120 DAS in comparison to control.

But, more significant increase in nitrite content was recorded by consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 30.22, 28.64 and 35.48 %, 35.71, 31.66 and 37.10 % and 41.76, 52.26 and 44.09 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, the highest increase in nitrite accumulation was found by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 46.15, 69.35 and 76.88 % in comparison to control after 40, 80 and 120 DAS, respectively. Like nitrate, accumulation of nitrite was found to increase in initial 40 days by 75.33 % which decreased to 69.35 % after 80 days by chemical fertilizer (Urea and DAP). At harvesting time (120DAS) 8.06 % of decrease in nitrite accumulation was recorded in comparison to control.

The trend of increase in accumulation of nitrite at harvesting time was Control< Urea and DAP< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of nitrite content was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.29.

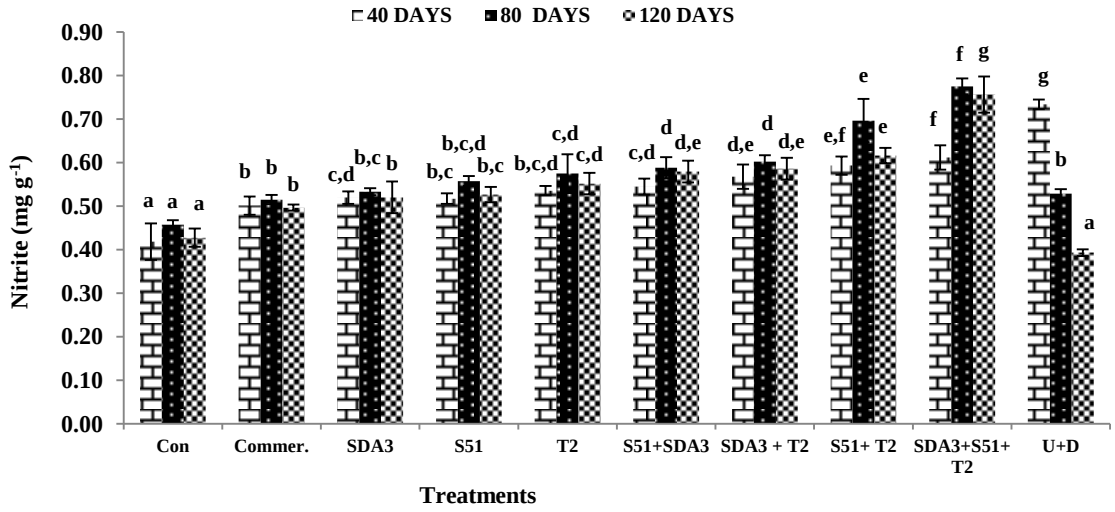

Fig. 4.29 Accumulation of nitrite (mg g^{-1}) in wheat (*Triticum aestivum* L.) plants from earthen pots applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.3.3 Accumulation of ammonium in wheat plants from earthen pots

In control plants, accumulation of ammonium was recorded to be 5.23, 5.79 and 5.60 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers increased its accumulation in plants of pot by 40.97, 32.47 and 15.05 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased it by 65.65, 96.91 and 83.81 %, 95.55, 113.44 and 103.39 % and 99.82, 291.60, and 282.36 % after 40, 80 and 120 DAS in comparison to control, respectively.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced accumulation of ammonium by 312.16, 348.63 and 365.90 %, 343.83, 540.16 and 532.62 % and 579.07, 656.30 and 652.55 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, maximum increase in ammonium accumulation was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 694.28, 743.13 and 727.15 % in comparison to control after 40, 80 and 120 DAS, respectively. Like nitrate, accumulation of ammonium by chemical fertilizer (Urea and DAP) was found to increase by 731.84, 134.09 and 103.68 % after 40, 80 and 120 DAS.

The trend of increase in accumulation of ammonium at harvesting time was Control< Commer.< *A. faecalis*< *M. phyllosphaerae*< Urea and DAP< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of ammonium content was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.30.

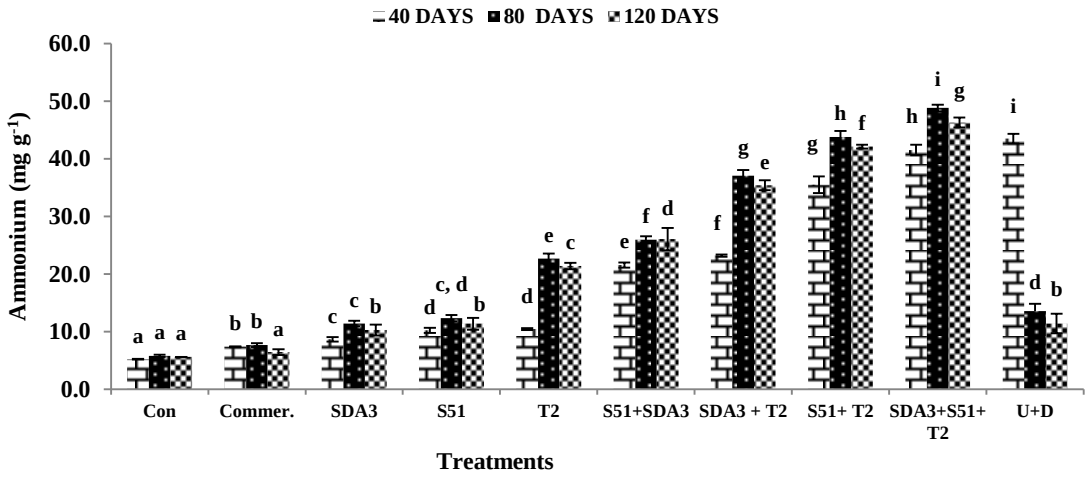

Fig. 4.30 Accumulation of ammonium (mg g^{-1}) in wheat (*Triticum aestivum* L.) plants from of earthen pots applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.3.4 Accumulation of phosphate in wheat plants from earthen pots

Phosphate content in control plants were recorded as 8.94, 10.81 and 10.08 mg g^{-1} after 40, 80 and 120 DAS, respectively. Commercial biofertilizers enhanced phosphate accumulation in plants of pot soil by 21.0, 9.41 and 14.51% in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased its accumulation by 25.62, 44.03 and 39.43 %, 67.62, 65.0 and 47.13 % and 68.68, 70.03 and 61.29 % after 40, 80 and 120 DAS in comparison to control, respectively.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* significantly enhanced its accumulation by 75.80, 125.29 and 100.32 %, 138.43, 149.82 and 134.35 % and 157.65, 220.59 and 222.08 %, respectively after 40, 80 and 120 DAS in comparison to

control. Whereas, maximum accumulation was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 165.48, 252.94 and 236.85 % in comparison to control after 40, 80 and 120 DAS, respectively. Like nitrate and ammonium, accumulation of phosphate by treatment of U+D was found to increase by 180.07, 28.82 and 11.04 % after 40, 80 and 120 DAS.

The trend of increase in accumulation of phosphate at harvesting time was Control< Urea and DAP< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of phosphate was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.31.

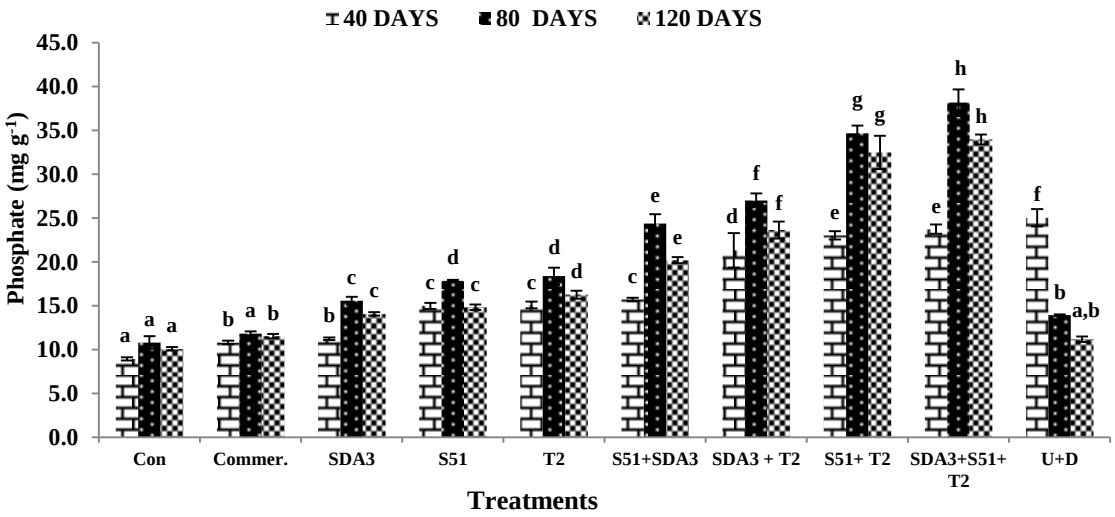

Fig. 4.31 Accumulation of phosphate (mg g^{-1}) in wheat (*Triticum aestivum* L.) from of earthen pots applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4 Alterations in physicochemical properties of experimental field soils by application of isolated microorganisms as biofertilizers

Application of isolated microorganisms as biofertilizers changed levels of initial concentrations of different physicochemical properties of experimental field soils through different degradation and solubilization mechanisms. The physicochemical properties of experimental field soils have been described in section 4.4.1 and values

have been presented in Table 4.8. Further, details of alterations on different physicochemical properties by application of isolated microorganisms as biofertilizers have been given below-

4.4.4.1 Effect of biofertilizers containing isolated microorganisms on nitrate levels of experimental field soil

The nitrate contents in experimental field soil of control were 2.66, 4.0 and 3.74 mg g^{-1} after 40, 80 and 120 DAS, respectively. The commercial biofertilizers increased level of nitrate in soil by 36.11, 14.81 and 4.95 % in comparison to control after 40, 80 and 120 DAS, respectively. After application of isolated microbes, individually increase at all levels were recorded. *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced the levels of nitrate by 101.39, 76.85 and 57.43 %, 131.94, 93.2 and 76.24 % and 151.39, 114.81 and 117.82 %, respectively in comparison to control after 40, 80 and 120 DAS.

Whereas, consortium of two isolates i.e. *faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced nitrate content by 164.86, 147.87 and 149.50 %, 174.76, 246.30 and 205.94 % and 295.83, 298.15 and 281.19 %, respectively after 40, 80 and 120 DAS in comparison to control. On the other hand, highest increase in nitrate content containing consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* was recorded as 419.44, 325.93 and 297.03 % in comparison to control after 40, 80 and 120 DAS, respectively. Furthermore, treatment of chemical fertilizer (Urea and DAP) also enhanced nitrogen content by 454.17, 48.15 and 36.14 % in comparison to control after 40, 80 and 120 DAS, respectively. The increase by chemical fertilizers in initial 40days were higher as 454.17 % which decreased after 80 (48.15 %) and 120 (36.14 %) days in comparison to control. The trend of increase in nitrate content by examined biofertilizers and chemical fertilizer was Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. In addition to this, nitrate content in experimental field soil were always higher than pot soil for all treatments. Thus, it may be concluded that the highest increase in the level of nitrate was obtained by the application of consortium

of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.32.

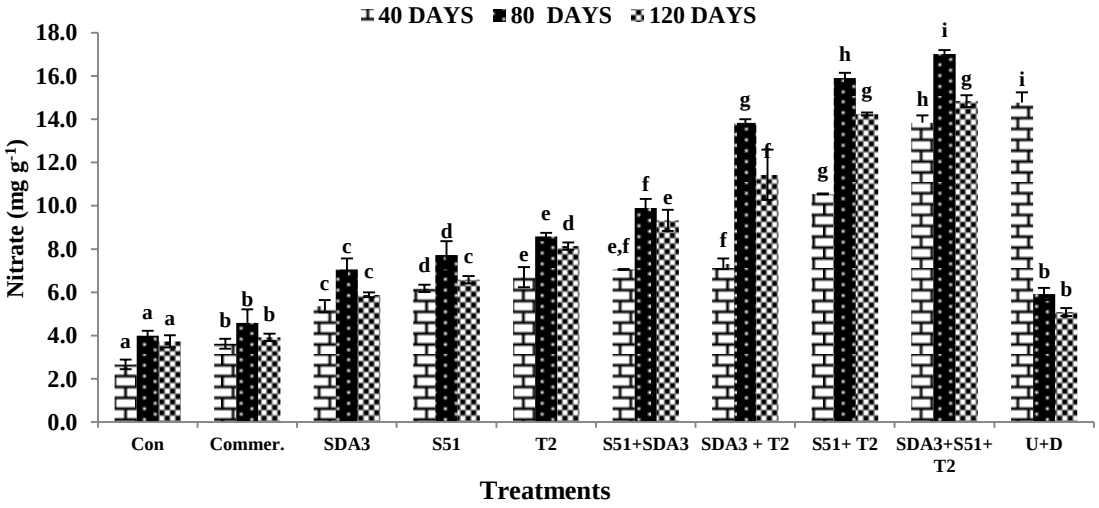

Fig. 4.32 Levels of nitrate (mg g^{-1}) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.2 Effect of isolated microorganisms on nitrite levels of experimental field soil

The concentrations of nitrite in experimental field soil of control were 0.084, 0.096 and 0.094 mg g^{-1} after 40, 80 and 120 DAS, respectively. Treatment of commercial biofertilizer (Commer.) enhanced nitrite content in field soil by 12.0, 9.83 and 4.96 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, application of isolated microbes i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly enhanced nitrite concentration in experimental field soil by 19.50, 16.59 and 16.38 %, 22.50, 23.19 and 20.0 % and 30.0, 27.64 and 26.03 % after 40, 80 and 120 DAS, respectively.

However, levels of nitrite were improved more significantly with treatment of consortium of two isolates, *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 36.95, 38.26 and 30.85 %, 42.0, 48.65 and 41.34 % and 46.35, 60.26 and 51.43 % in comparison to control after 40, 80 and 120 DAS, respectively. But, the highest increase in levels of nitrite was observed with the effect of consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 60.50, 68.21 and 62.95 %, respectively after 40, 80 and 120 DAS. The treatment of

chemical fertilizers i.e. Urea and DAP also enhanced the level of nitrate in pot soil in initial 40 and 80 days by 74.25 and 13.54 %, respectively but at harvesting time (10 days) decrease by 18.37 % was recorded in comparison to control. At last, the trend of increase in nitrite levels in pot soils was Control<Urea and DAP< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Like nitrate, nitrite concentrations with exception of U+D at 120 days, for all treatments were higher than pot soil. Thus, it may be concluded that the highest increase in the level of nitrite was gained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.33.

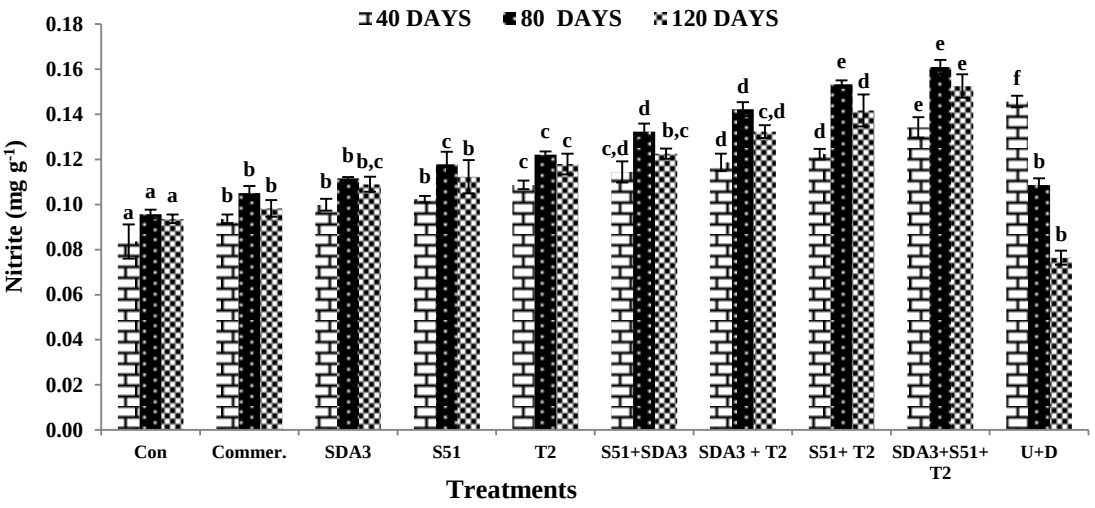

Fig. 4.33 Levels of nitrite (mg g^{-1}) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.3 Effect of biofertilizers containing isolated microorganisms on ammonium levels of experimental field soil

Ammonium contents in pot soil of control were 1.97, 2.11 and 1.92 mg g^{-1} after 40, 80 and 120 DAS, respectively. Commercial biofertilizer (Commer.) improved its concentration in experimental field soil by 38.98, 40.23 and 47.13 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly enhanced

ammonium levels in field soil by 60.34, 53.46 and 58.19 %, 91.02, 139.46 and 148.22 % and 96.44, 290.93 and 311.53 % after 40, 80 and 120 DAS, respectively.

Whereas, levels of ammonium were increased more significantly with treatment of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 303.73, 351.01 and 367.25 %, 334.98, 563.90 and 605.40 % and 573.34, 663.32 and 724.74 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, highest increase in levels of ammonium was observed with consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 660.31, 737.08 and 807.67 %, respectively after 40, 80 and 120 DAS. Likewise, treatment of chemical fertilizers U+D also enhanced level of ammonium in field soil by 712.90, 127.73and 114.63 %, respectively after 40, 80 and 120 DAS. Like nitrate and nitrite, ammonium concentrations for all treatments in experimental field soil were higher than pot soil. In addition to this, tendency of increase in ammonium levels in experimental field soils was same as that of nitrate and nitrite levels and highest increase was gained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.34.

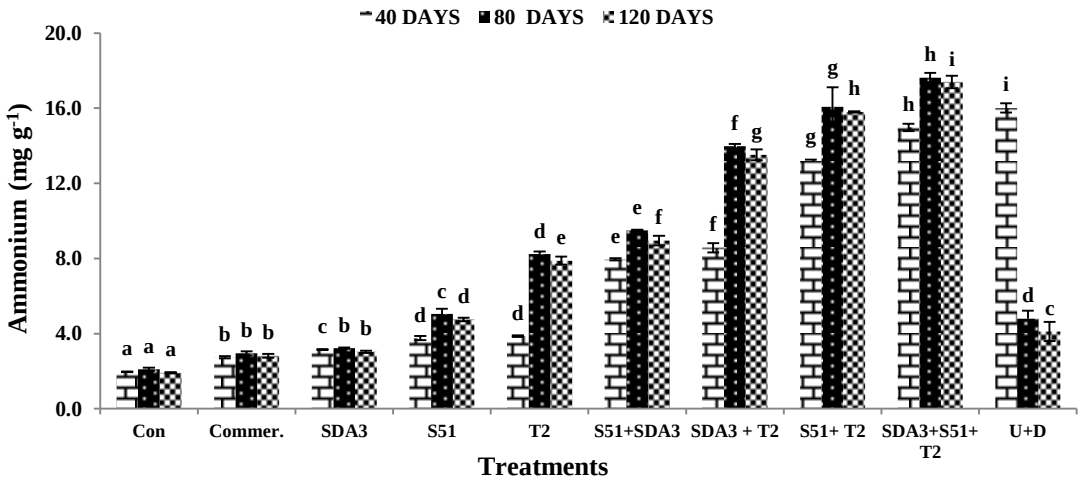

Fig. 4.34 Levels of ammonium (mg g^{-1}) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.4 Effect of biofertilizers containing isolated microorganisms on phosphate levels of experimental field soil

The concentration of phosphate in experimental field soil of control was 1.20, 1.55 and 1.45 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of market available biofertilizer (Commer.) enriched phosphate concentration in field soil by 20.91, 16.51 and 23.44 % in comparison to control after 40, 80 and 120 DAS, respectively. But, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved phosphate levels in field soil by 23.44, 20.34 and 25.14 %, 68.52, 56.17 and 59.59 % and 75.19, 62.95 and 72.09 %, respectively after 40, 80 and 120 DAS.

Whereas, more significant increase in levels of phosphate were observed with treatment of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 81.16, 93.69 and 96.12 %, 138.44, 145.21 and 129.97 % and 171.56, 174.84 and 180.88 % in comparison to control after 40, 80 and 120 DAS, respectively. However, highest increase in levels of phosphate was observed with consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 185.94, 207.02 and 200.52 %, respectively after 40, 80 and 120 DAS. Further, same tendency of increase in phosphate content was recorded as that of nitrate, nitrite and ammonium with treatment of chemical fertilizers (Urea and DAP). It boosted level of phosphate in experimental field soil by 197.50, 51.82 and 54.52 %, respectively after 40, 80 and 120 DAS. Like nitrate, nitrite and ammonium, phosphate levels for all treatments in experimental field soil were higher than pot soil. The tends of increase in phosphate levels in field soils was also same as that of nitrate, nitrite and ammonium levels and highest increase was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time as shown in Fig. 4.35.

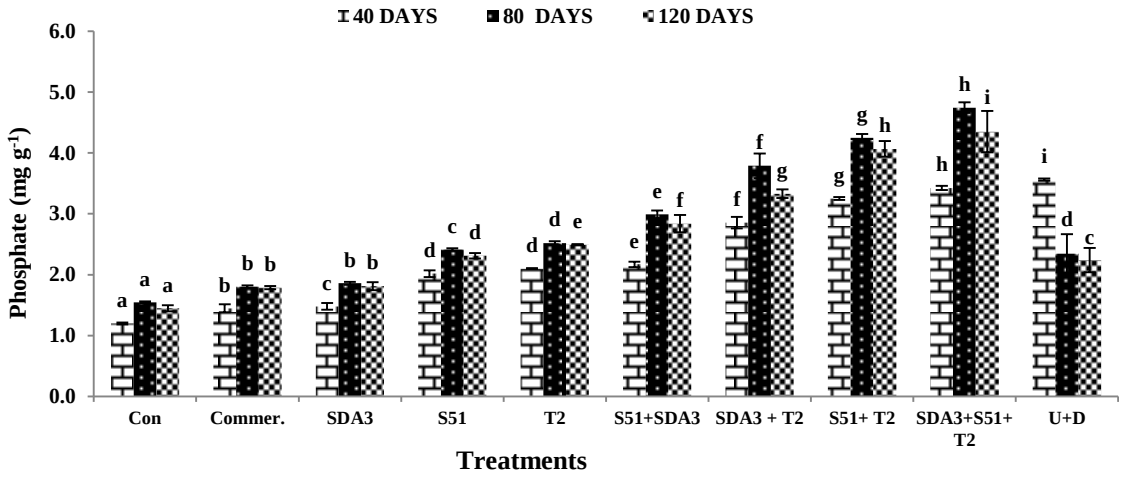

Fig. 4.35 Levels of phosphate (mg g^{-1}) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.5 Effect of biofertilizers containing isolated microorganisms on organic carbon levels of experimental field soil

The concentrations of organic carbon at seed sowing and harvesting time were 0.69 and 0.87 %, respectively in experimental field soil. Market based biofertilizer Commer. enhanced organic carbon by 52.76 % and chemical fertilizer i.e. Urea and DAP by 118.68 % in comparison to control. Whereas, treatment of isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved organic carbon by 137.47, 176.92 and 228.58 % respectively in comparison to control.

Further, more significant increase in carbon content was recorded by the application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 256.04, 284.62 and 343.56 %, respectively. The highest increase in carbon content was recorded by application of consortium of three isolates i.e. *A. faecalis, M. phyllosphaerae* and *T. virens* as 373.53 %. Further, organic carbon content of experimental field was always higher than pot field under the effects of all treatments. Unlike trends of increase in nitrate, nitrite, ammonium and phosphate levels, organic carbon was found to increase in a trend of Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A.*

faecalis, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, highest increase in carbon content of experimental field was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* as presented in Fig. 4.36.

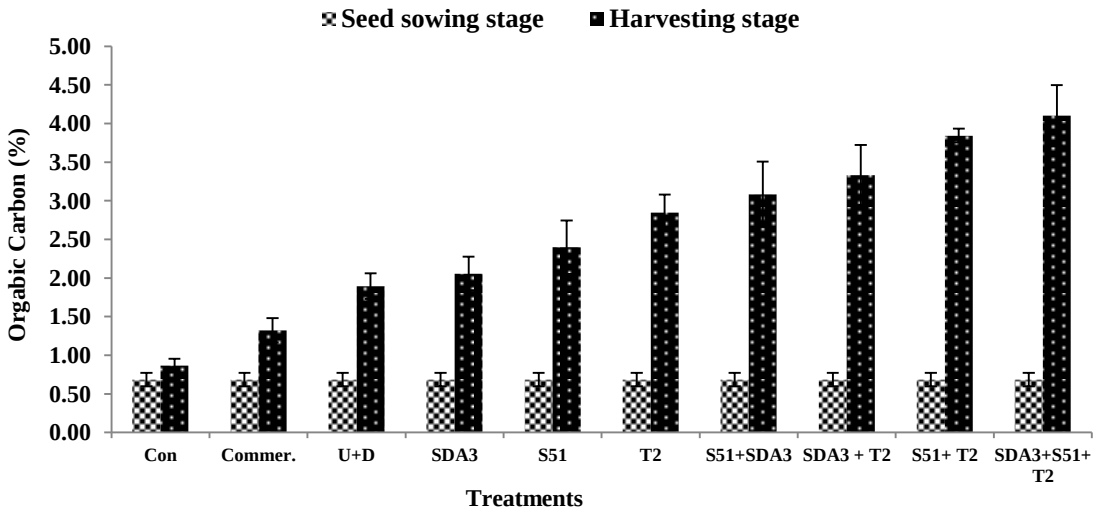

Fig. 4.36 Levels of organic carbon (%) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.6 Effect of biofertilizers containing isolated microorganisms on organic matter levels of experimental field soil

The concentrations of organic matter at seed sowing and harvesting time were 1.18 % and 1.49 %, respectively in experimental field soil. All other treatments increased organic matter in the same percentage as that of organic carbon, which has been decried in above section 4.4.3.5. Similar to organic carbon, organic matter of experimental field was always higher than pot field under the effects of all treatments. Like trends of increase in organic carbon, organic matter was found to increase same trend at harvesting time. Thus, highest increase in organic matter of experimental field was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* as presented in Fig. 4.37.

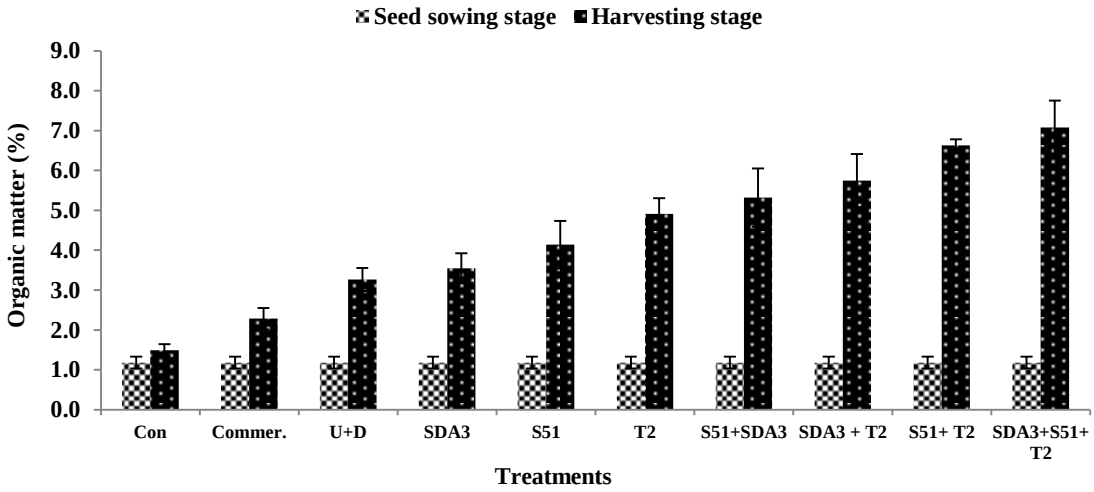

Fig. 4.37 Levels of organic matter (%) in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.7 Effect of biofertilizers containing isolated microorganisms on alkaline phosphatase activity in experimental field soil

Alkaline phosphatase activity (APA) was found to be 0.29 mg g^{-1} per hour at sowing time (zero day). At harvesting time, control soil reported slightly higher APA as 0.39 mg g^{-1} per hour in field soil which was 34.83 % higher in comparison to sowing time. The application of market based biofertilizer (Commer.) slightly increased its activity by 7.50 %. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* significantly improved APA 32.74, 57.72 and 89.34 % respectively in comparison to control.

Moreover, more significant increase in APA was observed by application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* by 116.71, 132.56 and 148.08 %, respectively at harvesting time in comparison to control. But, the highest increase of 156.44 % was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Unlike to all other treatments of biofertilizer, decrease of 10.49 % in APA was observed by chemical fertilizer U+D in comparison to control. Unlike trends of increase in nitrate, nitrite, ammonium, phosphate levels, organic carbon and organic matter, APA was found to increase in a trend of Urea and DAP< Control< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and

M. phyllosphaerae< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* in experimental pot soil. Further, in comparison to pot soils all treatments showed higher values of APA like other parameters e.g. nitrate, nitrite, ammonium, phosphate etc. Thus, highest increase in APA in experimental field soils was recorded by consortium of three isolates SDA_3+$S5_1$+T_2 as presented in Fig. 4.38.

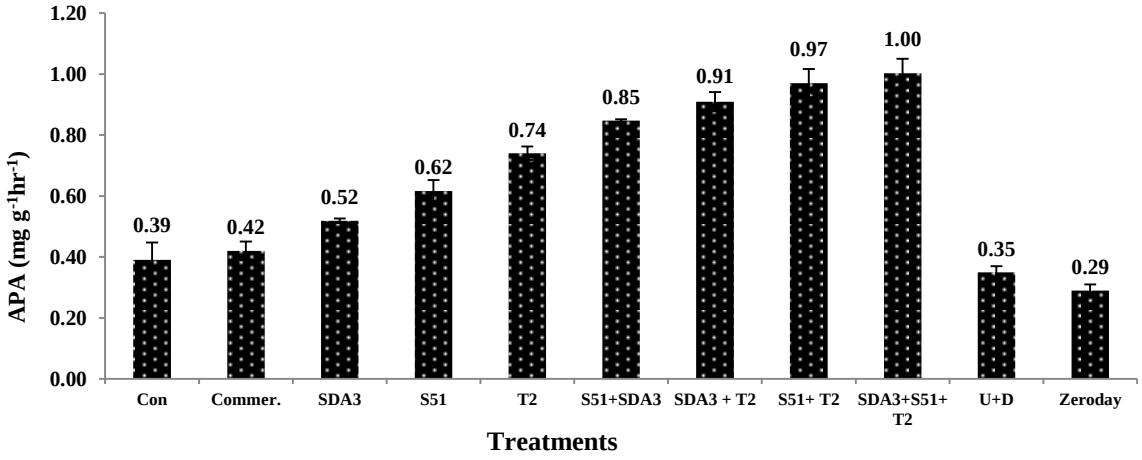

Fig. 4.38 Levels of alkaline phosphatase (mg g^{-1} hr^{-1}) activity in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.4.8 Effect of isolated microorganisms on dehydrogenase activity in experimental field soil

Dehydrogenase activity (DHA) was observed 8.54 µg TPF per 24 hours at sowing time (zero day). At harvesting time, DHA in control soil was 10.97 µg TPF per 24 hours at harvesting time which was 28.45 % higher than sowing time. Commercial biofertilizer (Commer.) only improved its activity by 4.26 % in comparison to control. Nevertheless, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* improved DHA by 12.25, 30.42 and 35.83 % respectively in comparison to control. Furthermore, significant increase in DHA was observed by application of consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* which were 40.24, 43.13 and 53.88 %, respectively at harvesting time in comparison to control. The highest increase of 62.91 % was

recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Similar to APA, decrease of 5.13 % in DHA was reported by chemical fertilizer (Urea and DAP) in comparison to control. DHA in experimental field was also higher than pot field soil. The trend of increase in DHA in experimental field soil was also same as that of APA. Lastly it may be concluded that the highest increase in DHA was recorded by consortium of three isolates (*A. faecalis*, *M. phyllosphaerae* and *T. virens*) as presented in Fig. 4.39.

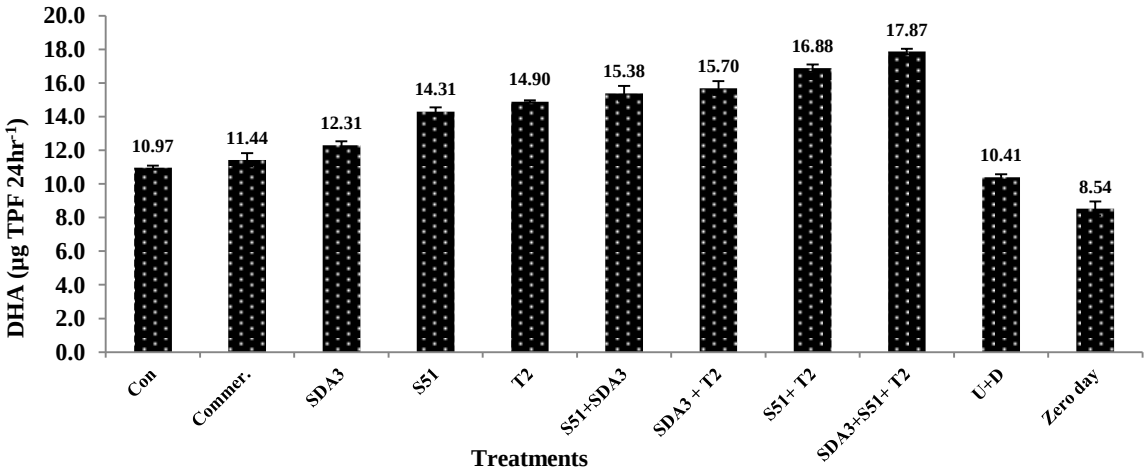

Fig. 4.39 Levels of dehydrogenase (µg TPF 24hr^{-1}) activity in soil of experimental field of wheat (*Triticum aestivum* L.) applied with different biofertilizer at seed sowing (zero day) and harvesting time (120 days). Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.5 Accumulation of nutrients in wheat (*Triticum aestivum* L.) of experimental field.

Similar to the pot plants, accumulation of nutrients i.e. nitrate, nitrite, ammonium and phosphate in experimental field plants treated with different biofertilizers and chemical fertilizers were also studied. The results have been described below-

4.4.5.1 Accumulation of nitrate in wheat plants from experimental field

The nitrate concentration in wheat plants from experimental field of control was 22.64, 33.97 and 31.76 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers increased accumulation of nitrate by 36.11, 14.81 and 4.95 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, application of individual isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased its concentration by 101.39, 76.85 and 57.43 %, 131.94, 93.52 and

76.24 % and 151.39, 114.81 and 117.82 %, respectively in comparison to control after 40, 80 and 120 DAS.

The consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced accumulation of nitrate more significantly by 164.86, 147.87 and 149.50 %, 174.76, 246.30 and 205.94 % and 295.83, 298.15 and 281.19 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, maximum increase in nitrate accumulation was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 419.44, 325.93 and 297.03 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, treatment of chemical fertilizer (Urea and DAP) also enhanced nitrate accumulation by 454.17, 48.15 and 36.14 % in comparison to control after 40, 80 and 120 DAS, respectively. It was observed that accumulation of nitrate by chemical fertilizers in initial 40days were very high which decreased after 80 and 120 DAS accordingly in comparison to control. Further, accumulation of nitrate content by effects of all treatments in plants of experimental field was always higher than plants of earthen pot soil.

The trend of increase in accumulation of nitrate was Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, highest accumulation of nitrate was obtained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time as shown in Fig. 4.40.

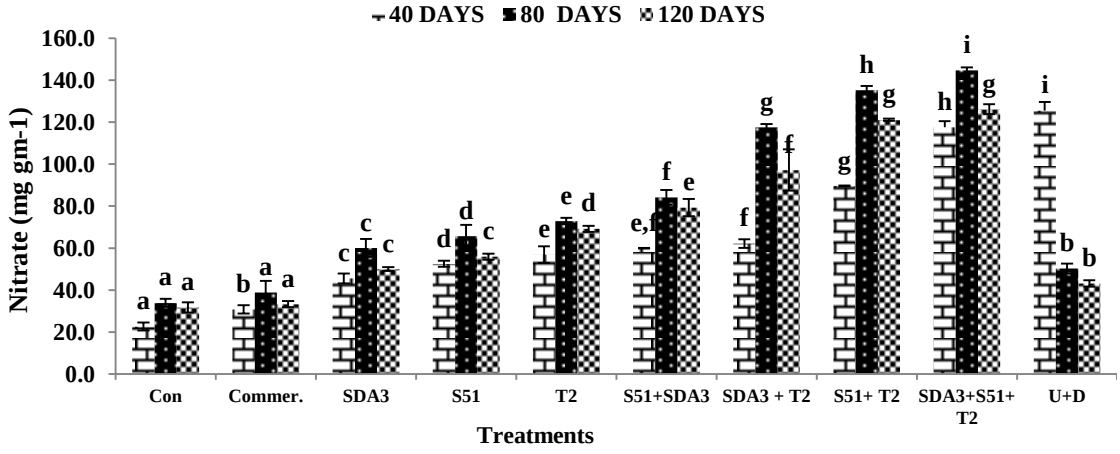

Fig. 4.40 Accumulation of nitrate (mg g^{-1}) in in wheat (*Triticum aestivum* L.) plants from experimental field applied with different biofertilizer at 40, 80 and

120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.5.2 Accumulation of nitrite in wheat plants from experimental field

The concentration nitrite in wheat plants from experimental field of control was 0.46, 0.53 and 0.51 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers increased its accumulation by 12.0, 9.83 and 4.96 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, application of individual isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased nitrite by 19.50, 13.54 and 11.29 %, 22.50, 23.19 and 20.0 %, 30.0, 27.64 and 27.72 %, respectively in comparison to control after 40, 80 and 120 DAS.

The consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced its accumulation more significantly by 36.95, 38.26 and 30.85 %, 42.0, 48.65 and 41.34 % and 46.35, 60.26 and 51.43 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, maximum increase in nitrite accumulation was recorded with the collective effects of consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 60.50, 68.21 and 62.95 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, treatment of chemical fertilizer U+D also enhanced nitrate accumulation by 74.25, 16.59 and 16.38 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, similar to nitrate, accumulation of nitrite in plants of experimental field was always higher than plants of earthen pot soil by effects of all treatments.

The trend of increase in accumulation of nitrite was Control< Commer.< Urea and DAP< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, highest accumulation of nitrite was obtained by consortium of three isolates i.e. *A. faecalis, M. phyllosphaerae* and *T. virens* at harvesting time as shown in Fig. 4.41.

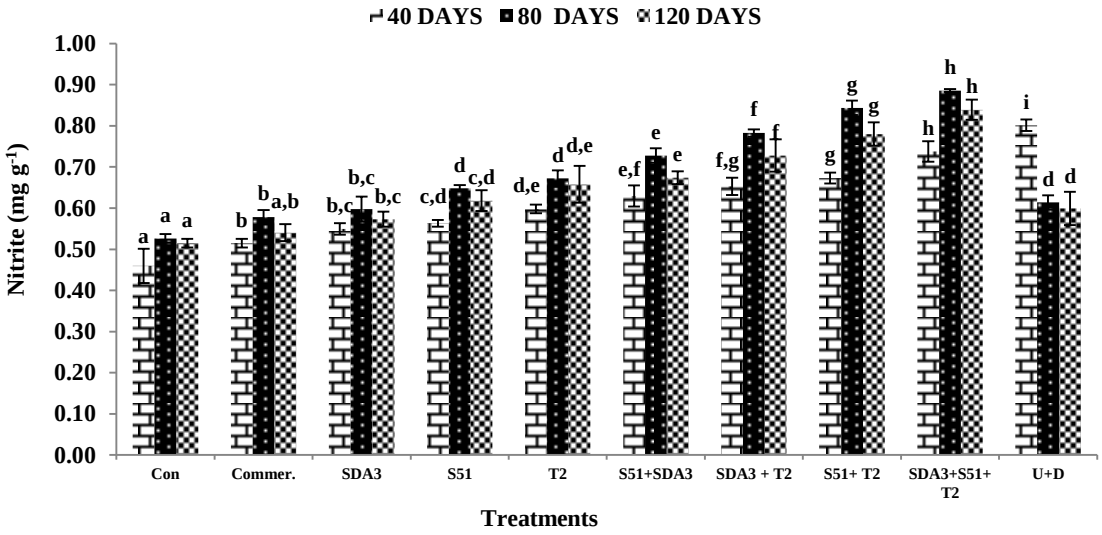

Fig. 4.41 Accumulation of nitrite (mg g^{-1}) in wheat (*Triticum aestivum* L.) plants from experimental field applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.5.3 Accumulation of ammonium in wheat plants from experimental field

In control plants, accumulation of ammonium was recorded to be 5.52, 5.90 and 5.37 mg g^{-1} after 40, 80 and 120 DAS, respectively. Application of commercial biofertilizers increased its accumulation in plants of experimental field by 38.98, 40.23 and 47.13 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* increased it by 60.34, 53.46 and 58.19 %, 91.02, 139.46 and 148.22 % and 96.44, 290.93 and 311.53 % after 40, 80 and 120 DAS in comparison to control, respectively.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced accumulation of ammonium by 303.73, 351.01 and 367.20 %, 334.98, 563.90 and 605.40 % and 573.34, 663.32 and 724.74 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, maximum increase in ammonium accumulation was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 660.31, 737.08 and 807.67 % in comparison to control after 40, 80 and 120 DAS, respectively. Like nitrate and nitrite accumulation of ammonium by chemical fertilizer U+D was found to increase by 712.90, 127.73 and 114.63 % after 40, 80 and 120 DAS. Further, similar to nitrate and nitrite, accumulation of ammonium in plants

of experimental field was always higher than plants of earthen pot soil by effects of all treatments.

The trend of increase in accumulation of ammonium at harvesting time was Control< Commer.< *A. faecalis*< Urea and DAP< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of ammonium content was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.42.

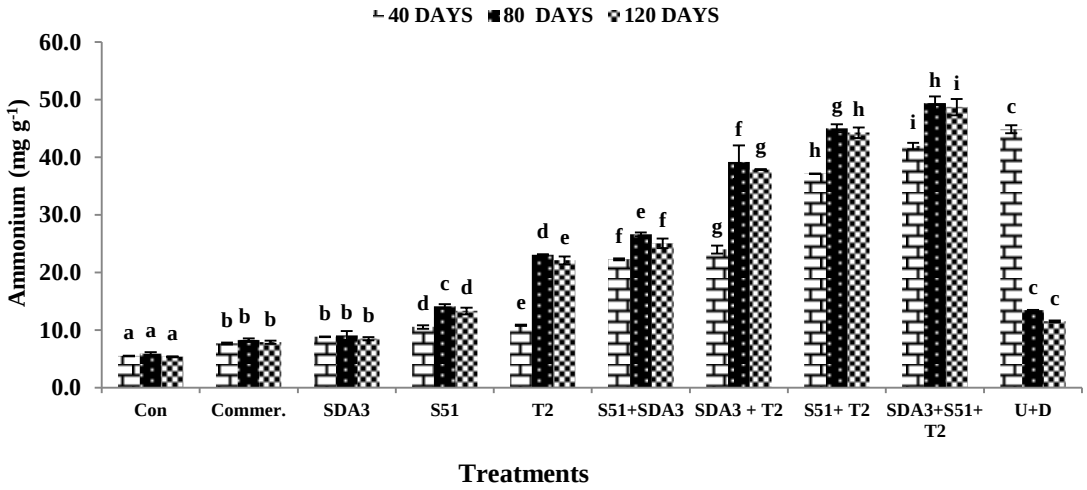

Fig. 4.42 Accumulation of ammonium (mg g^{-1}) in in wheat (*Triticum aestivum* L.) plants from experimental field applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.4.5.4 Accumulation of phosphate in wheat plants from experimental field

The concentration of in control plants was recorded as 10.18, 13.14 and 12.31mg g^{-1} after 40, 80 and 120 DAS, respectively. Commercial biofertilizers enhanced phosphate accumulation in plants experimental field by 20.91, 16.51 and 23.44 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, isolated microbes *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced its accumulation by 23.44, 51.82 and 54.52 %, 68.52, 56.17 and 59.59 % and 75.19, 62.95 and 72.09 % after 40, 80 and 120 DAS in comparison to control, respectively.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* significantly enhanced its accumulation by 81.16, 93.69 and 96.12 %, 138.44, 145.21 and 129.97 % and 171.56, 174.84 and180.88 %, respectively after 40, 80 and 120 DAS in comparison to control. Whereas, maximum accumulation was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* by 185.94, 207.02 and 200.52 % in comparison to control after 40, 80 and 120 DAS, respectively. Like nitrate and ammonium, accumulation of phosphate by treatment of U+D was found to increase by 197.50 and 20.34 % after 40 and 80 DAS. But at harvesting time (120 DAS) decrease by 25.47 % was recorded in phosphate accumulation in comparison to control. Further, except for U+D accumulation of phosphate in plants of experimental field was always higher than plants of earthen pot soil by effects of all treatments.

The trend of increase in accumulation of phosphate at harvesting time was Control< Urea and DAP< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* at harvesting time. Thus, the highest accumulation of phosphate was recorded by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting as shown in Fig. 4.43.

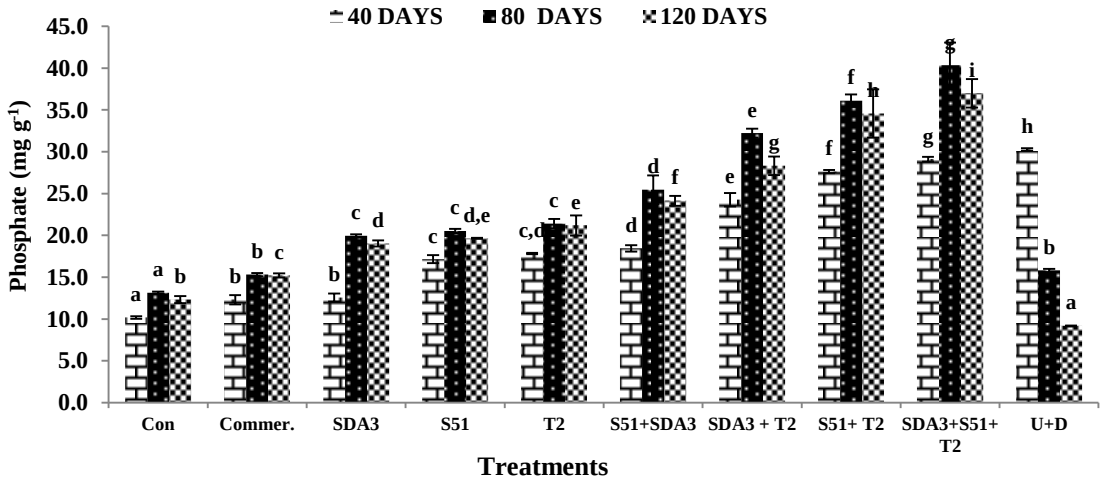

Fig. 4.43 Accumulation of phosphate (mg g^{-1}) in wheat (*Triticum aestivum* L.) from of experimental field applied with different biofertilizer at 40, 80 and 120 days after sowing. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.20)

4.5 Efficacy of best performing formulation of *Trichoderma* and PGPR will be studied on growth and yield of Wheat (*Triticum aestivum* L.) in experimental field.

The selection of best performing formulation of *Trichoderma* and PGPRs (*A. faecalis*, *M. phyllosphaerae*) was based on results acquired from section 4.2, 4.3 and 4.4. Details of the study have been given below-

4.5.1 Effect of selected isolated on growth and yield of wheat in experimental field

The same isolates, commercial biofertilizers and chemical fertilizers were applied in experimental field as that of earthen pots to confirm obtained results. The selected growth parameters (root and shoot length, number of root and leaves, fresh and dry weight) of wheat plants were same as that of earthen pots. Yield was also estimated as productivity of wheat in kilogram per hectare. The details of the findings have been given below-

4.5.1.1 Effect on root length of wheat in experimental field

The root length of control plants in experimental field was recorded to be 5.47, 7.63 and 9.23 cm after 40, 80 and 120 DAS in experimental field. The commercial biofertilizers (market based) showed 56.10, 42.36 and 24.55 % increase in comparison to control after 40, 80 and 120 DAS. Treatment of selected microbial isolated *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced root length by 62.20, 53.71 and 36.10 %, 81.71, 69.43 and 49.82 % and 69.63, 103.93 and 87.0 %, respectively after 40, 80 and 120 DAS, respectively.

The consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* showed 73.78, 106.11 and 89.17 %, 79.27, 103.06 and 97.83 % and 86.59, 111.79 and 98.92 % enhancement after 40, 80 and 120 DAS, respectively. But, co-inosculation of three microbes comprising of two rhizobacteria and one fungus i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* showed the highest increase in root length in comparison to control as 85.37, 129.26 and 110.83 % after 40, 80 and 120 DAS. Whereas, chemical fertilizer urea and DAP were showed 90.85, 144.54 and 128.52 % increase in root length in comparison to control after 40, 80 and 120 DAS. The results showed that consortium of microbes exhibited

higher potential of enhancing root length than individual microbial treatment. Further, in comparison to earthen pots, root length in experimental field was recorded to be high for all treatments for every stage.

The trend of enhancing root length at the time of harvesting was Control< Commer.< *A. faecalis*< *M. phyllosphaerae*< *T. virens*< consortium of *A. faecalis* and *M. phyllosphaerae*< consortium of *A. faecalis* and *T. virens*< consortium of *M. phyllosphaerae* and *T. virens*< consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*< Urea and DAP as shown in Fig. 4.44.

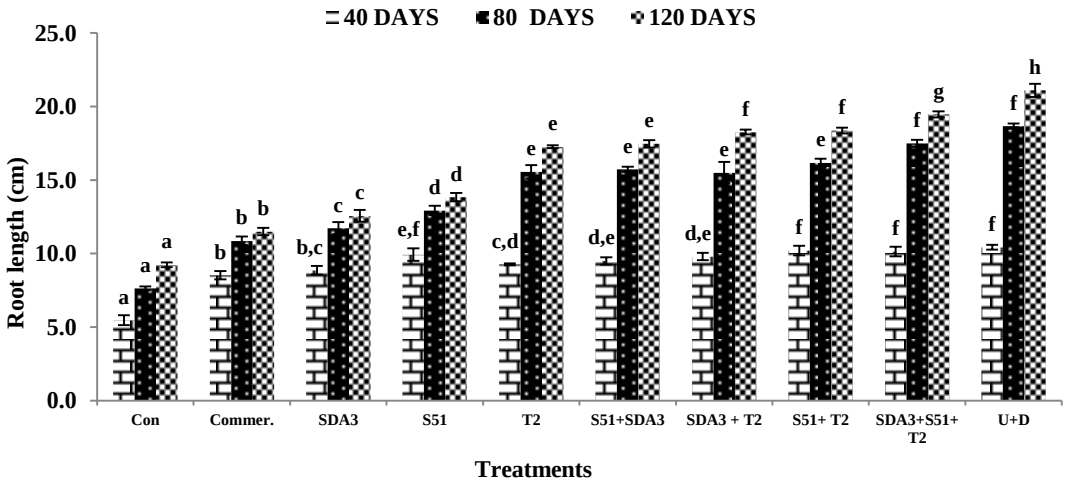

Fig. 4.44 Effect of different treatments on root length of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. The alphabets are significant differences between different treatments. [Where, Con= Control (without any treatment), Commer.= Commercial biofertilizer market based (recommended dose), SDA_3= *Alcaligenes faecalis*, $S5_1$= *Microbacterium phyllosphaerae*, T_2= *Trichoderma virens*, $S5_1+SDA_3$= Consortium of *Microbacterium phyllosphaerae* and *Alcaligenes faecalis*, SDA_3+T_2= Consortium of *Microbacterium phyllosphaerae* and *Trichoderma virens*, $S5_1+T_2$= Consortium of *Alcaligenes faecalis* and *Trichoderma virens*, $SDA_3+S5_1+T_2$= Consortium of *Microbacterium phyllosphaerae*, *Alcaligenes faecalis* and *Trichoderma virens* and U+D= chemical fertilizer Urea and di-ammonium phosphate (recommended dose)].

4.5.1.2 Effect on shoot length of wheat in experimental field

The average shoot length of control was recorded to be 27.47, 47.53 and 54.09 cm after 40, 80 and 120 DAS, respectively in experimental field. Treatment commercial biofertilizer enhanced shoot length of wheat plants by 21.09, 35.34 and 38.08 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, treatment of

individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced shoot length by 29.61, 29.03 and 38.58 %, 35.56, 31.56 and 39.77 % and 32.40, 31.42 and 40.63 % after 40, 80 and 120 DAS, respectively.

Higher enhancements in shoot length were observed by the treatment of consortium of two treatments i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* in comparison to control and individual treatments. It increased shoot length by 46.36, 39.44 and 41.07 %, 39.81, 32.82 and 42.46 % and 48.79, 40.60 and 44.22 %, after 40, 80 and 120 DAS, respectively. Further, highest increase in the sooth length of wheat plants were observed by the consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. The results showed 52.06, 42.01 and 46.19 % increase in shoot length after 40, 80 and 120 DAS. Whereas, treatment of chemical fertilizer (Urea and DAP) enhanced shoot length by 55.95, 43.48 and 51.46 % after 40, 80 and 120 DAS. Similar to root length, shoot length of wheat plants in experimental field were higher than earthen pots at almost every stage. The trend of increase in sooth length at time of harvesting was same as that of root length as shown in Fig. 4.45.

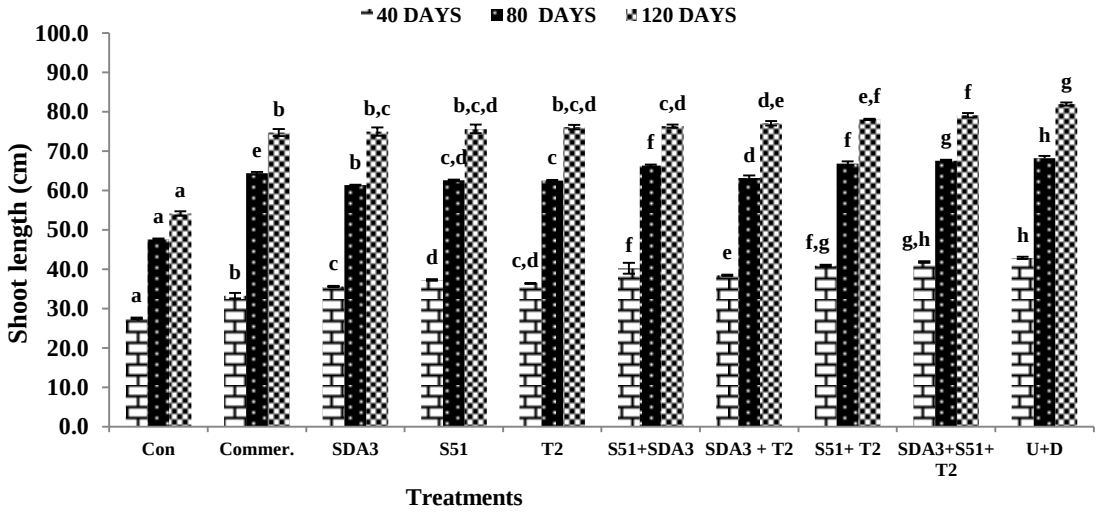

Fig. 4.45 Effect of different treatments on shoot length of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

4.5.1.3 Effect on number of roots of wheat in experimental field

The average number of roots in control wheat plants in experimental field was 8.67, 11.67 and 12.33 after 40, 80 and 120 DAS, respectively. Increase in no. of roots was observed by application of commercial biofertilizers as 11.54, 8.57 and 13.51 % in

comparison to control after 40, 80 and 120 DAS, respectively. Whereas, considerable higher increase in no. of roots were shown by the treatment of individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens*. These treatments enhanced no. of roots by 53.85, 51.43 and 48.65 %, 57.69, 57.14 and 59.46 % and 65.38, 82.86 and 75.68 % in comparison to control after 40, 80 and 120 DAS, respectively.

Likewise, consortium of two isolates like *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* exhibited higher increase in no. of roots in comparison to control as 76.92, 91.43 and 91.89 %, 88.46, 100.0 and 105.41 % and 92.31, 100.0 and 116.22 % after 40, 80 and 120 DAS, respectively. Further, the highest enhancement was recorded by the consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* which increased the no. of roots by 103.85, 117.14 and 127.03 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, treatment of Urea and DAP also enhanced root no. by 115.38, 128.57 and 129.73 % in comparison to control after 40, 80 and 120 DAS, respectively. In addition to this, similar to root and shoot length, no. of roots was also higher in wheat plants of experimental field in comparison to earthen pots at almost every stage for all treatments. The trend of increase in no. of roots was also same as root and shoot length at the time of harvesting as presented in Fig. 4.46.

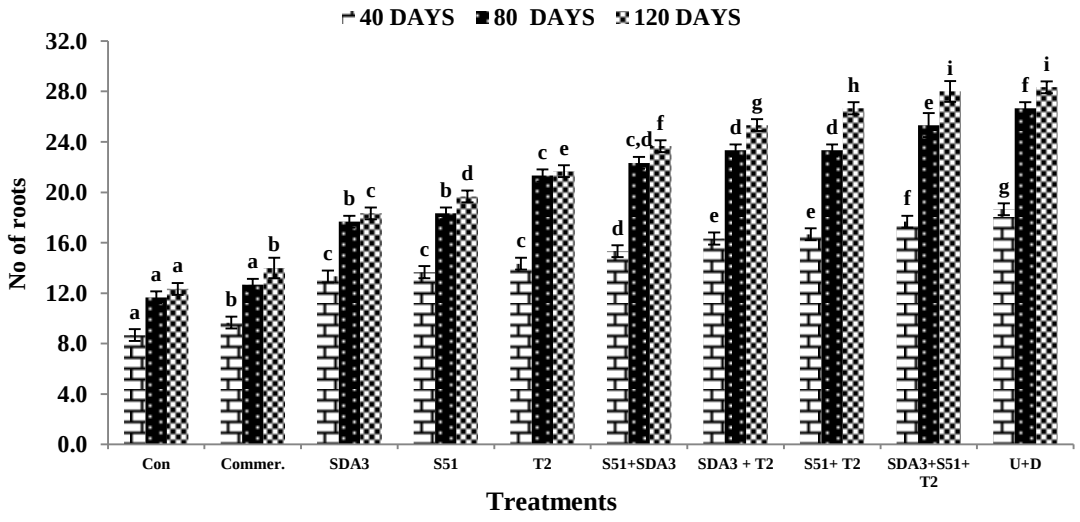

Fig. 4.46 Effect of different treatments on number of roots of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

4.5.1.4 Effect on number of leaves of wheat in experimental field

The average no. of leaves in wheat plants control was 4.33, 7.67 and 3.67 after 40, 80 and 120 DAS, respectively in experimental field. Application of commercial biofertilizers increased no. of leaves by 30.77, 52.17 and 118.18 % in comparison to control after 40, 80 and 120 DAS, respectively. Treatment of individual microbial isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* enhanced no. of leaves by 38.46, 52.17 and 127.27 %, 38.46, 6.52 and 127.27 % and 46.15, 65.22 and 127.27 % after 40, 80 and 120 DAS, respectively.

Whereas, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* showed higher increase in no. of leaves by 46.15, 56.52 and 136.36 %, 38.46, 56.52 and 154.55 %and 46.15, 60.87 and 145.45 % respectively, in comparison to control after 40, 80 and 120 DAS. But, the maximum increase in no. of leaves was obtained by consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*. This treatment enhanced the no. of leaves by 53.85, 78.26 and 181.82 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, chemical fertilizer (Urea and DPA) showed 61.54, 78.26 and 181.82 % increase after 40, 80 and 120 DAS. The results showed that no. of leaves of wheat plants grown in experimental field were higher in comparison to earthen pots like that root, shoot length and no. of roots for every treatment. The trend of increase in number of leaves of wheat plants in experimental field were also same as root and shoot length and number of roots at the time of harvesting as represented in Fig. 4.47.

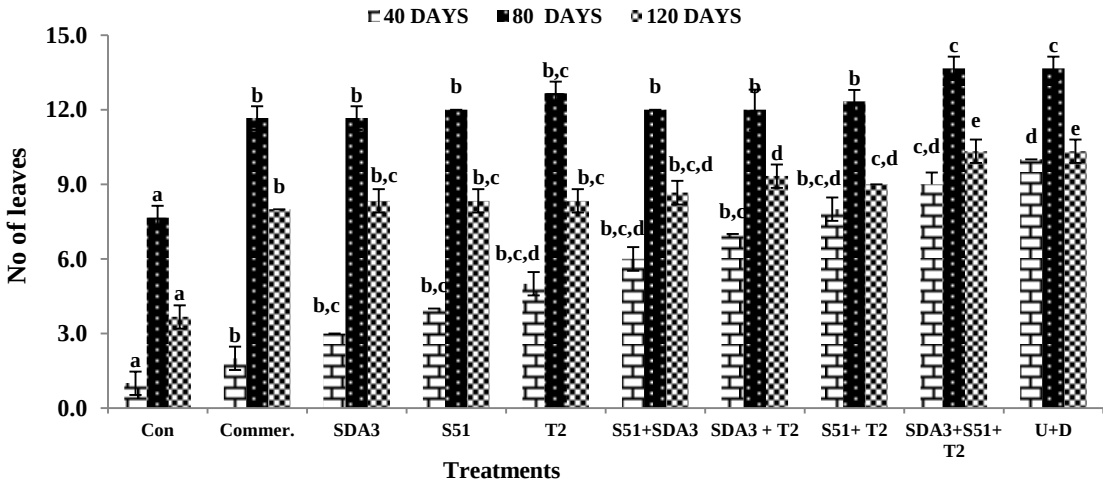

Fig. 4.47 Effect of different treatments on number of leaves of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

4.5.1.5 Effect on fresh weight of wheat in experimental field

The average fresh weight of control wheat plants were 2.87, 6.43 and 4.63 g plant^{-1} after 40, 80 and 120 DAS, respectively. The commercial biofertilizers showed increase in fresh weight in comparison to control plants by 108.14, 48.19 and 90.65 % after 40, 80 and 120 DAS, respectively. Further, selected isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* also enhanced fresh weight by 111.63, 50.26 and 92.81 %, 120.93, 60.62 and 105.04 % and 140.70, 80.78 and 120.50 % in comparison to control after 40, 80 and 120 DAS, respectively. Whereas, consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* enhanced fresh weights of wheat plants by 151.40, 89.64 and 135.83 %, 169.88, 99.38 and 144.82 % and 186.86, 106.74 and 156.76 % in comparison to control after 40, 80 and 120 DAS, respectively. But, the highest increase in fresh weight was recorded by consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 211.98, 120.73 and 164.60 % in comparison to control after40, 80 and 120 DAS, respectively. Treatment of chemical fertilizer (Urea and DAP) also enhanced fresh weight by 246.16, 133.63 and 172.95 %, in comparison to control after 40, 80 and 120 DAS, respectively. With some exceptions e.g. chemical fertilizer, all treatments at every stage showed higher fresh weight in wheat plants experimental field than earthen pots. The trend of increase in fresh weight were also same as root and shoot length, no. of roots and leaves at the time of harvesting as shown in Fig. 4.48.

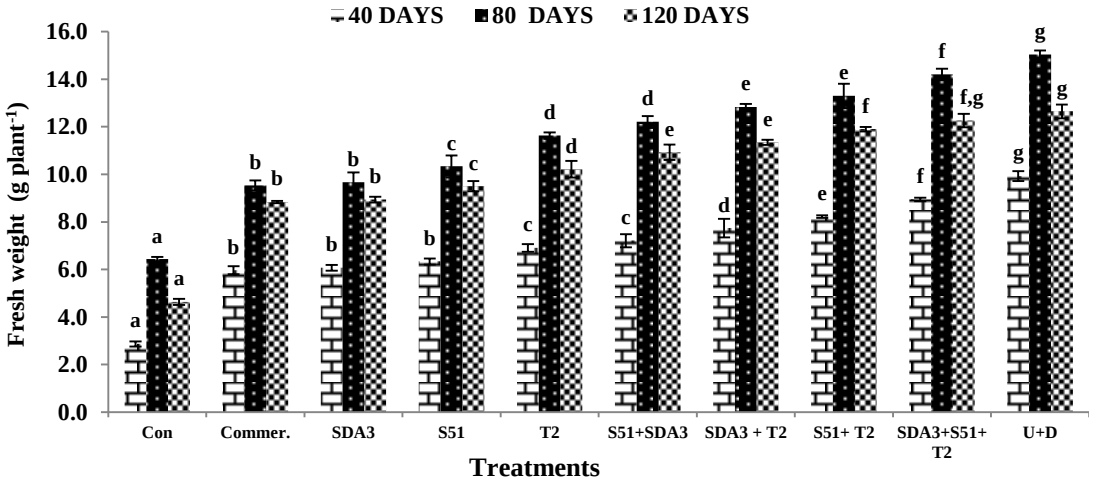

Fig. 4.48 Effect of different treatments on fresh weight of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

4.5.1.6 Effect on dry weight of wheat in experimental field

The average dry weight of control plants was 0.27, 1.06 and 2.73g plant^{-1} after 40, 80 and 120 DAS, respectively. The commercial biofertilizers enhanced dry weight by 191.46 , 73.19 and 21.95 % in comparison to control after 40, 80 and 120 DAS, respectively. Isolated rhizobacteria *A. faecalis*, *M. phyllosphaerae* and fungi *T. virens* significantly enhanced dry weight by 212.20,176.34 and 26.83 %, 287.80, 181.07 and 41.46 % and 314.63, 231.23 and 57.93 % in comparison to control after 40, 80 and 120 DAS, respectively.

Whereas, consortium of two isolates *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* significantly enhanced dry weight in comparison to control by 339.02, 205.68 and 59.76 %, 342.68, 259.94 and 62.20 % and 409.76, 243.53 and 65.85 % after 40, 80 and 120 DAS, respectively. Whereas, the highest increase was recorded by consortium of three individuals *A. faecalis*, *M. phyllosphaerae* and *T. virens* as 429.27, 533.12 and 79.39 % in comparison to control after 40, 80 and 120 DAS, respectively. Further, chemical fertilizer (Urea and DAP) also improved dry weight by 475.61, 579.18 and 94.88 % after 40, 80 and 120 DAS, respectively. The increase in dry weight was also higher for almost all treatments for plants of experimental field in comparison to earthen pots. The trend of increase in dry weight were also same as root and shoot length, no. of roots and leaves and fresh weight of plants of experimental field at time of harvesting as shown in Fig. 4.49.

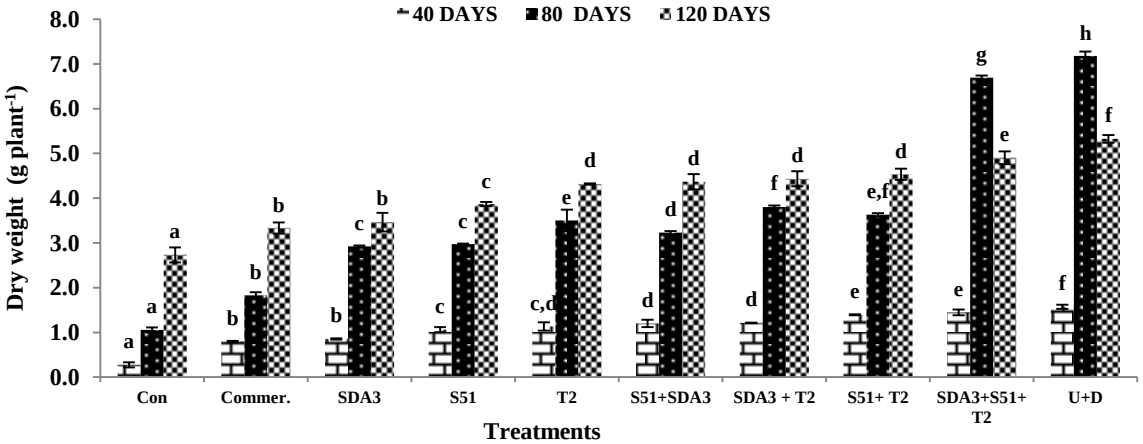

Fig. 4.49 Effect of different treatments on dry weight of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 days after sowing in pot experiment. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

4.5.2 Efficacy of best performing microbial isolates for growth and productivity of wheat in experimental field

The best performing isolates were identified on the basis of optimization process described in sec. 4.3. The details of findings have been presented in Table 4.9. The average root length of wheat plants of control was 9.23 cm at harvesting time. Two rhizobacteria *Alcaligenes faecalis* (SDA_3) and *Microbacterium phyllosphaerae* ($S5_1$) and one fungus *Trichoderma virens* (T_2) enhanced root length which became 12.57, 13.89 and 17.27 cm, respectively. Likewise shoot length also increased from 54.09 to 74.95, 75.60 and 76.06 cm with effects of *A. faecalis*, *M. phyllosphaerae* and *T. virens*, respectively. Likewise, no. of roots increased from 12.33 to 18.33, 19.67 and 21.67, no. of leaves from 3.67 to 8.33, 8.33 and 8.33 fresh weight from 4.63 to 8.93, 9.50 and 10.22 g $plant^{-1}$ and dry weights from 2.73 to 3.47, 3.87 and 4.32 g $plant^{-1}$ as given in Table 4.9. The productivity of control plants was 1.87 t ha^{-1} which was observed to increase by treatments of isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* to become 2.78, 2.87 and 2.89 t ha^{-1}. The percentage increase in the productivity was 48.82, 53.84 and 54.67 %, respectively with the effect of *A. faecalis*, *M. phyllosphaerae* and *T. virens* in comparison to control. Although, individually all three isolates improved growth parameters and productivity at harvesting time in comparison to control, but the highest enhancements and productivity were recorded by the fungus *T. virens* (T_2) (Fig. 4.50). The isolates of *T. virens* (T_2) showed highest increase in growth and productivity of wheat plants in comparison to *A. faecalis* (SDA_3) and *M. phyllosphaerae* ($S5_1$) at harvesting time. Thus, it can be concluded that the best performing isolate was, and *T. virens* T_2 followed by *M. phyllosphaerae* and *A. faecalis*.

Fig. 4.50 Effect of selected isolates on growth parameters of wheat (*Triticum aestivum* L.) in comparison to control (no fertilizer) uprooted from experimental field (where, 1=U+D, 2= T_2, 3=SDA_3, 4=Con, 5=Com, 6=$S5_1$, 7=$S5_1$ + SDA_3, 8=$S5_1$ + T_2, 9=SDA_3 +T_2 and 10=$S5_1$ + SDA_3 + T_2)

Table 4.9 Effects of individual isolates on growth and yield of wheat (*Triticum aestivum* L.) of experimental fields at harvesting time in comparison to control (no fertilizer)

Growth parameters	Con	SDA_3	$S5_1$	T_2
Root length (cm)	9.23 ± 0.17^{a}	12.57 ± 0.41^{c}	13.83 ± 0.29^{d}	17.27 ± 0.09^{e}
Shoot length (cm)	54.09 ± 0.56^{b}	$74.95\pm1.05^{b,c}$	$75.60\pm1.11^{b,c,d}$	$76.06\pm0.60^{b,c,d}$
No. of roots	12.33 ± 0.47^{a}	18.33 ± 0.47^{c}	19.67 ± 0.47^{d}	21.67 ± 0.47^{e}
No. of leaves	3.67 ± 0.47^{a}	$8.33\pm0.47^{b,c}$	$8.33\pm0.47^{b,c}$	$8.33\pm0.47^{b,c}$
Fresh weight (g plant^{-1})	4.63 ± 0.12^{a}	8.93 ± 0.12^{b}	9.50 ± 0.22^{c}	10.22 ± 0.35^{d}
Dry weight (g plant^{-1})	2.73 ± 0.17^{a}	3.47 ± 0.21^{b}	3.87 ± 0.05^{c}	4.32 ± 0.02^{d}
Yield (t ha^{-1})	1.87 ± 0.002^{a}	2.78 ± 0.001^{b}	2.87 ± 0.002^{c}	2.89 ± 0.003^{d}

*Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$. [Where, Con= Control (without any treatment), SDA_3= *A.*, $S5_1$= *M. phyllosphaerae* and T_2= *T. virens*)]

4.5.3 Efficacy of best performing formulation of *Trichoderma* and PGPRs on growth of wheat in experimental field

The best performance was recorded by the consortium of three isolates i.e. *A. faecalis, M. phyllosphaerae* and *T. virens* followed by consortium of two isolate i.e. *M. phyllosphaerae* and *T. virens, A. faecalis* and *T. virens* and *A. faecalis* and *M.*

phyllosphaerae. These formulations were applied for evaluating its effect on growth and yield of wheat (*T. aestivum* L.) in experimental field. The effects have been described in Table 4.10 as following-

Table 4.10 Effects of best performing combination of *Trichoderma* and PGPRs on growth parameters of wheat (*Triticum aestivum* L.) plants at harvesting time in experimental field in comparison to control (no fertilizer)

Growth parameters	Control	$S5_1$+SDA_3	SDA_3+T_2	$S5_1$+T_2	SDA_3+$S5_1$+T_2
Root length (cm)	9.23 ± 0.17^{a}	17.47 ± 0.25^{e}	18.27 ± 0.17^{f}	18.37 ± 0.19^{f}	19.47 ± 0.21^{g}
Shoot length (cm)	54.09 ± 0.56^{b}	$76.30\pm0.37^{c,d}$	$77.05\pm0.57^{d,e}$	$78.0\pm0.15^{e,f}$	79.07 ± 0.58^{f}
No. of roots	12.33 ± 0.47^{a}	23.67 ± 0.47^{f}	25.33 ± 0.47^{g}	26.67 ± 0.47^{h}	28.0 ± 0.82^{i}
No. of leaves	3.67 ± 0.47^{a}	$8.67\pm0.47^{b,c,d}$	9.33 ± 0.0^{d}	$9.0\pm0.47^{c,d}$	10.33 ± 0.47^{e}
Fresh wt. (g plant^{-1})	4.63 ± 0.12^{a}	10.93 ± 0.32^{e}	11.34 ± 0.11^{e}	11.90 ± 0.09^{f}	$12.26\pm0.28^{f,g}$
Dry wt. (g plant^{-1})	2.73 ± 0.17^{a}	4.37 ± 0.17^{d}	4.43 ± 0.17^{d}	4.53 ± 0.12^{d}	$4.90\pm0.14^{e,f}$
Yield (t ha^{-1})	1.87±0.002	2.90±0.002	2.91±0.001	3.10±0.002	3.14±0.002

*Data are presented as mean of twelve replicates with two determinations (n=12) ± S.D. is shown by bars. One way ANOVA is applied to determine statistical differences between means of various treatments at $p< 0.05$ (where, $S5_1$+SDA_3= Consortium of *M. phyllosphaerae* and *A. faecalis*, SDA_3+T_2= Consortium of *M. phyllosphaerae* and *T. virens*, $S5_1$+T_2= Consortium of *A. faecalis* and *T. virens*, SDA_3+$S5_1$+T_2= Consortium of *M. phyllosphaerae*, *A. faecalis* and *T.virens*)

As seen from Table 4.9, the maximum growth and yield of wheat plants were recorded for treatment of consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* followed by consortium of three isolates i.e. *M. phyllosphaerae* and *T. virens*, *A. faecalis* and *T. virens* and *A. faecalis* and *M. phyllosphaerae*. Root length of wheat plants in experimental field was enhanced by 89.17, 97.83, 98.92 and 110.83 %, respectively at harvesting time by the effect of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens*; and *A. faecalis*, *M. phyllosphaerae* and *T. virens*, respectively. Likewise shoot length was increased by 41.07, 42.46, 44.22 and 46.19 %, respectively in comparison to control. Whereas, no. of roots and leaves were enhanced by 91.89, 105.41, 116.22 and 127.03 % and 136.36, 154.55, 145.45 and 181.82 %, respectively after treatment of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens*; and *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Following same trend, fresh weight and dry

weight were also accelerated by 135.83, 144.82, 156.76 and 164.60 % and 59.76, 62.20, 65.85 and 79.39 %, respectively in comparison to control.

4.5.4 Efficacy of best performing formulation of *Trichoderma* and PGPRs on productivity of wheat plants in experimental field

The productivity of control was 1.87 t ha^{-1} which was enhanced by applying consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens*; and *A. faecalis*, *M. phyllosphaerae* and *T. virens* to become 2.90, 2.91, 3.10 and 3.14 t ha^{-1}, respectively (Fig. 4.51 and 4.52). The percentage increase in productivity of wheat in experimental field was 55.12, 55.89, 65.86 and 67.80 %, respectively in comparison to control with the effect of consortium of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens*; and *A. faecalis*, *M. phyllosphaerae* and *T. virens*. The enhancement in productivity by commercial biofertilizer was 8.22 %, which was low in comparison to collective effects of formulated consortia. Further, chemical biofertilizer (Urea and DAP) showed almost same productivity (3.27 t ha^{-1}) as that of consortia of *A. faecalis*, *M. phyllosphaerae* and *T. virens*. Therefore, application of consortia containing *A. faecalis*, *M. phyllosphaerae* and *T. virens* should be encouraged over chemical fertilizers as the productivity was nearly same and there were no side effects over environment also. Application of formulated consortia of *A. faecalis*, *M. phyllosphaerae* and *T. virens* may be recommended for commercial usage to enhance growth and yield of wheat.

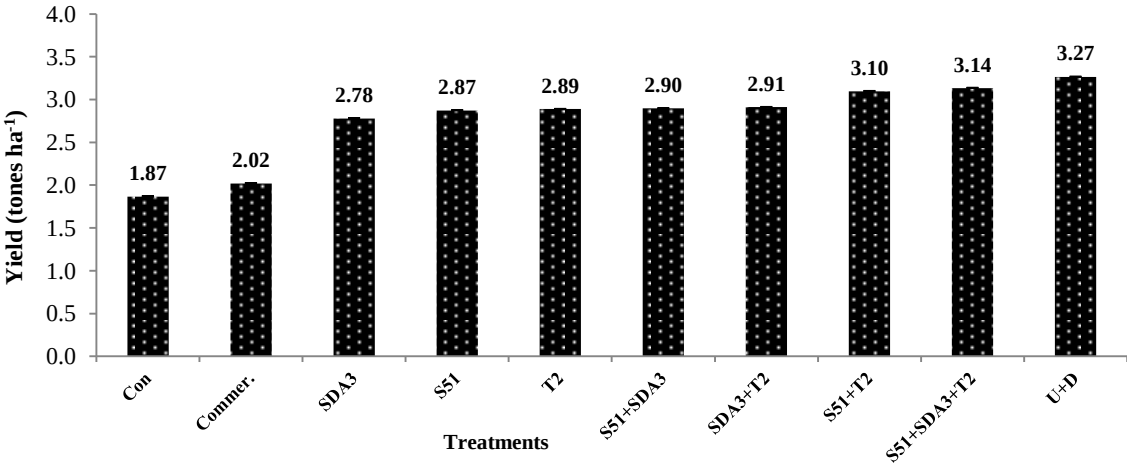

Fig. 4.51 Effect of different treatments on productivity of wheat (*Triticum aestivum* L.) plants at 40, 80 and 120 DAS in experimental field. Data are presented as mean of twelve replicates with two determinations (n=12) ±S.D. is shown by bars (remaining details are similar to Fig. 4.44)

Fig. 4.52 Demonstration of wheat (*Triticum aestivum* L.) at harvesting time in experimental field (a and b)

4.6 Estimation of cost benefit analysis of newly developed eco-friendly consortium vis a vis conventional chemical fertilizers.

The last objective of the study was to analyze whether the cost involved in the manufacturing and application of formulated biofertilizers provide benefits to the farmers and government or not. This analysis was done by analyzing cost benefit assessments of biofertilizer as newly developed eco-friendly consortium over control and chemical fertilizers. The details of the study have been summarized in Table 4.11.

4.6.1 Estimation of cost benefit analysis of newly developed eco-friendly consortium and individual isolates over control

As an estimate, input cost in (rs) ha^{-1} required for control was approximately 22,180 rs ha^{-1} that included field preparation cost, labor charges, seed purchasing cost, and irrigation cost ha^{-1}. The application of chemical fertilizers will add cost (i.e. about 5400 ha^{-1}) of it in addition to the amount of control. Thus, the total cost involved in application of chemical fertilizers (Urea and DAP) was ~27,180 rs ha^{-1}. The net benefits (ha^{-1}) acquired was based on net yield of wheat in (ton ha^{-1}) and selling income (ton ha^{-1}). Treatment of control provided net benefit of about 10572 rs ha^{-1} which was enhanced by application of chemical fertilizer and become 29645 rs ha^{-1}. This was about 180.41 % higher in comparison to control. Whereas, market based commercial biofertilizer (Commer.) required about 22420 rs ha^{-1} and gained about 22.30 % benefit with net benefit of ~12930 rs ha^{-1}. Whereas, treatment of *A. faecalis*, *M. phyllosphaerae* and fungi *T. virens* required about 22420, 22390 and 22390 rs ha^{-1}, respectively as input cost which was lesser than chemical fertilizer (29645 rs ha^{-1}). Whereas, the net benefit from treatment of *A. faecalis*, *M. phyllosphaerae* and fungi *T. virens* in the form of income was about 26260, 27835 and 28135 rs ha^{-1}, respectively. These were about 148.39, 163.29 and 166.13 % in comparison to control.

The consortium of isolated microbes showed higher gain in comparison to individuals and control. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* required input cost of about 22440 rs ha^{-1}. The net benefit gained as income was 28310, 28485 and 31910 rs ha^{-1}, respectively in comparison to control. The net income was about 167.78, 169.44 and 201.84 % in comparison to control. The maximum benefit was recorded by collective effects of two rhizobacteria and one fungus i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens*.

The input cost was about 22470 rs ha^{-1} and net income was 32480 rs ha^{-1}. The income was 207.23 % higher in comparison to control. Thus, the highest income was achieved by newly developed consortium of three individual *A. faecalis*, *M. phyllosphaerae* and *T. virens* followed by consortium of two individuals i.e *M. phyllosphaerae* and *T. virens*; *A. faecalis* and *T. virens* and *A. faecalis* and *M. phyllosphaerae*.

4.6.2 Estimation of cost benefit analysis of newly developed eco-friendly consortium and individual isolates over chemical fertilizers

The net benefit acquired by consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* were lesser than chemical fertilizers (Urea and DAP). Urea and DAP gained net benefit of about 29645 rs ha^{-1} whereas, *A. faecalis* and *M. phyllosphaerae* and *A. faecalis* and *T. virens* achieved only 28310 and 28485 rs ha^{-1} which was about 4.50 and 3.93 % lesser than chemical fertilizers. On the other hand, consortium of *M. phyllosphaerae* and *T. virens* and *A. faecalis*, *M. phyllosphaerae* and *T. virens* achieved net benefit in terms of income as 31910 and 32480 rs ha^{-1} which was 7.64 and 9.56 % higher in comparison to chemical fertilizers. Moreover, as the net benefits from newly form consortium were higher in comparison to chemical fertilizers, so application of these fertilizers should be encouraged over chemical fertilizers.

Table 4.11 Comparative cost benefit analysis of selected treatments over chemical fertilizers

Treatments	Input cost in (rs) ha^{-1}				Income (rs) ha^{-1}		
	Field preparation cost/labor charges/**	Seed purchasing cost	Fertilizers cost ha^{-1}	Irrigation cost ha^{-1**}	Net yield of wheat in (ton ha^{-1})	Selling income (ton ha^{-1})	Net benefit (rs ha^{-1})
Control (No fertilizer)	9300.00	3280.00	0.00	9600.00	1.87	32752	10572
Chemical fertilizer (U+D)	9300.00	3280.00	5400.00*	9600.00	3.27	57225	29645
Commercial (market based) biofertilizers (Comm.)	9300.00	3280.00	240.00	9600.00	2.02	35350	12930
***Alcaligenes faecalis* (SDA_3) based biofertilizer**	9300.00	3280.00	210.00	9600.00	2.78	48650	26260
***Microbacterium phyllosphaerae* ($S5_1$) based biofertilizer**	9300.00	3280.00	210.00	9600.00	2.87	50225	27835
***Trichoderma* based biofertilizer (T_2)**	9300.00	3280.00	210.00	9600.00	2.89	50575	28135
***Microbacterium phyllosphaerae* ($S5_1$)+*Alcaligenes faecalis* (SDA_3) based biofertilizer**	9300.00	3280.00	260.00	9600.00	2.90	50750	28310
***Alcaligenes faecalis* (SDA_3)+ *Trichoderma* (T_2) based biofertilizer**	9300.00	3280.00	260.00	9600.00	2.91	50925	28485
***Microbacterium phyllosphaerae* ($S5_1$)+*Trichoderma* (T_2) based biofertilizer**	9300.00	3280.00	260.00	9600.00	3.10	54250	31910
***Microbacterium phyllosphaerae* ($S5_1$) based biofertilizer + *Alcaligenes faecalis* (SDA_3) based biofertilizer +*Trichoderma* based biofertilizer (T_2)**	9300.00	3280.00	290.00	9600.00	3.14	54950	32480

*= Govt. rate, Labor charges day^{-1}= 300/- rs; *Urea (50 Kg) = 300/- rs; Biofertilizers Kg^{-1}= 100/- rs; *DAP (50 Kg) = 1450/- rs; *Irrigation 120/- rs h^{-1}

Chapter 5

Discussion

5. Discussion

Evolving sustainable, cost effective and ecofriendly procedure for enhancing the productivity of crops is a global need and is one of the major challenges in the field of agriculture at present (Meena et al. 2017). As an estimate the global food production must be amplified by at least 70 % upto 2050 as there is rising anxiety for food due to ever increasing population rates worldwide (Keinan and Clark 2012; Rahman et al. 2017). A recent estimate also presents that there has been an upsurge in world population at an frightening rate and is prophesied to rise over 2.4 billion by 2050 (Basu et al. 2017). To fulfill the nutritional demands of this growing population, the needy and greedy nature of humans has intensified the usage of chemical fertilizers to enhance the productivity of food crops. Thus, there is a growing interest in application of microorganisms with food crops and various previous studies have demonstrated different advantageous aspects of these microbes in growth and yields of various crops like wheat, rice, maize, onion, soybean etc. (Hasan et al. 2012; Tahir et al. 2013; Mahanta et al. 2014; Verma et al. 2015; Almaghrabi et al. 2014; Ng et al. 2015; Bunbury-Blanchette and Walker 2018). Microbial biofertilizers have emerged as a suitable alternative to meet these challenges in ecological way (Kundu et al. 2009; Kumar et al. 2014; Kumar and Singh 2018a).These biofertilizers are different formulations containing variety of living microorganism that have the potential to provide nutrients from through the biological procedures. Application of plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) as biofertilizers is an efficient alternative way out of the chemical fertilizers to maintain the sustainable growth and productivity of crops (Rahman et al. 2017; Zaidi et al. 2015; Kumar and Verma 2018). These microbes colonize in the roots and rhizospheric regions following inoculation onto seed or in soil and promote plant growth & development variably (Grobelak et al. 2015). Rhizosphere is a soil microenvironment directly surrounds the root system which is rich in encloses a number of free-living or symbiotic rhizospheric bacteria, fungi, insects and nematode etc. (Zeppenfeld et al. 2017). These organisms use to fix atmospheric N, produce siderophores, solubilize many essential minerals such as zinc and phosphorus, and produces plant hormones and synthesize some volatile compounds or catabolic enzymes which helps in plant growth and development (Dell'mour et al. 2012; Pérez-Montaño et al. 2014; Kumar S. et al. 2017; Renuka et al. 2017). Other indirect mechanisms of action of microbial plant growth promoters

involved in different processes like production of secondary metabolites that possess antibiotic qualities or antifungal substances, insecticides and immune-suppressants and stimulators of the plants defense system that eliminates attack of phytopathogens (Glick 2005; Sharma et al. 2017; Mhatre et al. 2019). The PGPFs compete with phytopathogens for space and nutrition and eliminate them by these antibiotic properties (Chandanie et al. 2009; Das et al. 2018; El-Sharkawy et al. 2018). These properties of PGPRs and PGPFs have benefitted the present study that helped in promotion of growth and productivity of foremost food crop wheat with the effect of application of biofertilizers containing these microorganisms. Pérez-Montaño et al. (2013) have reported the reports of FAO (http://faostat3.fao.org) which suggested that maize (*Zea mays*), rice (*Oryza sativa*) and wheat (*Triticum aestivum* L.) are foremost major cereals crops in terms of production (million tons) worldwide. Thus, wheat is the major cereal crop, feed, industrial raw material as well as is consumed by human, cattle and other herbivorous animals all across the world (Pérez-Montaño et al. 2013; El-Sharkawy et al. 2018). As estimation, the requirement for wheat in developing countries is predictable to upsurge by 60% by 2050 whereas climate change and global warming is also anticipated to disturb production negatively by 29 % in similar zones (Dixon et al. 2009; Basu et al. 2017). So, it is foremost task to enhance the productivity of wheat without causing burden on economy and environment which occur due to utilization of chemical fertilizers and pesticides.

The present study was focused on the isolation, characterization, optimization and application of PGPR and PGPF to enhance the growth and productivity of major food crop wheat (*T. aestivum* L.) as individual seed inoculants or consortium of multiple compatible selected microbial species with commercial biofertilizers and chemical fertilizer. Thirty three soil samples were collected from the soils of wheat fields around Lucknow city. Among all soil samples, twenty three bacteria and two *Trichoderma* spp. were isolated.

The biochemical depiction of selected bacterial isolates showed that isolates were positive for cellulase test. All bacterial isolates except for S_5, SS_4, M_1 and M_3 showed positive test for indole and nitrate (except S_2, S_5, $SS3_1$, $S8_Y$ and M_1) tests. Amylase test was positive for all bacterial isolates except for S_1, S_6, SS_1, $SS4_2$, $SS3_1$, $S5_1$, $S5_2$, $S8_Y$, SDA_1, SDA_2, SDA_3 and M_2. Whereas, among 23 bacterial isolates only

three i.e. $S5_1$, M_1 and M_3 showed positive citrate test and seven i.e. S_1, S_5, SS_1, $S5_1$, $S8_W$, M_2 and M_4 showed positive test for H_2S production. All bacterial isolates except S_5, SS_4, $SS3_1$, M_2 and M_3 showed positive lipase test and protease test was positive only for twelve bacterial isolates (S_2, S_5, S_7, S_9, SS_1, $SS3_2$, $S5_1$, $S8_Y$, SDA_1, SDA_2, SDA_3 and M_4).Whereas, all the isolated bacterial strains gave negative tests for urease and gelatin.

Among all isolated PGPMs three strains ($S5_1$, SDA_3 and T_2) were selected on the basis of good PGP activities like nitrogen fixation, phosphate solubilization, ammonia, siderophore and IAA production which were based on qualitative and quantitative tests. Phosphate solubilization and IAA production was tested quantitatively. SDA_3 and $S5_1$ gave 2.0 and 1.5 Psi for phosphate solubilization, respectively and T_2 secured 227µg ml^{-1}.Whereas SDA_3, T_2 and $S5_1$ produced 100.32, 99.78 and 99.84 µg ml^{-1} IAA respectively. However, nitrogen fixation, ammonia and siderophore production were observed qualitatively. The study represented that only the selected isolated strains showed good characteristics for all PGP tests which were essential criteria to select strains as biofertilizers.

Further, compatibility test among the PGPMs ($S5_1$, SDA_3 and T_2) that showed good PGP activities were also checked for their compatibility with each other to support the selection of these strains to be used in co-inoculant for pot and plot studies. The selected stains showed absence of inhibition zone around the colonies which signified that these were compatible with each other and may be used as biofertilizers in consortium.

Moreover, it has been reported that various phenotypic and genotypic methods were used to identify and characterize microbial isolates in previous years by different researchers (Mehnaz et al. 2010; Jida and Assefa 2012; Benmati et al. 2013; Spaepen et al. 2014; Luo et al. 2015). Morphological identification may be used for identification of PGPMs but molecular methods are supposed to be more reliable methods to authenticate different microbial studies as it is the basic requirement for microbial analysis at species level (Tahir et al. 2013; Kaur et al. 2013; Joe et al. 2016; Kumari et al. 2018). In this regard 16S and 18S rRNA gene sequencing technique was used for identification of bacterial and fungal isolates. Among three selected isolates two $S5_1$ and SDA_3 were bacterial strains which were identifies as *Microbacterium*

phyllosphaerae ($S5_1$), *Alcaligenes faecalis* (SDA_3) and one fungal strain identified as *Trichoderma virens* (T_2).

At last, these three compatible strains were examined for their efficiency to be used as consortium to enhance the growth and productivity of wheat crop on the basis of their good PGP activities. These strains were firstly examined in earthen pots individually as well as in consortium for their potential to enhance growth and productivity of wheat. The best performing formulations were further used in the experimental fields. The results obtained from selected isolates were compared with control (no treatment) and market based commercial (*Azoto* plus) biofertilizers.

Previous studies of Tahir et al. (2013); Meena et al. (2015); Grobelaket al. (2015); Joe et al. (2016); Kumari et al. (2018) etc. were in accordance with the present study. Meena et al. (2015) had also utilized same process to isolate PGPR strains from pea nodules, by culture-dependent standard plate technique. The morphological and biochemical analysis was basis of selection of total of 19 rhizobacterial isolates. Among these, four PGPR isolates that showed good growth in comparison to reference strain at lower temperature were selected for physiological and biochemical description. These strains also produced IAA (maximum of 1.4 µg ml^{-1}) and solubilized phosphate similar to present study. Likewise, Grobelaket al. (2015) has also applied similar biochemical and morphological characterization of bacterial isolates for isolation of 45 bacterial strains from roots of *Agrostis capillaris*. Most of the isolated bacteria were Gram-negative. Joe et al. (2016) have also reported similar method of isolation and phylogenetic analysis by 16S rRNA gene sequences of *Acinetobacter* spp. and *Bacillus* spp. from roots of *Phyllanthus amarus*. These were reported to be salt tolerant endophytic and phosphate solubilizing bacteria. The same process of isolation was also reported by Kumari et al. (2018) who isolated thirty nine strains of rhizobacteria from roots of mung bean plants. The cultural, morphological and biochemical characteristic were used to identify the strains as *Pseudomonas*, *Bacillus*, and *Acinetobacter*. These gave negative results for indole test, H_2S production, lactose utilization and positive for glucose and citrate utilization. They also characterized these stains on molecular levels done by 16S rDNA gene sequencing analysis and submitted to NCBI GenBank using BLAST (basic local alignment tool) analysis. Isolation of PGPRs genera including *Enterobacter, Azospirillum* and *Bacillus* has also been

reported by Tahir et al. (2013). Kaur et al. (2013) have also characterized *Alcaligenes Faecalis* by biochemical and 16 S rDNA analysis similarly to the present study.

The results were in accordance with Cortivo et al. (2017) who have also reported also that the association of plant and microorganisms should be adequately compatible to endorse the multiplication of PGPMs to promote different signals which preceded their colonization (Videira e Castro et al. 2016). Recently Saritha et al. (2019) have suggested to avoid usage of multiple strains of PGPMs in biofertilizers if they might not be compatible with each other. Whereas, Joe et al. (2016) have reported that incompatible strains of *Acinetobacter* sp. and *Bacillus* sp. failed to promote growth of medicinal plant *Phyllanthus amarus* under co-inoculation conditions. They strictly recommended evaluation of compatibility before using co-inoculation of bacterial strains as biofertilizer. Naseem and Bano (2014) have reported consortia of inocula of three bacterial strains *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2), and *Alcaligenes faecalis* (AF3) tolerated drought conditions when used as PGPR for maize plants which enhanced different growth parameters. They have sequenced the isolated strains by 16S rRNA techniques similar to present study.

The isolated strains were used as biofertilizer to improve the growth and productivity of wheat. The positive outcomes of present study have proved that applied biofertilizers may be successfully applied at ground levels to enhance the productivity of major food crops of the world 'wheat'. The results of present study indicated that different isolates of *Trichoderma* and PGPRs alone or as a consortium and its similar commercially available products significantly ($p<0.05$) improved all plant growth parameters i.e. root and shoot length, no. of root and leaves, fresh and dry weight in earthen pot conditions and in net house conditions (Fig. 4.9-4.14). Among different isolates, two *Microbacterium phyllosphaerae* ($S5_1$) and *Alcaligenes faecalis* (SDA_3) were plant growth promoting rhizobacteria (PGPR) and one *Trichoderma virens* (T_2) was plant growth promoting fungus (PGPF). The reason of such a good improvement in the crop productivity may be collective efforts of two rhizobacteria and one fungus which might have added to the plant growth promoting mechanisms of wheat in multiple ways after its inoculation as suggested by Mahanta et al. (2014) for strains *Pseudomonas striata* and *Glomus fasciculatum*. Likewise, Upadhyay et al. (2012) have also reported enhanced dry weight i.e. 26 % of wheat plants when co-inoculated

Arthrobacter sp. and *Bacillus subtilis* at 2 dS m^{-1} of salinity level and 40 % when co-inoculated at 6 dS m^{-1} of salinity levels in pot experiments under greenhouse conditions. The PGPR and PGPF both have potential to competitively colonize in plant roots and its rhizospheric regions and promote plant growth by tumbling population of injurious soil organisms (Kumari et al. 2018; Meena et al. 2017; Wang et al. 2017). The PGPR also stimulate plant growth by various indirect and direct mechanisms like enhanced secretion of plant growth regulators (e.g. auxin, cytokinins, ethylene and gibberellins), phosphate solubilization activities, upsurge nutrients uptake and suppressed different fungal, bacterial, viral and nematode pathogens (Mukhtar et al. 2017; Kumar and Verma 2018). Phytohormone IAA is responsible for growth promotion of root and shoot with plant biomass by enhancing stimulating cell division and elongation of plant which are secreted by these microbes including *Trichoderma* spp. (Marulanda et al. 2009; Vacheron et al. 2013; Martínez-Medina et al. 2014; Kaushal and Wani 2016; Basu et al. 2017). Whereas, *Trichoderma* spp. also have the ability to directly affect pathogens through space and/or nutrient competition, antibiosis, mycoparasitism and/or indirectly through initiation of plant resistance against abiotic and biotic stresses (Nawrocka et al. 2013; Zeilinger et al. 2016; Mendoza-Mendoza et al. 2017; Sharma et al. 2017; Wang et al. 2017; El-Sharkawy et al. 2018).

The length of root and shoot, number of roots and leaves and fresh and dry weights were found to increase in order of Control< Commer.< *A. faecalis* < *M. phyllosphaerae* < *T. virens* < consortium of *A. faecalis* and *M. phyllosphaerae* < consortium of *A. faecalis* and *T. virens* < consortium of *M. phyllosphaerae* and *T. virens* < consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens* < Urea and DAP in earthen pots. In initial 40 days after sowing (DAS), the increase in growth due to application of microbial biofertilizer was less pronounced which increased gradually till 40-80 DAS and became stagnant thereafter. It may be due to the enhanced activities of microbes after inoculation which gradually increased and became static after reaching its peak. The same pattern of microbial impacts was recorded in experimental fields also that of Mäder et al. (2011) have reported that dual culture of wheat with PGPR (*Pseudomonas jessenii* and *P. synxantha*) and natural arbuscular mycorrhizal fungus (AMF) in form of consortium increased grain yield of wheat by 41% as compared to un-inoculated controls. It has also been reported that consortium of

Providencia sp., *Anabaena* sp. and *Calothrix* sp. enhanced 18.6% protein content of wheat plants in field experiments (Rana et al. 2012). The enhancements of roots and shoots in comparison to controls may be due to production of IAA by selected strains of PGPR and PGPFs especially *Trichoderma* spp. (Mendoza-Mendoza et al. 2017). Yield of wheat and barley was increased with the application of a consortium of bacteria and fungi over the application of single strain (Turan et al. 2012). Further, synthesis of phytohormones, solubilization of minerals and antifungal activities may also be the governing factors as suggested by Glick (2012) and Grobelak et al. (2015). According to Spaepen and Vanderleyden (2011) IAA shows important role in rhizobacteria and plant interactions which in turn govern defense mechanisms of plants against phytopathogenic bacteria. Further, it controls development of lateral roots, enhancement of primary root length and root hairs (Vacheron et al. 2013). El-Sharkawy et al. (2018) have recently reported that of the direct contact of *Trichoderma* isolates individually or in combinations with arbuscular mycorrhizal fungi inhibited germination of wheat pathogen *Puccinia graminis* and increased yield and productivity of wheat. Wang et al. (2017) have reported that plant dry matter yields of sweet sorghum plants applied with urea+viable *Trichoderma viride* (T) biofertilizer and urea+nonviable *T. viride* (TS) biofertilizer treatments were expressively higher as compared with conventional fertilizer (CK) urea. T and TS biofertilizer improved plant biomasses by 91.23% and 61.08%, respectively in comparison to CK. In addition to this, Isfahani and Besharati (2012) also detected a significant rise in length, fresh and dry weight of roots and shoots and yield of inoculated cucumber plants with collective effects of biofertilizers containing *Pseudomonas* sp. and *Pantoea agglomerans* and chemical fertilizers under field conditions. Further, Bayoumi et al. (2018) found that application of *Trichoderma* spp. improved vegetative growth traits and chlorophyll content of onions which may be due to their synergistic effects of *Trichoderma harzianum* and *T. viride*. It improved the growth and yield of onion in comparison to fungicide application which was traditionally practiced by farmers.

Grobelak et al. (2015) have reported sprouting of seeds for 48 h on plates in bacteria suspension and inoculation of plants after two weeks showed highest yield and significantly increased the shoot biomass of fescue and rape plants. The reason may be the activities of endophytic bacteria which beneficially affected plant growth. Further, the chemical fertilizers provided essential nutrients to crops but also polluted the

environment in terms of N fertilizers (Cortivo et al. 2017; Kumar and Verma 2018). According to Pérez-Montaño et al. (2014) plants embrace nitrogen from soil as nitrite, nitrate or ammonia and phosphorus as phosphate. These forms of N and P are not in ample amount in different soils and chemical fertilizers are normally gone astray during rainfall or through leaching. But, application of inoculated strains of rhizobacteria and fungus not only promoted all growth parameters but also improved the sustainability of agro-ecosystems with enhanced production in greener way (Mhatre et al. 2019). According to Kumar et al. (2014), Meena et al. (2015), Joe et al. (2016) these microorganisms helped in plant nutrition by employing symbiotic and non-symbiotic N fixation, enhanced the accessibility of nutrients like N, P, Fe, S etc. in the rhizosphere and enhancing root surface area by production of phytormones (IAA, cytockinin, gibberellin etc.). Whereas, according to Inagaki et al. (2015), seed inoculation with two diazotrophic bacteria strains may improve leaf area, stem diameter and relative chlorophyll content, but had no effect on dry matter yield of maize plants.

The effectiveness of PGPR and PGPF communities also depends upon several other factors, such as genotype of the host plant, plant age, soil characteristics (pH, texture, moisture etc.) and agronomic management (Cortivo et al. 2017). The favorable soil environments and other environmental conditions such as temperatures, pH, salinity and metallic concentration of soil, water holding capacity and mineral contents are all critical factors that affect rhizospheric colonization (Fentahun et al. 2013; Arrese-Igor et al. 2011). In addition to this, synthetic chemical inputs, such as nitrogen and phosphorus fertilizers, herbicides and pesticides may diminish microbiological populations as well as contaminate food chain and pollute environmental resources (Berg 2009; Cortivo et al. 2017; Rahman et al. 2017).

It was found that physiochemical and biological properties of the agricultural field played important roles in terms of translocation of nutrients, water and supply of oxygen to support growth and development of crops (Turner et al. 2013). The qualities of PGPR and PGPF to colonies in the roots and internal tissues of plants are essential for translocation of nutrients (Cortivo et al. 2017; Wang et al. 2017).

The uptake and accumulation of plant nutrients like nitrate, nitrite, ammonium and phosphate were revealed in the study as nutrient uptake is indispensable for growth

and development of wheat and other crops (Verma et al. 2014; Cortivo et al. 2017). These plant nutrients are used to be present in the soil in quite larger amounts but used to be unavailable for plants as they are required to be in insoluble forms for the plant. PGPR and PGPF help in the solubilization of plant nutrients and make these available to plants (Bhattacharyya and Jha, 2012; Meena et al. 2015; Wang et al. 2017). The results of present study were positive in enhancing N and P uptake in forms of nitrate, nitrite, ammonium and phosphates with the effects of microbial activities. The results demonstrated that the concentration of nitrate, nitrite, ammonium and phosphate in wheat plants of control was 25.16, 0.43, 5.60 and 10.08 mg g^{-1}, respectively at harvesting time in earthen pots. But, in experimental field the concentrations of nitrate, nitrite, ammonium and phosphate were higher in comparison to pots which may be due availability of larger surface for interaction of isolated microbes. It was found to be 31.76, 0.51, 5.37 and 12.31 mg g^{-1} at harvesting time, respectively. The results indicated that with the effect of selected isolates of rhizobacteria and fungi loss of N in the form of ammonia and nitrate were reduced. These were accumulated in plants which may be confirmed by the results which showed accelerated production of these within plants (Mandal et al. 2016; Wang et al. 2017).Whereas, application of individual isolated microbes SDA_3, $S5_1$ and T_2 increased uptake and accumulation nitrate, nitrite, ammonium and phosphate concentration in wheat plants of pots by 62.25, 110.0 and 104.13, 21.61, 23.12 and 29.03, 83.81, 103.39 and 282.36 and 39.43, 47.13 and 61.29 %, respectively in comparison to control at harvesting time. On the other hand, application of individual isolates SDA_3, $S5_1$ and T_2 in experimental field enhanced uptake and accumulation of nitrate, nitrite, ammonium and phosphate in wheat plants by 57.43, 76.24 and 117.82, 11.29, 20.0, and 27.72, 58.19, 148.22 and 311.53 and 54.52, 59.59 and 72.09 %, respectively at harvesting time.

As expected, consortium of two isolates i.e. *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and *T. virens* more significantly ($p<0.05$) increased nitrate uptake and accumulation nitrate, nitrite, ammonium and phosphate by 186.19, 227.75 and 333.75, 37.10 and 44.09, 365.90, 532.62 and 652.55 and 100.32, 134.35 and 222.08 %, respectively after 40, 80 and 120 DAS in comparison to control in earthen pots. Likewise, enhanced uptake and accumulation of nitrate, nitrite, ammonium and phosphate in wheat plants by co-inoculation of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; and *M. phyllosphaerae* and

T. virens was also recorded in experimental fields. These were about 149.50, 205.94 and 281.19, 30.85, 41.34 and 51.43, 367.2, 605.40 and 724.74 and 96.12, 129.97 and 180.88 % lower in comparison to earthen pot. The reason behind such consequences may be distribution of nutrient over larger areas in experimental field in contrast with smaller areas of earthen pots. The application of biofertilizers enhanced the content of nitrate, nitrites, ammonium and phosphate in wheat plants which demonstrated the assimilation nutrients that helped in development of plants (Wang et al. 2017). Co-inoculation of PGPR and PGPF also aided in enhancing the growth of the wheat plants Chandanie et al. (2009) have also reported enhancement on the plant shoot, root dry weight and plant growth in cucumber (*Cucumis sativus* L.) plant with co-inoculation of AMF *Glomus mosseae* with PGPF *Penicillium simplicissimum* GP17-2 and *Trichoderma harzianum*. Likewise, Etesami and Alikhani (2016) have found that co-inoculation of endophytic (*Pseudomonas putida* REN5) and rhizospheric bacteria (*Pseudomonas fluorescens*) reduced application rates of N-fertilizer for rice plants. Further, Tanwar et al. (2013) have also suggested combined application of PGPF and arbuscular mycorrhizal fungi (*Trichoderma viride, Glomus mosseae* and *Acaulospora laevis*) with PGPR (*Pseudomonas fluorescens*) along with 50% small doses of P fertilizer during seedling transplantation increased overall growth and yield of pepper. According to Martínez-Medina et al. (2014), Li et al. (2015), Sharma et al. (2017) and Bayoumi et al. (2018) significant contribution of *Trichoderma* spp. in growth promotion may be attributed to destruction of phyto-pathogens related to root and foliar diseases, enhanced uptake and solubilization of essential nutrients, improved root elongation and root hair development, alteration in composition of microflora around roots and upsurge in plant growth hormones (IAA and gibberellic acid) production. These have the potential to produce antimicrobial and antibacterial compounds like benzoic acid, methylhydantoin, mevalonolactone etc. (Arqués et al. 2015; Sharma et al. 2017), lytic enzymes like chitinases (Sharma and Shanmugam 2012), β-glucanases (Djonović et al. 2007), proteases (Sharma et al. 2017) etc. These compounds target pathogens, pests and foreign bodies and manage disease control. Further, Cortivo et al. (2017) have reported that application of consortia of PGPR and N-fixing bacteria anticipated different chances to increase root growth in wheat and as well as its resilience towards environmental stresses, and aided in reduction of N losses from agricultural ecosystems which partially saved fertilizer within crop rotations and ultimately enhanced productivity.

When, PGPRs and PGPFs are applied to larger fields, it build up a more complex situations for proper working of microbes as well as their effect on the growth and developments of crops. At such situations, various other factors like interactions of PGPR and resident soil microbioma, fertility, soil and climatic status are involved which may either positively or adversely affect the productivity (Cortivo et al. 2017). The results were in good agreements with Wang et al. (2017) who have reported biofertilizer containing *Trichoderma viride* enhanced nitrification in the soil. Further, these results were also in accordance with previous study of Meena et al. (2015) who have reported successful phosphate solubilization by PGPR strains namely *Pseudomonas, Bacillus, Enterobacter* and endosymbiotic *Rhizobium*. Likewise, El-Hadad et al. (2011) recognized that application of PSB *Bacillus megaterium* improved shoot length, dry weight of shoot and root and N, P, K content of tomato plant and decreased rhizospheric population of *Meloidogyne incognita*. Some of the researchers have also reported contradictory results as Joe et al. (2016). They have found that co-inoculation of *Bacillus* sp. PVMX4 with *Acinetobacter* sp. ACMS25 produced minute reduction in germination ratio, vigour index, P content, radical scavenging and antioxidant activities which might be due to failure of both strains act synergically, when inoculated together.

Further, the highest increase in nitrate, nitrite, ammonium and phosphate accumulation containing consortium of three isolates *A. faecalis*, *M. phyllosphaerae* and *T. virens* was recorded as 365.0, 76.88, 727.15 and 236.85 % in comparison to control at harvesting time, respectively in wheat plants of earthen pots. Similar results were also observed in case of experimental fields which were about 297.03, 62.95, 807.67 and 200.52 %, respectively at harvesting time, in comparison to control. The trend of increase in accumulation of all nutrients were Control< Commer. < Urea and DAP < *A. faecalis* < *M. phyllosphaerae* < *T. virens* < consortium of *A. faecalis* and *M. phyllosphaerae* < consortium of *A. faecalis* and *T. virens* < consortium of *M. phyllosphaerae* and *T. virens* < consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*.

Thus, the highest accumulation of nitrate, nitrite, ammonium and phosphate was obtained by collective effects of consortium of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* at the time of harvesting. The positive effects of co-

inoculation of isolates were based upon the compatibility of microbes with each other and soil and climatic conditions (Videira e Castro et al. 2016). According to Fentahun et al. (2013) the extreme or adverse climatic conditions like higher temperatures, pH, salinity and heavy metal contamination are crucial factors in root colonization and inhibit its development. Further, enhanced nutrient uptake and accumulation may be due to effects of microbial activities that sobulized minerals and helped in its uptake by wheat plants (Kumar et al. 2014). Previous studies shown positive effects of PGPR and PGPF on growth and yield of different crops like wheat (Chandanie et al. 2009; Hasan et al. 2012; Zhang et al. 2012; Foroutan 2013; Tahir et al. 2013; Verma et al. 2015; Mukhtar et al. 2017; Kumar and Singh 2018; Kumar et al. 2018), maize (Almaghrabi et al. 2014), rice (Lavakush et al. 2014;Elekhtyar 2015; Ng et al. 2015; Kantachote et al. 2016), pea (Meena et al. 2015), onion (Bayoumi et al. 2018; Bunbury-Blanchette and Walker 2018), mung beans (Jain et al. 2012; Ahmad et al. 2013; Hosseini et al. 2014; Kumari et al. 2018), tomato (Jabnoun-Khiareddine et al. 2009; Tucci et al. 2011; Molla et al. 2012; Li et al. 2015), soybean (Kang et al. 2014; Mahanta et al. 2014; Masciarelli et al. 2014; Munda et al. 2018), cabbage (Turan et al. 2014) oil yielding plants (Grobelak et al. 2015), etc. and the findings of present study were in accordance with these study.

Control< Commer. < Urea and DAP < *A. faecalis* < *M. phyllosphaerae* < *T. virens* < consortium of *A. faecalis* and *M. phyllosphaerae* < consortium of *A. faecalis* and *T. virens* < consortium of *M. phyllosphaerae* and *T. virens* < consortium of *A. faecalis*, *M. phyllosphaerae* and *T. virens*

The maximum enhancements in all growth parameters were recorded by co-inoculation of three isolates i.e. *A. faecalis*, *M. phyllosphaerae* and *T. virens* in comparison to control for pot and experimental fields both. This may be due to enhanced biological N fixation by microbes present in the consortium as well as enhanced uptake and accumulation of essential nutrients (Chandanie et al. 2009). Further, some variations were observed in result of field conditions like accumulation of nitrite and phosphate levels and fresh weight of wheat plants under effect of Urea and DAP in field conditions. These may be attributed to prevailing abiotic and biotic conditions that can be controlled in green house conditions for pot experiments but

cannot be controlled in experimental fields (Pérez-Montaño et al. 2013; Sharma et al. 2017).

Regardless of all upsurges in growth and productivity of wheat or other crops, the key factor of successful interactions of PGPRs and PGPFs as biofertilizers is final yield or productivity which is directly associated with survival, nutrient uptake and reproduction of host plants (Baum et al. 2015; Cortivo et al. 2017; Renuka et al. 2017). The individual isolates enhanced productivity in pots by application of *Trichoderma virens* as 1.32 g plant^{-1} followed by *Alcaligenes faecalis* (1.31 g plant^{-1}) and *Microbacterium phyllosphaera* (1.26 g plant^{-1}) in comparison to control (0.79 g plant^{-1}). Whereas, collectively the consortium of three isolates i.e. *Trichoderma virens*, *Alcaligenes faecalis* and *Microbacterium phyllosphaera* secured highest yield as 1.84 g plant^{-1}. These results may be correlated with the uptake and accumulation of nutrients in the plants. Sayyed et al. (2010) have reported increase in root and shoot length and yield of groundnut (*Arachis hypogaea*) with the application of *Alcaligenes faecalis* under pot culture conditions. Likewise, Manjunath et al. (2011) have reported enhancement in the growth parameters and yield of wheat by treatment of *Providencia* sp., *Alcaligenes* sp. and *Anabaena torulos* individually and in combinations under pot conditions. The grain yield was reported to be 5.64 g pot^{-1} with the treatment of *Alcaligenes* sp.+ 2/3 N+ full dose of P and K. Mäder et al. (2011) have also reported enhancement in productivity of wheat by application of consortium of AMF with *Pseudomonas* strains (*Pseudomonas jessenii*, R62 and *Pseudomonas synxantha*, R81) in addition with phosphorus fertilizer. Whereas, in the present study no chemicals fertilizer was used with PGPMs and the grain yield was about 1.26 g plant^{-1} for individual treatment of *Alcaligenes faecalis* and 1.84 g plant^{-1} for consortium of *Alcaligenes faecalis, Microbacterium phyllosphaerae* and *Trichoderma virens* which was significantly higher. According to Cortivo et al. (2017) growth and yield of wheat were highly reliant on input levels as well as nitrogen use efficiencies of plants. PGPRs and PGPFs helped in breakdown of nutrients present in the soil and made it available to plants to utilize these in their different metabolic, catabolic and biochemical mechanisms like cell division, transpiration and photosynthesis, energy transfer, signal transduction etc. (Lavakush et al. 2014; Waadt et al. 2015; Mukhtar et al. 2017). Previous studies of Cortivo et al. (2017); Defez et al. (2017); Wang et al. (2017) etc.

have reported increase in N and P accumulation by biological N-fixation done by PGPRs and PGPFs. The results of present study were in accordance with these studies.

At last, the main and important findings of the present study was enhancement in growth and productivity of wheat plants with the application of isolated plant growth promoting rhizobacteria *Alcaligenes faecalis* and *Microbacterium phyllosphaera* and fungi *Trichoderma virens*. The maximum yield was recorded by application of consortium of these three strains which was about 3.14 tons ha^{-1} and net benefit was about 32480 rs ha^{-1}. Such enhancements in yield may be due to accumulation of nutrients (nitrate, nitrite, ammonium and phosphate) in plant parts (Mahanta et al. 2014). The used biofertilizers also reduced the loss of N, P and other nutrients to the environment which ultimately results in soil and water pollution (Rawat et al. 2012; Kumar et al. 2014). Thus, it helps to overcome the problem of environmental stress caused by environmental contamination (Kumar and Verma 2018). Whereas, Cortivo et al. (2017) examined commercial biofertilizer comprising of consortia of PGPR and N-fixing bacteria (*Azospirillum* spp., *Azoarcus* spp. and *Azorhizobium* spp.) which affected root and shoot growth, accumulation of nitrogen and grain yield of wheat. However, the present study has reported enhancements in growth parameters and grain yields of wheat with individual as well as co-inoculation of selected strains of microorganisms. Whereas, Mäder et al. (2011) have reported enhancements in wheat yield by consortium of AMF and *Pseudomonas* strains with chemical fertilizer (N and P). Whereas, in the present study growth and yield of wheat was enhanced with the effects of isolated PGPMs individually as well as in consortium.

Further, biofertilizer also do not require additional application of different chemicals like insecticides, herbicides and weedicides to protect the crops as defense mechanisms against these invaders already exist in the microbes present in the biofertilizers (Rahman et al. 2017). Possible agronomic benefits may be achieved by poor farmers in the form of savings on purchasing of chemical fertilizers and insecticides, herbicides and weedicides (Zaidi et al. 2015).

The important finding of present study was that wheat crop exhibited greater nutrients (N and P) accumulation with help of PGP activities of rhizobacteria and fungus which reduced nutrient losses to the environment from agricultural filed in the

form of run off. This is a first-time report on the potential of selected combinations of PGPR *Microbacterium phyllosphaerae* ($S5_1$) and *Alcaligenes faecalis* (SDA_3) and PGPF T*richoderma virens* (T_2) as plant growth-promoting microorganisms used as biofertilizer for improving growth and yield of wheat. Its application will not only help in sustainable agriculture, but will also reduce thw costs involved in the rejuvenation of environmental systems and will surely protect the environment against different kind of pollutions (Meena et al. 2015; Cortivo et al. 2017; Wang et al. 2017). Other possible agronomic benefits consist of savings on fertilizers and minimizing the cost involved in chemical fertilizers.

As the price of fertilizers continues to skyrocket, controlling costs is a major issue for conserving rights and future of farmers. Since biofertilizers helps in making traditional fertilizers more effective, farmers can reduce the amount of NPK applied in the cultivation (Zaidi et al. 2015). One of the major advantages of biofertilizers is reduction in cost and usage of other chemicals to enhance the productivity (Goljanian-Tabrizi et al. 2016).The main reason for reduced cost of biofertilizers is the use of cheaper carriers as the manufacturing of biofertilizer essentially requires it and these carriers are usually very cheap as compared to chemical fertilizer.

These new formulated biofertilizers may be commercialized as many of these have been already available in markets since the discovery of rhizobia in 1886 (Deaker et al. 2004; Vinale et al. 2008; Mukherjee et al. 2013; Kumar and Verma 2018). These products should be promoted by governments over usage of chemical fertilizers for sustainable and ecofriendly agricultural practices. The success of commercialization of biofertilizers depends upon positive collaboration of interdisciplinary technical organizations and private industries and its extension to the stakeholders (Tabassum et al. 2017).The security and constancy, extended shelf life, low stock costs and availability of career materials with economical and feasible market, reliable and consistent actions are the prerequisites for the commercial success of biofertilizers (Mhatre et al. 2019). Further, despite of auspicious results in lab and greenhouse conditions, the field experiments showed lack of consistency and reliability which may be due to alterations in edaphic properties from site to site and time to time for different crops (Pérez-Montaño et al. 2013). Further, the lab conditions used to be controlled under greenhouse whereas natural fields cannot be governed and are

influence by different abiotic and biotic stresses prevailing in the atmosphere (Vimal et al. 2017).

Thus, with the better understanding of beneficial application of PGPR and PGPF as biofertilizers, biopesticides and biocontrol agent, it should be encouraged over chemical fertilizers (Zaidi et al. 2015). Further, advance research and development in the field of biofertilizers will become an efficient technique to cope up the problem of increased production of food crops like wheat, rice, maize, barley etc. to fulfill the needs of ever growing population. These are not only ecofriendly but also cost efficient to be consumed by farmers without worrying about its rate. Collective efforts of molecular and biotechnological tools along with traditional methods will help in better understanding of rhizospheric science to increase the competence of biofertilizer managing policy. In addition to this, genetic engineering tools may also be utilized to improve biocontrol competence of PGPR and PGPFs by over expressing different antiphytopathogenic characters synergistically for well-organized pest management. Moreover, potential of co-inoculation of effective bacterial and fungal strains can be explored for reduction in detrimental impacts of different biotic and abiotic stresses on plants (Finkel et al. 2017). In future, research should be done to explore formulation of nano-encapsulated, natural metabiotics and usage of their cell fragments as biofertilizers which proposes an attractive strategy with minimum environmental problems (Sharma et al. 2017). The commercialization of PGPR based biofertilizers should be considered more precisely by different governmental agencies. Future research should be focused on to optimize better growth conditions, safety, constancy and longer shelf lives of biofertilizers containing PGPR and PGPF products. These requirements are crucial for commercial success of biofertilizers.

As per our review of literatures done so far, we found that most of the previous studies were concentrated on the application of *Trichoderma virens* as biocontrol agents. Lesser people have used *T. virens* as PGPF for promoting growth and yield of wheat. But, in the present study we have successfully applied *T. virens* as PGPF for enhancing growth and yield of major food crop wheat. Further, two selected bacterial isolates *Microbacterium phyllosphaerae* and *Alcaligenes faecalis* have also not been applied as PGPR for improving growth and yield of wheat. In addition to this, we have also successfully applied consortium of these three strains as biofertilizer which in turn

intensified growth of wheat plants in comparison to market based commercial biofertilizers. The formulation utilized in the present study increased yield of wheat in comparison to the other strains like *Pseudomonas jessenii*, *Pseudomonas synxantha*, arbuscular mycorrhizal fungi and P fertilizer (Mäder et al. 2011); *Azospirillum* spp., *Azoarcus* spp. and *Azorhizobium* spp. (Cortivo et al. 2017), applied in previous studies.

Moreover, as the net benefits from newly form consortium were higher in comparison to chemical fertilizers, so application of these fertilizers should be encouraged over chemical fertilizers. Further, application of chemical fertilizers although increases productivity and gives superficial benefit to farmers, but at broader level as health of environmental perspectives, it causes pollution of soil and water. This will cost higher to rejuvenate polluted soil and water by governmental agencies. So, application of biofertilizers should be preferred over chemical fertilizers to overcome its drawn backs.

Chapter 6

Summary & Conclusion

6. Summary and Conclusions

According to FAO (2017), it is difficult to feed the monitoring global populations of up to 11 billion or more by 2100 which will require more than 70 % additional food in comparison to the present production level. Thus, it will be the greatest challenge to fulfill the needs of such a huge population with deteriorating soil, declining water supply for irrigation and other environmental concerns e.g. sensitivity of agriculture to climate change especially global warming. The natural agriculture system was modified by the scientists with the adoption of conventional chemical fertilizers in the starting of green revolution in 1960 and later on the application of pesticides, herbicides and insecticides started habitually and enormously to control pest and disease in agricultural as well as horticultural crops. These practices have resulted in over exploitation of soil ecosystems which in term created unsustainability agriculture. Thus, to minimize these negative impacts of green revolution package and the effective alternatives of chemical fertilizers and pesticides etc. are to be discovered and established in the market the microbes e.g. bacteria and fungi which make available the various nutrients e.g. nitrogen, phosphorus, potassium, zinc and iron etc. provide phytohormones (IAA, gibberellin and cytokinin etc.) secrete antibody and enzymes and help the plant and rhizosphere in pest and disease control have been attempted in various crop. However, the stability of microbes in the agricultural fields after application and its succession in new agro-climatic conditions are the major challenge.

The present study focused on isolation from the native wheat fields and its characterizations for affectivity as biofertilizers for wheat cultivation in the similar agro- climatic conditions. Twenty three bacteria and two fungi were isolated from thirty three soil samples collected from different agricultural fields around Lucknow city. The isolated bacterial and fungal strains were studied for their morphological, biochemical and plant growth promoting activities

Most of the bacterial isolates showed positive test for indole (except for S_5, SS_4, M_1 and M_3) and nitrate (except S_1, S_2, $SS3_1$ and M_1) test. Amylase test was recorded positively for most of the bacterial isolates except for S_1, S_6, SS_1, $SS4_2$, $SS3_1$, $S5_1$, $S5_2$, $S8_Y$, SDA_1, SDA_2, SDA_3 and M_2. Whereas, among 23 bacterial

isolates only $S5_1$, M_1 and M_3 showed positive citrate utilization test and S_1, S_5, SS_1, $S5_1$, $S8_W$, M_2 and M_4 showed positive test for H_2S production. All bacterial isolates except S_5, SS_4, $SS3_1$, M_2 and M_3 showed positive lipase test and protease test was positive only for twelve bacterial isolates (S_2, S_5, S_7, S_9, SS_1, $SS3_2$, $S5_1$, $S8_Y$, SDA_1, SDA_2, SDA_3 and M_4). However, all isolated bacterial strains were negative for urease and gelatin tests. Different plant growth promoting activities like phosphate solubilization, IAA production, nitrogen fixation, siderophore production and ammonia production were differentially shown by all the isolates. The three isolates two rhizobacterial and one fungal isolates which were possessing multiple and high plant growth promoting activities like Nitrogen fixation, phosphate solublization, siderophore , IAA and ammonia production and were compatible in the consortium. These three isolates were characterised by 16S RNA and 18SRNA molecular identification and phylogenetic tree (Fig 4.6, 4.7 and 4.8).

The two bacteria and one *Trichoderma* spp. selected for further study were evaluated for their in-vivo effects with seed coating. The seed were coated with $1x10^8$ ml^{-1} c.f.u. for bacteria, $2x10^8$ ml^{-1} spore and plants were grown in earthen pots in net house for the entire session. *Alcaligenes faecalis* rhizospheric bacteria isolated from rhizosphere of the native wheat fields. So significantly increase in root and shoot length, number of root hairs and leaves, fresh and dry weights and grain weight per plant. *Microbacterium phyllosphaerae* showed significant increase in root and shoot length, number of root hairs and leaves, fresh and dry weights and grain weight per plant. The fungus *Trichoderma virens* also showed more pronounced increase in plant growth and yield parameters over control. The maximum increase in plant growth was measured in number of leaves and biomass production which significantly converted grain weight per plant by application of these bacterial and fungal biofertilizer isolated from the native soil.

Different combinations of either of *A. faecalis* and *M. phyllosphaerae* with *T. virens* strains and both with the fungus were attempted to understand the effect of consortia on the growth and productivity of wheat in earthen pots. It is noticed that consortia of *A. faecalis* and *M. phyllosphaerae*; *A. faecalis* and *T. virens*; *M. phyllosphaerae* and *T. virens* and *A. faecalis*, *M. phyllosphaerae* and *T. virens* were more effective than single isolates. The best enhancement of growth and productivity

was obtained when a consortium of the two bacterial and one fungal isolate was used collectively.

The microbial biofertilizer in individual or in consortia increased the level of nitrate, nitrite, ammonium, phosphate, organic carbon, organic matter and activities of alkaline phosphatase as well as dehydrogenase activity in soil which are indicative of its enhanced fertility and microbial activity. The application of biofertilizer in single microbe or in consortia prepared from wheat field enhanced the levels of nitrate, nitrite, ammonium and phosphate in wheat plants on 40, 80 and 120 days after sowing (DAS) which indicate that the enhanced biomass production and yield is a consequence of uptake of nutrients made available by newly prepared biofertilizers in the plants and their metabolisms. The biofertilizers prepared from the native isolates as single strain biofertilizer or consortium of two or three strains were also applied in experimental plots of 2x2 m^2 in the experimental field laboratory Environmental Science at Babasaheb Bhimrao Ambedkar University, Lucknow. The same dose of biofertilizers as applied in earthen pots was also applied in the experimental plots as seed coatings.

The activity of native microbes as biofertilizer was maintained in the experimental plots too as it was observed in in-vitro plant growth promoting activities in earthen pots. Root and shoot length, number of root hairs and leaves, fresh and dry weights of wheat plants increases significantly on the measurement at 40, 80 and 120 DAS after sowing, which was more pronounced on application of consortium of three microbes, two rhizobacteria and one fungus and *A. faecalis*, *M. phyllosphaerae* and *T. virens* yield per hectare. The yield per hectare was also improved on application of these native biofertilizers which was better on the application of consortia in comparison to the single species. The effects of these native microbial biofertilizers were better and more consistent in comparison to the commercial biofertilizer (*Azoto* plus) obtained from Biotech Park, Lucknow. However the chemical fertilizers Urea and DAP on recommended dose yielded slightly more biomass and grain yield in comparison to control, commercial biofertilizer and newly prepared biofertilizers used in this study which was prepared from the native rhizospheric microbial isolates obtained from the wheat fields nearby Lucknow city. Soil fertility was also improved by the amendment of these fertilizers. However, organic carbon and microbial

activities all found more on application of native biofertilizer in comparison to the application of chemical fertilizer; Urea and DAP.

Therefore, the following specific conclusion were drawn from this study-

1. Plant growth promoting rhizobacteria and soil fungi isolated from the rhizosphere of native wheat field are found more effective than the commercially available biofertilizers.
2. The consortia of compatible microbes are more effective to promote growth and productivity of wheat than single isolates of the same microbial strains.
3. In-vitro plant growth promoting activities were effective in in-vivo conditions in earthen pots and experimental field based experiments for cultivation of wheat (*T. aestivum*).
4. These microbes are enhancing availability of nitrate, nitrite, ammonium and phosphate in soil and plant parts which is subsequently increasing the growth, productivity and yield of wheat in pot and field conditions.
5. Our result indicates that a big bank of microbial species is available in agricultural fields which can be isolated and characterized for various uses. New generation biofertilizers can be prepared by making consortia of microbes with multiple activities and use of chemical can be avoided to maintain the agricultural sustainability.

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Publications

Publications

Research Papers

Kumar M and Singh RP (2017) Enhancement in growth promotion and production of wheat (*Triticum aestivum* L.) by application of a native strain of *Trichoderma virens* (T_2) in pot condition. International Journal of Science, Technology and Society, 3(2):52-67

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Kumar M and Singh RP (2019) Plant growth promoting and organic waste degrading activities of a native rhizobacterial strain of (*Alcaligenes faecalis*) for Wheat (*Triticum aestivum* L.) cultivation**.** Indian Journal of Environmental Protection, 39(4):333-338

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Kumar M and Singh RP (2017) Characterization of *Trichoderma virens* (T_2) as plant growth promoting fungus and its application on wheat crop (*Triticum aestivum* L.) for enhancing production**.** International Symposium on Microbes for Sustainable Development: Scope & Applications. 58 Annual Conference of Association of Microbiologists of India (AMI) from 16-19 November

Kumar M and Singh RP (2016) Isolation and characterization of drought tolerant plant growth promoting rhizobacteria (PGPR) from Wheat (*Triticum aestivum* L.) Rhizosphere**.** International Symposium on "Microbes and Biosphere: What's New what's Next. 57 Annual Conference of Association of Microbiologists of India (AMI) from 24-27 November

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Plant Growth Promoting And Organic Waste Degrading Activities Of A Native Rhizobacterial Strain Of *Alcaligenes faecalis* For Wheat Cultivation

Mahesh Kumar and Rana Pratap Singh

Babasaheb Bhimrao Ambedkar University, Department of Environmental Science, School for Environmental Sciences, Lucknow - 226 025

A native isolate of rhizospheric *Alcaligenes faecalis* was isolated from agricultural soil of peri urban area of Lucknow and characterized by morphological and molecular characteristic using 16s RNA. It showed in-vitro, plant growth promoting activities by producing indole 3-acetic acid (IAA), hydrogen cyanide, siderophore, ammonia and showed phosphate solubilization activities potential. An earthen pot experiment was conducted under net house conditions with the isolated strain ($1x10^8$ CFU/mL) which increased root length (28, 33 and 67%), shoot length (30, 45 and 55%), fresh weight (54, 67 and 90%), dry weight (55, 77 and 55%), number of root hairs (92, 69 and 38%), number of leaves (46, 63 and 58%) at an interval of 40, 80 and 120 days after sowing (DAS) in wheat (*Triticum aestivum* PBW 343). The weight grain per plant was also increased by 68% over control.

KEYWORDS

Biofertilizer, Plant growth promoting bacteria, Sustainable agriculture, Wheat

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crop which provides 20% of daily dietary and protein calories for about 4. 5 billion people [1]. To obtained a high yield of wheat, a significant amount of chemical fertilizers are applied throughout the world [2]. It causes environmental contamination in agro-ecosystems, nitrate pollution in water and emission of NOx which building up the greenhouse gases in the atmosphere [3]. Due to the lack of nitrogen and phosphorus in the soil, the farmer often uses urea and diammonium phosphate (DAP) to enhance the wheat yield [4]. The NOx emitted by the agricultural field add to global warming problem for a long time due to its persistence and higher warming potential 298 times higher that of carbon dioxide [5, 6]. Agriculture contributes about 42% of growing emissions of N_2O in the air. The beneficial microorganisms referred to as plant growth promoting rhizobacteria (PGPR) are important to provide soil nutrient to crops without compromising the soil fertility and environmental sustainability [7]. Many rhizospheric microbes, such as *Azotobacter chroococcum, Microbacterium phyllosph-aerae* have been reported to provide nutrients to the wheat crop after their application in the wheat fields [8, 9]. Kandil and Baris reported an increase in the wheat yield by applying *Azotobacter, Azospirillum, Bacillus sp*ecies and *Bacillus megaterium* M3 and consortium of *Bacillus subtilis* OSU142, *B. megaterium* M3, *Azos-pirillum brasilense* Sp245, respectively over control [10,11]. It has been found that native strains of known and unknown PGPRs can be a more effective nutrient provider to wheat crop fields and can easily adapt to the local agro-climatic conditions. We report the role of a native strain of *A. faecalis* isolated from peri-urban agricultural fields near Lucknow which showed strong plant growth promoting activities and enhanced growth and productivity of wheat in pot conditions.

2. MATERIAL AND METHOD

2. 1 Isolation and purification of bacteria from the collected soil samples

Healthy wheat plants were randomly uprooted from the soil from their rhizosphere region (about 4 inch deep) at Mohanlalganj, Lucknow. The root system was ploughed and the rhizosphere soil samples were mixed and kept in an icebox in sterilized polyethylene bags and maintained at 4°C in the laboratory till further study. The microbe was isolated by serial dilution method (range of 10^{-6}–10^{-8}) [12]. The diluted samples (0.1 mL) were spread on the nutrient agar (NA) and assayed for the growth of the bacterial colonies. The purification of the colonies was done on the same solid medium

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RESEARCH ARTICLE

Increase in Growth and Productivity of Wheat (*Triticum aestivum* L.) Applied with a Native Strain of *Trichoderma brevicompactum* in Earthen Pots

Mahesh Kumar[1] • Sridevi Tallapragada[2] • Rana Pratap Singh[3]*

Abstract This study was conducted to isolate and characterise a native plant growth promoting strain of *Trichoderma brevicompactum* from the rhizosphere of wheat plants to evaluate its potential as biofertiliser for wheat (*Triticum aestivum* L). The isolated fungus showed plant growth promoting activities (PGPA) as positive test for production of siderophore, indole-3-acetic acid (IAA) and solubilisation of phosphate. The strain was identified as *T. brevicompactum* on the basis of morphological and molecular characterisation by 18S rRNA partial gene sequencing. Fifteen seeds of wheat (*T. aestivum* L. cv PBW 343) were primed with *T. brevicompactum* (2×10^8 conidia/ml) for 30 min and sown in earthen pot at the rate of 15 seed per 8 kg soil. The growth parameters of wheat for example, root and shoot length, number of root hairs and fresh as well as dry weights were measured at 40, 80 and 120 days after sowing, Results showed that the seeds primed with *T. brevicompactum* inoculum before sowing produced significantly higher biomass over their non-primed controls. Productivity parameters like length of spikelet (35.82%) and grain weight per plant (82.05%) were also significantly higher in plants raised form the primed seeds. Treatment of *T. brevicompactum* strain enhanced soil nutrients in terms of organic carbon, organic matter, nitrate, nitrite, ammonium and phosphate in soil of the rhizospheric region at depth of 0–12 cm. The data indicated that this native strain of *T. brevicompactum* can be developed as plant growth promoting biofertiliser for cultivation of wheat in addition to its well described role as an effective biopesticide.

Keywords: Biofertiliser, Plant growth promoting fungus, Sustainable agriculture, *T. brevicompactum,* Plant growth promoting activity

1. Introduction

The current world population of 7.6 billion is expected to reach 8.6 billion in 2030, 9.8 billion in 2050 and 11.2 billion in 2100 according to a new report of United Nations. With roughly 83 million people being added to the world's population every year, food security is increasingly threatened in many developing countries in the tropics, where traditional agricultural systems become unsustainable due to demographic pressure and use of agrochemicals. Providing food and nutritional security for increasing population with growing demand of food in an eco-friendly way is a major concern of the day. Food anxiety will also increase due to heat, flood and drought stresses expected to get induced by the climate change and anthropogenic pollution in 21st century (Mäder *et al.*, 2011). Availability of plant nutrients is essential for crop growth and high yield. The nitrogen (N), phosphorus (P) and potassium (K) are major nutrients often get limiting for the growth of plants, if found deficient. Due to lack of N and P in the soil, farmers often over load urea and diammonium phosphate (DAP) to enhance the yield of cereals like wheat (Zheng *et al.*, 2016). Excessive use of chemical fertilisers, resulting in many serious environmental problems due to their loss in atmosphere and leaching in ground water and surface water resulting of nitrate and phosphate pollution (Kumar *et al.*, 2014).

[1]Research Scholar, [3]Professor/Dean Academic Affairs, Department of Environmental Science, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India
[2]Chief Environmental Expert, WAPCOS, C11-189, Chanakyapuri, Satya Marg, New Delhi, India
*Corresponding author email id: dr.ranapratap59@gmail.com

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SHORT COMMUNICATION

Plant Growth Promoting Activities of *Microbacterium phyllosphaerae* in Wheat (*Triticum aestivum* L.)

Mahesh Kumar[1] • Rana Pratap Singh[2]*

Abstract A native strain of *Microbacterium phyllosphaerae* was isolated from agriculture field of Lucknow Uttar Pradesh India and characterized for its plant growth promoting activities, that is, indole-3-acetic acid (IAA) production, phosphate solubilization activities, ammonia and siderophore production. The isolated rhizobacteria was identified and characterized using 16S rRNA sequencing and applied to soil in earthen pots sown with wheat (*Triticum aestivum* L.). A significant increase in the root length, shoot length, fresh weight, dry weight, length of spikelet and grain weight were observed in the plants grown with *M. phyllosphaerae* in earthen pots as compared with the plants grown without the seed priming. The results indicate that application of *M. phyllosphaerae* as a biofertilizer produced more biomass and enhanced grain weight per plant of wheat by 61% over control. Further studies are going on to evaluate the field performance of this native soil microbe as biofertilizer for the cultivation of wheat in north Indian agro-climatic conditions.

Keywords: Biofertilizer, Rhizospheric bacteria, Sustainable agriculture

1. Introduction

Food anxiety is expected to increase due to heat and drought stress induced by climate change and anthropogenic pollution in the 21st century (Mäder *et al.,* 2011; WHO, 2018). A large population depends upon cereals like wheat, barley and rice etc. for their nutritional requirements. Wheat consumes a significant amount of chemical fertilizers. The chemical fertilizers result in contamination of water by leaching and enhance built up of NOx as an emission, adding to greenhouse gases and a resultant global warming (Elkoca *et al.,* 2010; Kumar *et al.,* 2013, 2014a; Tongwane *et al.,* 2016).

As eco-friendly and alternative plant nutrient, plant growth promoting rhizobacteria (PGPR) can facilitate plant growth and productivity directly or indirectly, hence they are used as biofertilizers in plant agriculture (Kumar, 2016). In this study, we report isolation and characterization of a native strain of *Microbacterium phyllosphaerae* from the rhizospheric soil of the local agricultural field, which was characterized to possess PGPR activities. An attempt has been made to explore the role of this microbe for its growth promoting activities *in vivo* during cultivation of wheat in the earthen pots. No report is available on the role of this rhizobacteria as PGPR as per our database.

2. Materials and Methods

2.1 Isolation and Purification of Bacteria from the Collected Soil Samples

Soil samples were collected from rhizospheric region from about of 0–12 cm deep soil of wheat and other agriculture crops from, peri-urban and rural areas of district Lucknow, UP, India. The root system was ploughed out and the rhizospheric soil samples were mixed and kept in the ice box in sterilized poly bags to brought to the laboratory and placed in a refrigerator at 4°C till future studies. This bacterium was isolated by serial dilution of supernatant obtained from soil samples. It was diluted to the extent in which dilution factor ranged from 10^{-5} to 10^{-8}. The diluted samples (0.1 ml) were spread on the nutrient agar (NA) used for growth of the bacterial colonies. The purification of the colonies was done on the same solid medium with repeated plating (Aneja, 2003).

[1]Research Scholar,[2]Professor, Department of Environmental Science, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India
*Corresponding author email id: dr.ranapratap59@gmail.com

RESEARCH ARTICLE

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Enhancement in growth promotion and production of wheat (*Triticum aestivum* L.) by application of a native strain of *Trichoderma virens* (T2) in pot condition

Mahesh Kumar and Rana Pratap Singh*

Department of Environmental Science, School for Environmental Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow- 226025, Uttar Pradesh, India

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Corresponding author:
Rana Pratap Singh

Email:
*cceseditor@gmail.com

ABSTRACT

An endemic strain of *Trichoderma virens* was isolated from wheat field of village Nagram Lucknow, Uttar Pradesh, India. This strain showed positive test for production of indole 3-acetic acid, phosphate solubilizing activities and siderophore production. The strain was identified on the basis of morphological and molecular characterizations by 18S rRNA partial gene sequencing as *Trichoderma virens.* Fifteen seeds were primed with conidia of *Trichoderma* at the rate of $2x10^8$ conidia/ml of *Trichoderma* and grown. The root length, shoot length, number of tillers, fresh weight and dry weight were increased at 120 days, over control in which no additional nutrient source was applied. The plants applied with *Trichoderma virens* produced more significant biomass. The fungus enhanced length of spikelet and grain weight per plants by 35% and 69%, respectively. The data indicated that the endemic strain of this fungus can be used as plant growth promoting microbe for cultivation of wheat in this region in addition to its well described role as effective for enhancing wheat production.

INTRODUCTION

Wheat is the most important staple food crop used to support humanity as provides more calories in the diet. Wheat is second most important cereal crop in India, the second producer of wheat in the world. Due to the deficiency of nitrogen and phosphorus in soil, farmers often over load urea and di-ammonium phosphate (DAP) or nitrogen, phosphorus and potassium (NPK) in agricultural fields during wheat cultivation in northern part of India . The use of chemical fertilizers and synthetic herbicides and pesticides has significantly influenced the environment by increasing pollution and destruction (Molla et al., 2012). To improve crop production, fertilizers and synthetic pesticides have been used without concern for environmental problems and soil health (Elkoca et al., 2010). Besides, the soluble chemical fertilizers enhance plant production and yield very significantly but require heavy irrigation as a support measure. It is already reported that the excessive use of chemical fertilizers are unsustainable for ecosystem and also, uneconomical (Singh et al., 2006; 2008a; 2010). Few of these problems can be solved by the use of microorganism, as these are natural, useful and ecological product (Mirzakhani et al., 2009).

Although, reactive nitrogen (nitrate, nitrite and ammonia) specie have been reported in leaves and other parts of plant including wheat (Dahiya el al. 2004; Sanchez-Bragado, et al. 2017). But, only 30-50 % of applied nutrients are obtained by the plant and rest is lost either through runoff or leeching in the surface or ground water (Zhou et al., 2014). Moreover, a significant amount is emitted as NOx gases and in building up the level of these gases which contribute significantly in greenhouse gas (GHG) effects. The nutrients taken by the plants are also not assimilated completely. To cope up these problems the rhizospheric microbes are used as significant assimilator of nutrients to the plants (Hoyos-Carvajal et al., 2009). Many plant growth promoting microbes have been reported to be used as biofertilizer in wheat and other crops (Sharma et al., 2012). *Trichoderma* sp. has been reported to be a very potential soil microbe which can easily be isolated and applied in the agriculture field for bio control of plant disease (Buensanteai et al., 2010). *Trichoderma* sp. has been reported as to be potential promoter of plant growth and development (Gravel et al., 2007; Shaban and El-Bramawy, 2011).

Trichoderma sp. has been reported to be a very potential soil microbe which can easily be isolated, proliferated and applied in the agricultural fields for bio-control of various diseases (Jabnoun-Khiaredinne et al. 2009; Foroutan, 2013; Zhang et al., 2014), degradation of organic matter (López-Mondéjar et al., 2011; Molla et al., 2012) and