Developing a novel crop system with microbial inoculants for high income and multiple ecosystem services

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Submitted by

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SCHOOL FOR ENVIRONMENTAL SCIENCES

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University)

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2022

Dedicated to

My Beloved Parents

DECLARATION

This is to certify that the material embodied in the present work entitled "**Developing a novel crop system with microbial bioinoculants for high income and multiple ecosystem services**" is based on candidate's original research work. It has not been submitted in part or full for any other diploma or degree of any university. The indebtedness of the candidate to others has been duly acknowledge at relevant places. I hereby also undertake that the thesis is essentially free from all kinds of plagiarism.

Rotimishra.

Name and Signature

(Candidate)

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This is to certify that the thesis entitled "**Developing a novel crop system with microbial bioinoculants for high income and multiple ecosystem services**" submitted by Ms. Roli Misra 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.

This thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfy all the requirements as stipulated in Doctor of Philosophy (Ph.D.) regulation 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.

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PREFACE

Agriculture is the primary source of income for many individuals and the primary source of revenue for most developing countries. Although we are currently self-sufficient in our food needs but this may not be the case in the near future as our population grows because, our land resources remain fixed, with little or no room for further expansion. In addition, the unsustainable agricultural practices such as the excessive and injudicious use of chemical pesticides and fertilizers coupled with continuous climate change had resulted in depletion of soil quality that promoted fertile lands conversion into degraded and marginal lands. Nearly 80% of Indian farmers have marginal or degraded land holdings of 1-2 hectares or less and approximately 6.73 million ha land categorized as marginal due to high alkalinity/ salinity which has lost its fertility for conventional agriculture. Such land necessitates long-term economic support for farmers as well as better crop management techniques. Many chemical and biological techniques including cultivation of salt tolerant cultivars have been develop for improved soil quality as well as enhanced crop productivity but the future functionality of today's cropping systems in terms of resilience, adaptability to climate change, multifunctionality of agricultural landscapes, provisioning of ecosystem services, and biodiversity has been questioned on simplified cropping. Hence, highly diverse crop system can help to establish more sustainable agricultural systems, develop value chains for minor crops, and contribute to socioeconomic advantages.

This study attempted the ecological and economic cultivation of Indian licorice, stevia, and chilli as a high-paying diverse cropping system with enhanced soil fertility of slightly saline soil. The role of plant growth promoting microbes were also assessed in supporting the growth and productivity of designed crop community as well as improved soil fertility for sustainable agricultural production without use of agrochemicals.

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Roli Misra

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LIST OF ABBREVIATIONS

Alkn Phos	Alkaline Phosphatase
AMF	Arbuscular mycorrhizal Fungi
ANOVA	Analysis of Variance
BD	Bulk Density
C	Chilli
CAS	Chrome Azurol Sulphonate
CEV	Crop Equivalent value
CFU	Colony Forming Unit
CIMAP	Central Institute of Medicinal and Aromatic Plants
C-Seq	Carbon Sequestration
CSI	Calcium Solubilization Index
DF	Degree of Freedom
DHA	Dehydrogenase
DMRT	Duncan's multivariate Test
DNA	Deoxyribose Nucleic Acid
$dS\ m^{-1}$	Decisimen per meter
DW	Distilled Water
EC	Electrical conductivity
ESP	Exchangeable sodium potential
FAO	Food and Agriculture Organization
FAOSTATS	Food and Agriculture Organization Corporate Statistical Database
G	Gram
g/cm^3	Gram per Cubic Centimeter
$g\ cc^{-1}$	Gram per Cubic Centimeter
GA	Glycyrrhizic Acid
GDP	Gross Domestic Product
HCN	Hydrogen Cyanide
HDTMA	Hexadecyl-trimethyl ammonium brome
HPLC	High Performance Liquid Chromatography
HYVs	High Yielding Varieties
IAA	Indole-3 Acetic Acid
ITS	Internal Transcript Spacer
K_{avl}	Available Potassium
$Kg\ ha^{-1}$	Kilogram per hectare
L	Indian Licorice
LC	Indian Licorice-Chilli
LER	Land Equivalent Ratio
LS	Indian licorice-Stevia
LSC	Indian licorice-Stevia-Chilli
MAI	Monetary Advantage Index
MBC	Microbial Biomass Carbon
$Mg\ l^{-1}$	Megagram per liter
MSOC	Mass of Soil Organic Carbon
NA	Nutrient Agar

ND	Nutrient Detectable
N_{avl}	Available Nitrogen
NB	Nutrient Broth
NCBI	National Centre for Biotechnological Information
NE	North East
NFM	Nitrogen Free Malate
nm	Nanometer
NS	Not Significant
NSSO	National Sample Survey Organization
OC	Organic Carbon
OECD-FAO	Organization for Economic Cooperation and Development
P_{avl}	Available Phosphorus
PCO_2	Partial pressure of carbon di oxide
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PEG	Polyethylene Glycol
PGPB	Plant Growth Promoting Bacteria
PGPMs	Plant growth promoting microbes
$PGPMs^-$	Without Plant Growth Promoting Microbes
$PGPMs^+$	With Plant Growth Promoting microbes
PGPR	Plant Growth Promoting Rhizobacteria
ppm	Parts Per Million
PSI	Phosphate Solubilization Index
RCBD	Randomized Completely Block design
RS	Ralegarh Siddhi
S	Stevia
SAR	Sodium Absorption Ratio
SC	Stevia-Chilli
SOM	Soil Organic Matter
TDB Y^{-2}	Total Dry Biomass per two years
TN	Total Nitrogen
TOC	Total Organic Carbon
UNEP	United Nations Environment Program
US-EPA	United States Environmental Protection Agency
USSL	US Salinity Laboratory
UV	Ultraviolet
v/v	Volume by Volume
w/v	Weight by Volume
ZSI	Zinc Solubilization Index

Introduction

Agriculture, as a production system, quality of food and food diversity has been facing an increasing problem, on account that the farmers are quitting agriculture; and India is no exception to it (Nag et al., 2018). The world's population is predicted to grow by about 35 percent to 9.7 billion by 2050, owing to an increased demand for agricultural products such as food, feed, fiber and fuel etc. The recent survey of National Sample Survey Organization (NSSO, 2013) advocates that 64.1 per cent of rural people engaged in the agricultural sector live in poor socioeconomic situations (Nag et al., 2018). Indian agriculture supports nearly 50 percent of the employment but contributes only 15 percent to the Gross Domestic Product (GDP). A country's economic growth is largely determined by its industrial production including agricultural industry. Agriculture, is the primary source of income for many individuals and the primary source of revenue for most of the developing countries. It is the spine of the economic operation of several nations. In addition to providing foods and raw materials, agriculture also contributes to employment opportunities for a large number of the unskilled, semiskilled and skilled population. Though India is currently self-sufficient in its food needs, this may not be the case in the near future as our population grows because, our land resources remain fixed, with little or no room for further expansion. Instead, our agricultural land is shrinking due to the establishment of industries, the expansion of towns and cities, and the construction of homes and other infrastructure. As a result, in order to avoid a scenario of food shortage and agricultural sustainability in the country akin to that of the pre-Green Revolution era, agricultural management need to be changed in a sustainable way. The agriculture sector is highly vulnerable to climatic changes, shrinking arable land due to degradation, desertification and conversion of agricultural lands into commercial landscape for infrastructure development to accommodate and provide modern facilities to a growing population (Meena et al., 2017a).

1.1 Degraded/ Marginal Land

Low productivity and strong restrictions on agricultural operations characterize as degraded / marginal land (Kang et al., 2013). If the cost of production is barely covered by the combination of yields and prices, land is considered as degraded (Dale et al., 2010). An area where cost effective production is not viable under the given environmental conditions, agriculture regulations, cultivation techniques as well as macroeconomic and legal situations are considered as marginal or degraded land. (Schroers, 2006). Marginal lands are unsuitable for field crops due to low crop productivity caused by edaphic or climatic constraints (Gelfand et al., 2013). Since 1960, the amount of arable land per capita has declined from 0.41 to 0.21 hectare (FAO 2009) and the pressure on the remaining land will increase as the number of animals and human population will grow, as well as the demand for commercial crops and bioenergy supplies to replace fossil fuels will increase (OECD-FAO 2009; Tirado et al. 2010). These rising demands will almost certainly necessitate the conversion of new land to agricultural use and/or increased productivity on existing land.

Land degradation is one of the world's most serious environmental issues, and it will get worse unless immediate action is taken. Around a quarter of the world's total land area has been degraded. Highly degraded soils are found especially in semi-arid areas (Sub-Saharan, Africa, Chile), areas with high population pressure (China, Mexico, India) and regions undergoing deforestation (Indonesia) (UNEP, 2002). The most significant cause of land degradation in India is water erosion which contributes alone is about 56% followed by the wind erosion (28%), chemical degradation 12%) and physical degradation (4%)

(Figure -1.1).

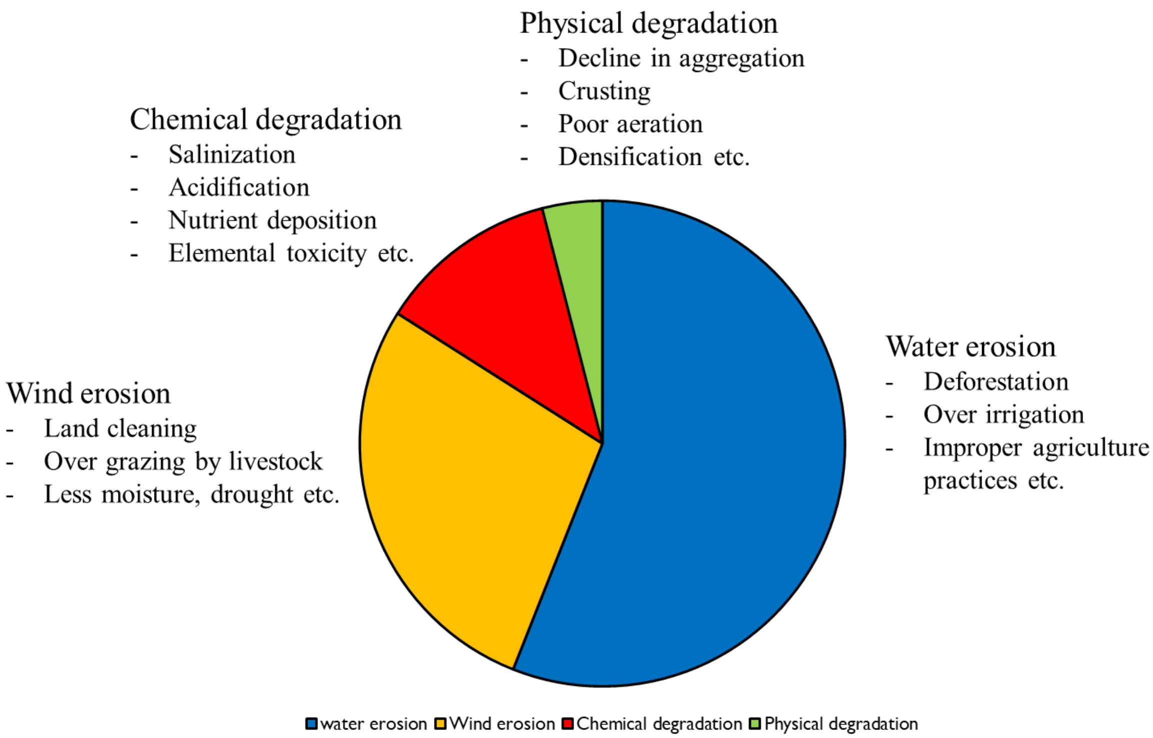


Figure – 1.1 Global cause of land degradation in India

Land degradation due to excessive use of chemical fertilizer is a vital threat to land resources, resulting in large economic and associated environmental consequences (Katkar, 2019). Nearly 80 percent of the farmers in India belong to the marginal or small land holding category with 1-2 ha land or even less. Around 25% of the world's total land area has been degraded. By 2050, 95 percent of the land area might be damaged, if current trends will continue. Land degradation affects 3.2 billion people worldwide, primarily rural communities, smallholder farmers and the impoverished. In India, approximately 6.73 million ha land has been categorized as marginal land due to the high alkalinity/ salinity with Gujrat followed by Uttar Pradesh and it has lost its fertility for conventional agriculture. It is estimated that at current rate, 11,7 million hectares of land will be affected by 2025 (Singh et al., 2018). For 10,000 years, soil salinity/alkalinity, the second most common cause of land degradation after soil erosion, has been a source of deterioration in agricultural communities. Every day, around 2000 acres of arable land is lost to production owing to salinization around the world (Shahid et al., 2013).

1.2 Management techniques for restoration

Rebuilding of such kind of degraded/ marginal lands, as long as economic support to farmers with productivity is of undue concern (Singh et al., 2020). Many biological and chemical practices are in use to improve the quality of marginal/degraded land (Gill et al., 2009). Crop residue incorporation, green manuring with dhaincha, sunhemp, application of organic manure, compost and spent wash press mud compost as well as some land configuration, tillage and drainage improvements have been implemented for restoration of degraded agroecosystem with some limitations (Katkar et al., 2019). Several salt/alkali tolerant cultivars of key crops, such as rice, wheat, maize, sorghum and mustard have also been developed that may be grown (Tahjib – Ul -Arif et al., 2018; Genc et al., 2019; Nawaz et al., 2020; Rajabi Dehnavi et al., 2020; Mohamed et al., 2020). Aside, various bushes, biofuel crops e.g., Jerusalem Artichoke, Trees (Acacia, Date palm, Jujube, fig etc.) and agro-techniques for their successful development on marginal lands have been identified (Zou et al., 2020). Useful grasses (Lemon grass, Vetiver, Palma rosa, Sudan, Bermuda, Canary etc.) are also known to be planted in degraded fields to aid in the reclamation of such soils through root activity and the addition of leaf litter (Merwad et al., 2017; Ravelombola et al., 2018; Singh et al., 2018; HUANG Rui-dong.,2018; G, Singh, 2018; Al Kharusi et al., 2019; Chen et al., 2019; Ye et al., 2020; Kumar et al., 2020; Sadder et al., 2021; Madhhesiya et al., 2021; Weston et al., 2021). Recent breakthroughs have been made in selecting species with high biomass and protein levels, of environmental conditions, such as salt and drought.

1.3 Diversified cropping

The future functionality of today's cropping systems in terms of resilience, adaptability to climate change, multifunctionality of agricultural landscapes, provisioning of ecosystem services, and biodiversity has been questioned due to the simplification of farming systems and growing environmental problems (Rusch et al. 2016; Lichtenberg et al. 2017). Highly diversified cropping can help to establish more sustainable agricultural systems, develop value chains for minor crops, and contribute to socioeconomic advantages (Meynard et al. 2018; Feliciano 2019). Diversification is a relatively new concept which can be used for diverse cropping, mixed cropping, crop combination and intercropping etc. There is no apparent distinction between the concepts of diversification and 'diversity' in many different circumstances (Hufnagel et al., 2020).

Diversified cropping is a key agroecological paradigm that can help in improving resource efficiency, reduce pests and diseases, diversify income streams, and boost production resilience (Carolina et al., 2021), It has the ability to improve soil quality and increase system output (Figure -1.2). In a variety of crop rotations, improved soil qualities such as higher soil water uptake and storage and a bigger population of beneficial soil organisms may boost yield tolerance to drought and other severe growing conditions (Shah et al., 2021). According to Davis et al. (2012), diverse cropping systems produced equivalent or even better yields than simplified systems while having reduced environmental impacts. A few studies, reported on diverse cropping system with little inputs are found to be highly productive than mono cropping with excessive inputs (Awasthi et al., 2014; Carruthers et al., 2019). Polycultures were found to sustain more biomass production, carbon sequestration and reduction in nitrogen leaching up to two-times higher in comparison to the monocultures in various studies (Dybzinski et al., 2008; Fornara and Tilman, 2008; Tilman et al., 2014). Likely, intercropping with increased crop diversity can be considered as an attempt to improve the delivery and stability of ecosystem services with enhanced crop productivity (Kremen et al. 2012; Wezel et al. 2014; Garbach et al. 2017). On the peripheries of modern intensive agriculture, it could be very beneficial in resource limited or low input agriculture systems. Growing two or more plant species as an intercrop or diverse crop in the same unit area at the same time can be a promising path for ecological and sustainable intensification by allowing greater yield stability and food security with minimum inputs (Brooker et al., 2015; Raseduzzaman and Jensen, 2017).

Figure – 1.2 Benefits of highly diverse cropping system

Growing research suggests that degraded lands have a lot of promise for restoration, potentially most efficiently through perennial cropping systems that can provide extra ecosystem services at the same time. When perennial crops are used in a system, they have the potential to improve the amount and diversity of organic inputs returned to the soil (Mosier et al., 2021). Oil palm, rubber, cocoa, coffee, and other perennial tree crops have been planted in large areas of natural and regenerated forest. Many farmers in tropical regions rely on these crops, which are farmed on big scale plantations as well as by smallholders. Perennial tree cover protects the soil from erosion better than annual crops, according to popular belief. Tree crops, on the other hand, may take several years to close their canopy, whereas most annual crops give sufficient cover within six weeks of sowing (A. hartemink, 2006).

Biodiversity enhances agro ecosystem functions by allowing farms to develop their own soil fertility, plant health and sustained yields but intensification of perennial system can negatively affect ecosystem services like diminished pollinator habitat and deteriorate water quality with higher external inputs for biomass generation (Hendrickson and Sanderson, 2017). So, we hypothesized, the combination of perennial and seasonal crop system would be more beneficial approach to use marginal or degraded land in a sustainable way. However, the emerging commercial crops of industrial value in combination of perennial and short duration seasonal crops in the same unit area have not been attempted previously. Aromatic and medicinal plants can sustain various adverse conditions prevailing on the marginal lands and no economical loss ascribing disease occurrence or cultivation issue in the aromatic plants has been reported yet (Khan et al., 2020).

1.4 Introducing novel cropping pattern for sustainable agriculture

Indian farmers are still using century old techniques when it comes to farming, all these techniques are obsolete now and they will have to change themselves with the time. Famers can change their fortune by mending the ways of their farming practices. The government had constituted an Inter-ministerial committee in April 2016 to examine issues relating to "Doubling of Farmers Income" and recommend strategies to achieve the same. The committee has submitted report in September 2018. The committee on Doubling Farmers Income recognizes agriculture as a valued enterprise and has identified seven major sources of growth, viz., Improvement in crop and livestock productivity, Resource use efficiency or savings in the cost of production, increase in cropping intensity, Diversification towards high value crops, Improvement in real prices received by farmers and shift farm to non-farm occupations.

Changing crop pattern with new crop combinations of high valued industrial and seasonal crops that has never been adopted yet through diversification is a new and novel approach for sustainable agriculture (Siddiqui and Afzal, 2018). It has the huge potential to result in better and more stable yields, as well as increased profitability and agro-ecosystem resilience in the long run (Urruty et al. 2016; Raseduzzaman and Jensen 2017; Meynard et al. 2018; Rosa-Schleich et al. 2019; Liu et al. 2019; Renard and Tilman 2019). In present study we have cultivated three different types of crops in the same unit area at the same time as mixed crop for higher agricultural sustainability to the existing agro climatic condition. Cropping of new different kinds of crops as combination crop at same unit area with microbial inputs is the novelty of the current study.

Abrus precatorius L. (Family- Fabaceae) and *Stevia rebaudiana* Bertoni

(Family- Asteraceae) commonly known as Indian licorice and Mithi Patti are perennial, herbaceous and economically important medicinal plants that are in huge demand in the pharmaceutical, cosmetics and food industries owing to their low-calorie content and 50-300 times higher sweetness than sucrose are alternative to cane sugar (Jain et al.,2009; Bhatia and Siddiqui, 2013; Samuel et al., 2018; Rojas et al., 2018; Rather et al., 2019). These are found to develop better roots in sandy loam soils at alkaline pH and grows well in tropical climate (Lulie et al., 2014 and Sheikh and Hedge, 2017). To fulfil the huge requirements of raw material by drug and cosmetic industries for extraction of its active components, there is a need to promote the cultivation of medicinal aromatic plants for enhanced biomass through proper nutrient management and cultural techniques on such kind of marginal lands (Kumar et al., 2017). Considering the current scenario, Indian licorice (*Abrus precatorius* L.) and stevia (*Stevia rebaudiana* B.) need to be introduced as a component crop in India.

Chilli (*Capsicum annuum* L.) has been selected as a food crop in the present study which is a fruit vegetable widely cultivated in the world, are one amongst the healthy diet constituting vitamin C, fibres, carbohydrates, rich protein and mineral salts (Fe, Ca and P) (Sanati et al., 2018). Among the selected crops, Indian licorice is already in use as a rotating crop for reclamation of degraded land (Kushiev et al., 2005). Likely, stevia and chilli have been predominantly cultivated with other staple and vegetable crops as an intercrop to increase productivity and economic return (Tarafder et al., 2003; Singh et al., 2007; Lulie et al., 2014; Begum et al., 2015; Ahmed et al., 2016; Kumari et al., 2019; Mulyono et al.,2019) but cultivation of Indian licorice, stevia and chilli as diverse cropping system have never been applied on degraded land for restoration as well as higher socioeconomic benefits.

1.5 Microbial amendments as fertilizer

It has been stated that use of chemical fertilizers and pesticides have been indiscriminately used not only in food crops but also in medicinal and aromatic crops, may cause long term sustainability problems and may affect the quality of active compounds in the crops used for medicinal and pharmaceutical purposes (Pereira et al., 2019). However, they adversely affect native beneficial micro-organisms of the soil with high residual contaminations in the plant which subsequently raise questions on the quality of its useful metabolites. So, it is suggested to avoid chemical fertilizers and pesticides for the cultivation of medicinal and aromatic plants (Arora et al., 2018). Uses of biofertilizers are one of the major facets of sustainable agriculture to reduce or eliminate the use of chemical fertilizers for enhanced crop production and improved soil properties (Blanchet et al., 2016 and Douds et al., 2017). Biofertilizers, that use microorganism, provide nutrients to the soil as well as plant by phosphorus solubilization, nitrogen fixation and plant growth stimulation through the manufacture of growth-promoting chemicals. Biofertilizers differ from chemical and organic fertilizers in that they do not directly feed nutrients to crops and are cultures of particular bacteria and fungus. They are also extremely simple to install and have a low cost of ownership (Suhag, M., 2016).

The microbial community is the most abundant and diversified creature on Earth (Locey and Lennon, 2016), and it shapes environmental conditions by driving biogeochemistry, the nutrient cycle, and decomposing natural and manmade compounds (Locey and Lennon, 2016). (Wilmes and Bond, 2006 and Wagg et al., 2014). The ecological functions are mostly regulated by microbial enzymes (e.g., salt solubilizer, dehydrogenase and phosphatase) and secondary products (e.g., IAA and siderophores) released by microbes in response to their environmental conditions (Wilmes and Bond, 2006). These beneficial microbes can be used as biofertilizers, biopesticides and biostimulants. These microbes have the potential to replace agrochemicals which shows various negative impacts on agricultural produce, health, biodiversity and ecosystem functioning (Kopittke et al., 2019; Geisen et al., 2019). Applications of agriculturally beneficial microbes popularly known as plant growth-promoting microbes (PGPMs) or bioinoculants have been reported to be highly effective in the cultivation of agricultural crops e.g., chilli as well as in medicinal plants (Tahami et al., 2017; Hyder et al., 2020; Raza et al., 2021). Some microbial bioinoculants have recently been reported to increase the stomatal density and level of a secondary metabolite in certain medicinal plants including stevia (Gupta et al., 2011 and 2021; Cakmakci et al., 2020) and can prove to be a reliable ecological input for the cultivation of medicinal and aromatic plants (Gupta et al., 2011; Nagachandrabose, 2018). Hence, plant growth promoting microbes (PGPMs) have been applied to enhance the productivity and yield of such crops which are considered as an ecological intervention for restoring soil carbon pool of agroecosystems and increase in the crop productivity as well as soil fertility (Akhazari et al., 2015; Tahami et al., 2017; Gouda et al., 2018; Khan et al., 2019). Biofertilizers with beneficial microbiomes have developed as a new and environment friendly method for boosting soil fertility and plant growth in sustainable agriculture practices (Bertola et al., 2019; Murgese et al., 2020; Fasusi et al. 2021). The beneficial soil microbes having multiple plant growth-promoting traits are used as good biofertilizers as single or as in consortium for agricultural sustainability (Rastegari et al. 2020a; Kour et al. 2020b, c; Rana et al. 2020b; Soumare et al., 2021).

Microbial consortia improve more useful traits in crops as compared to individual microbial strains due to the inclusion of a diverse set of biological and plant growth promotion controlling mechanisms and in addition, a decrease in disease caused by pathogens (Ju et al., 2019; Bradacova et al., 2020; Sunita et al., 2020; Behera et al., 2021). Consortium of PGPMs favored more plant growth promotion and secondary metabolites as compare to individual microbial strains (Kalaiyararsi and Victoria 2016; Rajasekhar et al., 2016; Schoebitz et al., 2016; Jayashree and Jagadeesh 2017). Microbial consortium of several AMF and PGPMs has been assessed for sustainable cultivation of chilli (Thilagar and Bhagyraj, 2015). The effect of microbial inoculants as a consortium are well investigated on different plants but it has never been assessed on cultivation of Indian licorice and stevia. Hence, in present study, consortium of four PGPMs (Bacteria- *Bacillus pseudomycoides* and *Bacillus firmus;* Fungi- *Aspergillus luchuensis* and *Aspergillus tamarii*) was firstly assessed to its efficacy on plant growth promotion and secondary metabolites production in *Abrus precatorius* L. as compare to individual strains application and uninoculated control (Pot study). Further, consortium as a best performing biofertilizer over single strain was used for field experiment and its efficacy was assessed on growth and productivity of cropping system as well as soil fertility of marginal land.

The major objectives of this established diverse cropping system are to convert degraded land into productive land by planting a group of crops which can survive in the available soil condition possessing low carbon and slightly basic pH. Established crop pattern suitable for the given micro-climate can be one novel approach to utilize such kind of land for enhancing the carbon sinks, other ecosystem services and value-added products useful for livelihood and additional income generation for the local farmers. In the present study, we hypothesized, 1- Highly diverse crop system i.e., Indian licorice, stevia and chilli (LSC) would promote more above ground plant biomass, carbon assimilation, crop yield in terms of crop equivalent yield value (CEV) and land equivalent ratio (LER) over less diverse crop system as two and three crop combination and sole crops with the microbial amendment. 2- Intercrop of Indian licorice and stevia (LS) could promote more economic advantage in terms of monetary advantages index (MAI) because both are as considered cash crops. Soil cultivated with sole Indian licorice or in combination of Indian licorice will be more fertile as compare to other non-licorice crop combinations. 3- Microbial amendments would promote addition above ground and belove ground benefits over non-inoculated controls.

The objectives of the study:

In light of the above perspective the following specific objectives were planned for their study.

1. Isolation and characterization of climate resilient Plant Growth Promoting micro-organism (PGPMs) from dry and semi-arid plateau.
2. To assess effect of water, and salt stresses on survival and reproductive potential of selected useful microorganisms.
3. Bioassay of selected single microbe and consortium on growth and metabolite production of Indian licorice in earthen pots.
4. To assess growth and productivity of other suitable commercial crops with Indian licorice along with its impacts on soil fertility in absence and presence of microbial inoculants.

5. Cost Benefit Analysis of this constructed crop cultivation system in terms of soil restoration and economic outcome.

Review of Literature

2.1 Problems of degraded land in world and India

World agriculture has been severely affected due to global warming within this century. By 2080, global agricultural productivity is projected to decline between 3 and 16%, and it is predicted that there would be an average of 10–25% decline in agricultural productivity in developing countries (Pal et al., 2019). Further, the rise in sea level could lead to loss of farmland by inundation and increase salinity of groundwater in coastal areas. The effects of climate change on agriculture are both, direct as well as indirect (Pal et al., 2019). With the increasing growth of population, there is a greater demand for food. Thus, world agriculture has to be intensified but land availability is still limited or even decreasing at increasing rate of urbanization. The per capita availability of land in India has decreased from 0.48 ha in 1951 to 0.12 ha in 2016 and projected to further reduce to 0.10 ha by 2025 (Paul et al., 2019). The improper use or poor management, usually for agriculture, industrial and urban purposes decline the soil health and leads to soil degradation. It could be physical, chemical or biological decline in terms of loss of organic matter, decline in soil fertility, and structural conditions, erosion, adverse changes in salinity, acidity or alkalinity and toxic effects of chemicals and pollutants etc. In addition to this all-out drive, soil health has also become major interest in developing areas, where the extensive production system has been intensified (Sinha et al. 2013). Intensive agriculture, that is cultivation of high-yielding cereals and other crops which lead to nutrient depletion from the soil and use of chemical fertilizers and pesticides without any proper dosage again resulted in soil health degradation (John et al. 2001).

2.1.1 Impacts of current agricultural practices

India became self-reliant in food grain production with green revolution and after this, there is increase in the use of chemical fertilizers and irrigation water to meet the nutrients and water demand, respectively, of high yielding varieties (HYVs) of crops. However, the excessive use of chemical fertilizers coupled with decrease in use of organic manure and over exploitation of ground water, causes deterioration in the quality as well as quantity of natural resources (Figure -2.1). Fertilization, which is widely used in agricultural systems to enhance crop production in shadowing of economic benefits, strongly influences soil biogeochemical cycles (Han et al, 2016). The harmful consequences for the crop system from external nitrogen additions are considerable, since it can greatly accelerate soil acidification and salinization /increased alkalinity through nitrogen transformation processes such as nitrification and groundwater contamination due to reactive nitrogen accumulation (Guo et al. 2010; Shen et al.2011; Zhao et al. 2014; Han et al.2015). Most of the farmers here have no idea about the amount of pesticide required per acre of land. Rampant usage of pesticide not only impacts the fertility of the soil but also increases the cost of farming in many folds.

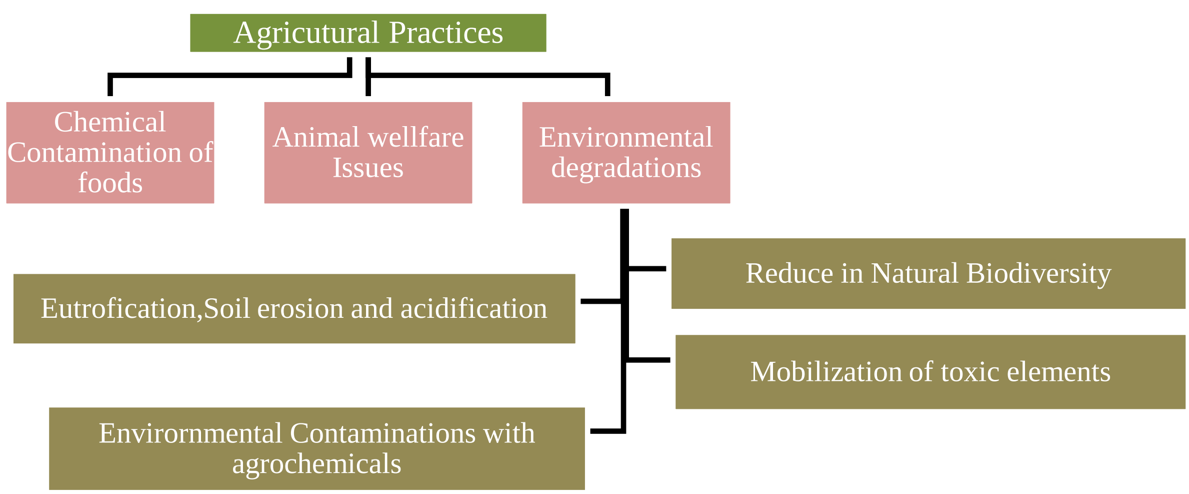


Figure-2.1 Impacts of Current Agricultural practices

2.2 Classification of degraded land

2.2.1 Worldwide land distribution

Globally about 25% of the total land area has been degraded. If this trend continues, 95% of the land area could become degraded by 2050. Globally, 3.2 billion people are affected by land degradation, especially rural communities, smallholder farmers, and the very poor. The world population is projected to increase by about 35 percent to 9.7 billion in 2050, with rising demands for agricultural products including food, feed, fiber and fuel. Global estimates of total degraded area vary from less than 1 billion ha to over 6 billion ha, with equally wide disagreement in their spatial distribution (Gibbs and Salmon 2015). Figure 2.2 (https://earthbound.report/2017/02/01/a-map-of-global-soil-degradation/) shows worldwide land distribution in terms of degradation. Most of the part of India comes under very degraded land category.

Figure- 2.2 worldwide land distribution

2.2.2 Salt affected soil and their distribution

Soil degradation resulting from salinity, sodicity/ alkalinity, or a combination of both is a main obstruction to optimal utilization of soils in the world. It is a vital threat to land resources, resulting in large economic and associated social consequences.(Katkar, 2019). Soil salinity, the second major cause of land degradation after soil erosion, has been a cause of decline in agriculture societies for 10,000 years. Globally about 2000 ha of arable land is lost to production every day due to salinization. Salinization can cause yield decreases of 10-25% for many crops and may prevent cropping altogether when it is severe and lead to desertification (Shahid et al., 2018). All soils contain a certain quantity of water- soluble salts, which are indeed essential for healthy growth of plants. If the quantity of soluble salts in a soil exceeds a certain threshold value (which in turn depends on the geochemical and environmental conditions, physicochemical properties of soil, and chemical composition of salts causing salinity), the growth of the most plants is adversely affected. Such soils are designated as salt-affected.

2.2.2.1 Classification of salt affected soil

2.2.2.1.1 Classification of saline and sodic soil as per Indian system

The US Salinity Laboratory Staff in 1954 (USSL 1954) originally proposed the three categories of salt-affected soils on the basis of SAR, ESP, pH and Electrical conductivity of the soil saturation paste extract, i.e., saline, saline-alkali, and alkali soils. The term ‘‘alkali’’ was discarded later on to be replaced with ‘‘sodic’’ and these soils contain sufficient exchangeable sodium (ESP>15) to affect the physical behavior of soils and interfere with growth of the most of the crops (Table- 2.1, Dagar et al., 2016).

2.2.2.2 Distribution of salt affected soil in India

As per recent estimates documented by Singh et al.2018, nearly 953 million ha area is affected by salinity and sodicity in the world. In which Australia followed by north and

Table-2.1 Characterization of saline and sodic soil as per Indian system

Characteristic	Saline soil	Alkali/Sodic soil
pH	<8.2	>8.2
ESP	<15	>15
EC	>4 dS/m	Variable, mostly<4 dS/m
Nature of salts	Neutral, mostly Cl and SO_4 of Na, Ca and Mg. HCO_3 may be present but CO_3 is absent	Capable of alkaline hydrolysis, prevalence of HCO_3 and CO_3 of Na

central Asia and south America have the maximum problem of high salt concentration in the root zone soil (Figure 2.3) and state wise India is at top with highly salinity and sodicity affected area. As per data reported in the same, approximately 6.73 million ha otherwise productive area is constituted by salty lands in which Gujarat followed by UP has found maximum area affected by salinity and sodicity (Table 2.2, Figure 2.4). It has been predicted that with this rate,11.7 million ha area will be affected by 2025 (G. Singh, 2018). Biodiversity loss, ecosystem deterioration, desertification of previously productive farm land, increasing number of dead and dying plants at the early stage of growth because they are very less tolerant to salts, declines in crop yield and productivity, contamination of drinking water, lowering the biological activity, weakened road and building foundation of salts within natural soil structure are some severe damages caused by salinity/sodicity (Shahid et al., 2013). Effect of high salt concentration in soils are marked in plants which exhibit physiological changes including stomata closure, hyper osmotic shock, inhibition of cell division, and photosynthesis; however, the most common effects are nutrient

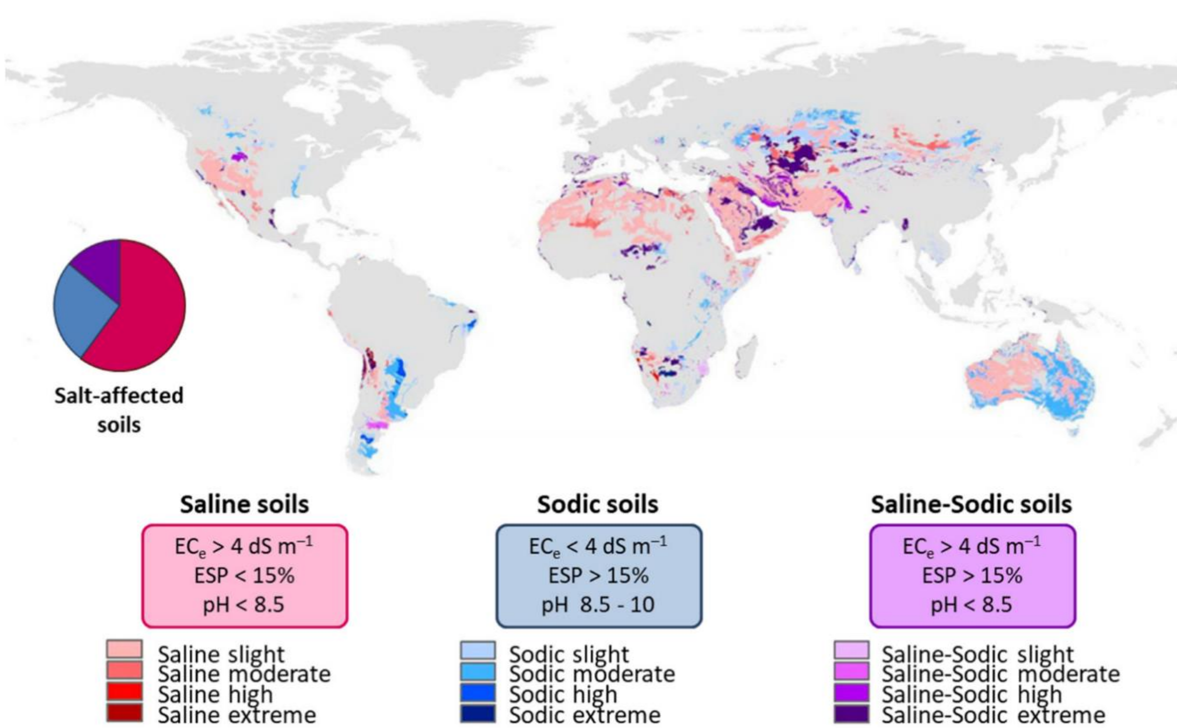


Figure-2.3 Classification of world distribution of salt affected soils under different levels of saline stress. *Saline soils (Pink range) encompass 60% of the total salt affected soils, sodic soils (blue range) include 26%, and saline-sodic soils (purple range) include the remaining 14%. Three main parameters are considered for the classification: electrical conductivity on the saturated soil extract (ECe), exchangeable sodium percentage (ESP), and pH (Romano et al., 2020)*

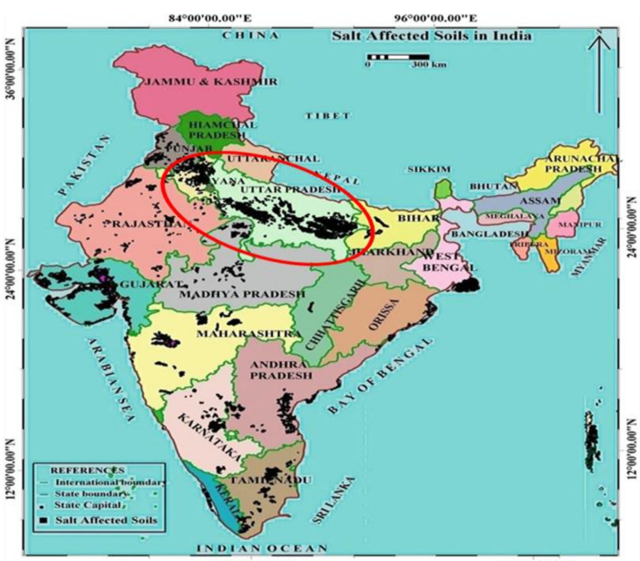
imbalance, low osmotic potential and toxicity of specific ions such as Na^+ and Cl^-, resulting in plant growth inhibition or death (Aslam et al., 2011).

2.3 Approaches for abatement

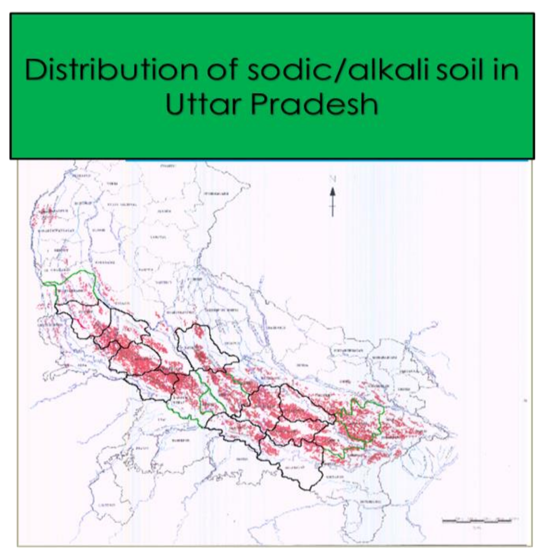
Agricultural practices play an important role in enhancing the crop productivity but such practices are not adequate under changing climatic conditions due to negative effect on crop growth, yield, soil quality and nutritional quality of agricultural produce. To avoid the risk of reduced or limited crop yields, farmers often apply more chemical fertilizers and pesticides than needed due to their relatively low prices (Cordell et al. 2011; Struik et al.

Table - 2.2 Extent of salt-affected soils in different states of India ('000 ha)

State	Saline soil	Sodic soil	Total
Andhra Pradesh	77.60	196.61	274.21
Gujarat	1680.57	541.43	**2222.00**
Haryana	49.16	183.40	232.56
Karnataka	1.90	148.14	150.03
Madhya Pradesh	0	139.72	139.72
Maharashtra	184.09	422.67	606.76
Orissa	147.14	0	147.14
Rajasthan	195.57	179.37	374.94
Tamil Nadu	13.23	354.78	368.02
Uttar Pradesh	21.99	1346.97	**1368.96**
West Bengal	441.27	0	441.27
Total	2956.81	3770.66	**6727.47**



Distribution of salt-affected soils in India (Based on the map prepared by NRSA Hyderabad, CSSRI Karnal, and NBSS & LUP, Nagpur 1997)



Source – Modified from Dagar et al., 2016

Figure - 2.4 Distribution of salt affected soils in India

around the world which resulted a widespread environmental impact, so there are substantial agreements done for a global transition to farming practices that ensure about food safety and nutrition, and protect the ecological services on which agriculture depends (Barrett., 2010 and FAO.,2014b). These agreements promoted several alternative approaches that harnesses biodiversity (such as nutrient cycling, pest control, or pollination) and ecosystem services to reduce vulnerability and achieve resilience to climate change and productive farms (Wazel et al., 2015 and tittonell and Giller, 2013). These prospective aim to either replace external chemical inputs (such as fertilizers, pesticides) with ecosystem services, or search for complementary interactions (Bommarco et.al., 2013). These agricultural management processes could be substituted for biological functions, which affect the ecosystem's natural balance (Kibblewhite et al. 2008) and lead to deterioration of soil quality (Paul et al., 2019). There is an increasing interest on the impact of cropping systems on soil quality to assess the physical, chemical and biological properties of soil in relation to crop production (Paul et al., 2019). Here soil health is the synonyms of soil quality.

Degraded land is one of the most vicious environmental factors limiting the productivity of crop plants. Several studies are reported in India that extremely carbon depleted soils like salt-affected soils have quite high potential for carbon sequestration through vegetation and plantation of suitable tree and grasses species with good management practices like rain water harvesting (Singh et al., 2018). Several salt tolerant varieties of major crops like rice, wheat and mustard have been developed which can be grown either without or with half the recommended dose. Besides these, several salt tolerant trees, grasses, bushes, biofuel crops also have been identified and agro-techniques for their successful cultivation in salty lands. It is also reported that trees and grasses are established in salty lands for reclamation of such soils by root activity and addition of leaf litter (G, Singh, 2018). There have been recent advances in selecting species with high biomass and protein levels in combination with their ability to survive a wide range of environmental conditions, including salinity and drought. Other management options are also available for amendment and sustainable productivity of salt-affected swell–shrink soils. The use of amendments (gypsum), land configuration, tillage, and improvements in drainage and some other innovative approaches include crop residue incorporation, green manuring with dhaincha, sunhemp, and application of organic manure, compost, and spent-wash press-mud compost need to be instituted for improvement of soil quality (Katkar, 2019). Combination of chemical amendments and organic conditioner, along with bio-inoculants, has been found to be beneficial in improving the air–water relationship in sodic soils. Agricultural production in arid and semiarid regions is restricted by soil salinity, sodicity/alkalinity, poor water resources, limited rainfall, and loss in soil fertility, which may be constrained to a localized area or sometimes extends over the whole of the basin. (Katkar, 2019). Development and modification of appropriate technologies for reclamation and management of such lands seems promising option to accomplish future food and nutritional security as well as adoption and mitigation strategy to cope with predicted future climate change scenario(G. Singh, 2018).

Leaching and chemical or organic amendments are the most frequently used methods for salt- affected soil remediation. Leaching involves the application of excess water to promote the movement of soluble salts from the surface soil to deeper soil strata. The application of amendments to agricultural soils can help to build soil resilience against potential soil threats such as soil compaction, erosion, soil fertility or organic matter (OM) loss (Amoah-Antwi et al., 2020). One option is natural/mined gypsum, which has been used for decades to improve soil structure (Hamza and Anderson, 2002, Herrero et al., 2009, Chen and Dick, 2011, Chi et al., 2012). From a chemical perspective gypsum is proven to be an excellent source of Ca and S for plant nutrition and improving crop yield. Ca is essential for most nutrients to be absorbed by plant roots. Without adequate calcium, uptake mechanism would fail, it helps stimulate root growth. (Dick et al., 2006; Batool et al., 2015; Walia and Dick, 2016). The Ca present in gypsum binds OM to clay, thereby protecting it and preventing carbon (C) losses (Walia and Dick, 2018). Application of gypsum as amendment for the reclamation of saline and alkali/sodic soils, mitigating subsoil acidity and Al toxicity is also a widely used practice (Oster and Frenkel, 1980; Shainberg et al., 1989; Toma et al., 1999; Farina et al., 2000; Dick et al., 2006). Flocculation or aggregation and porosity, is needed to give favorable soil structure for root growth and air and water movement. In terms of physical quality, the Ca in gypsum increases the aggregation potential of soil particles making the soil more resilient against compaction (Watts and Dick, 2014; Walia and Dick, 2019). Compared with limestone, gypsum is 200 times more soluble when applied to soil at a neutral pH (EPRI, 2006). Its solubility allows movement of Ca and S through the soil profile into rooting zones (Chen and Dick, 2011).

Deep tillage to break up the horizon high in adsorbed sodium is a common technique used for the reclamation of degraded soils. Tillage practices showed positive effects on soil properties and crop yields. It provides highest OM accumulation, the maximum root mass density (0-12 cm soil depth), and the improved chemical properties

(Alam et al., 2014). Tillage can improve the soil's physical condition, and can bring a saline layer to the surface. However, it can also create a plow layer through the continuous use of the plow. Care must, thus, be exercised in using tillage (Shahid et al., 2018).

Land configuration techniques such as ridges and furrows and broad bed and furrow can play a vital role to overcome soil related problems by providing easy and uniform germination as well as good growth and development of plants. Land configuration plays a major role in minimizing soil erosion and improving water and nutrient use efficiency of field crops (Ramesh et al., 2020). It is particular useful in areas having poor quality of irrigation water because it helps to avoid direct contact of young plants with saline irrigation water. Raised bed method of sowing has been found helpful to minimize the effect of temporary water logging and salt injury of plants (Joshi et al., 2018). Babu et al.,2020 evaluated the productivity, produce quality, the profitability of baby corn, and soil properties under different land configurations comprising flatbed, ridge and furrow, and broad bed and furrow and found the soil of broad bed and furrow had a higher pH, organic carbon content, organic carbon pools, microbial biomass carbon, and enzymatic activities (dehydrogenase, fluorescein diacetate, and acid phosphatase) compared to soils of other land configurations.

The availability of phosphorus (P) to plants for uptake and utilization is messed up in alkaline and calcareous soils due to formation of poorly soluble calcium phosphate raw materials and hence fixation and precipitation of applied P. Plants grown on such soils can be stunted with shortened internodes and poor root system due to P deficiency (Mahmood et al., 2013). Simply adding P fertilizer at normal rates in these soils may not result in optimal yield and crop quality (Stark and Westermann, 2003). Mostly rice is grown after wheat crop in rice-wheat area of northern Punjab. A considerable area under wheat is being harvested by combine harvester which leaves behind a large amount of loose straw in the field. Crop residues are good source of plant nutrients, primary source of organic carbon added to the soil and the important component for soil health and the stability of agricultural ecosystem (Iqbal et al., 2011). The management practices of rice residue impact the soil physical and chemical properties and productivity of crops (Uddin et al., 2016). The studies reported that rice-wheat sequence gave the yields of 7 t ha^{-1} of rice and 4 t ha^{-1} of wheat which resulted in removal of more than N 300, P 30 and 300 kg ha^{-1} from the soil and produced the residue about 7-10 t ha^{-1} yr^{-1} of rice and wheat in the study area. In south Asia, rice crop is produced the residue of 9-10 t ha^{-1} and the farmers of this area need to manage the residue while wheat sowing (Singh and Bimbraw, 2019).

Green manuring is the simple, effective and cheap technology for management of salt affected soils. The high pH, excess exchangeable Na, high calcium carbonate, low organic matter content and adverse physical properties of alkali soils influence the transformation and availability of native and applied nutrients. Nutrient release and loss mechanism of applied nutrients are also adversely affected in these soils. The crops grown in these soils invariably suffer from poor germination, crop stand, and nutritional disorders that finally results in low yields. Application of green manure enhances the reclamation of saline and sodic soils by improving physical and chemical properties of soil by markedly decreasing soil pH. Plant litter incorporation improves aggregation, aeration and water retention. Application of green biomass helps to curtail the evaporation from soil surface and thereby decreases salt concentration in the root zone profile which results in arresting sub soil sodicity. *Gliricidia sepium*, *Crotalaria juncea*, S*esbania aculeata* ,*Vigna* *unguiculata, Leucaena leucocephala, Vigna radiat*a are some common green manure crops.

Green manures are forage or leguminous crops that are grown for their leafy materials needed for soil improvement. A green manure crop can be cut and then ploughed into the soil or simply left in the field for an extended period prior to tilling for next crop. Although only leguminous crops are considered as green manure crops, there are a few exceptions too. The growing and turning of green manure crops enriches soil organic matter vis-à-vis additional nutrients to the soil besides reclamation of saline and /or sodic soils. In addition to the supply of nutrients, green manuring crops can help to scavenge the leftover nutrients from the preceding crop. It also increases soil drainage and water retention capacities that help to prevent leaching losses, soil erosion, and weed problems too as these are also incorporated (Shirale et al., 2018). Soil salinity restricts plant growth due to high salt content which leads to creation of osmotic stress in the root zone. The application of green biomass increases the release of salts into soil solution as result of mineral dissolution due to increase in partial pressure of carbon dioxide and organic acids which leads to leaching of salts below the root zone and creates favorable environment in the root zone of crop plants. The production of organic acids (amino acid, glycine, cysteine and humic acid) during mineralization of organic materials by heterotrophs and nitrification by autotrophs cause a decrease in soil pH. The applied green manures during decomposition produce CO_2, which dissolves in water to produce carbonic acid. This acid increases the solubility of calcium carbonate minerals by lowering the pH and dissolving the calcium carbonate and forming a host of complex calcium ion pairs, thus increasing soil solution Ca_2^+ concentration which replaces Na^+ on exchange complex and thus cause reduction in exchangeable sodium percentage (ESP). Application of green manures also reduces concentration of certain cations and anions such as Cl^-, $SO_4{}^{2-}$, $HCO_3{}^-$ and $CO_3{}^{2-}$

2.4 Limitations of existing approaches

Gypsum application has some limitations like, except Sulphur (S) nutrition, crop benefits from gypsum are thought to be indirect, thus requiring 3 to 5 years or more to reap yield benefits from changes in soil chemical and physical properties. Its application will not benefit all types of soils. Applying gypsum to sodic soils, clayey soils with poor drainage, soils containing acidic subsoils, and soils where there is deficient Ca and S will likely be most beneficial. Although yield responses have been noted with gypsum, producers will have to balance the cost and benefits of using gypsum in their crop management system (Watts and Dick., 2014). The use of gypsum for effective management of salt affected soils is well documented however its availability, cost and transportation are some of the serious issues that prevent small and marginal farmers from using gypsum.

Leaching technique is restricted to saline soils as its effects on SAR is limited and is even counterproductive, since it reduces soil stability. Leaching depends on water availability and quality, as well as soil drainage and water table depth (Qadir et al., 2000). Another disadvantage of leaching is that such treatment reduces total nitrogen (TN), total organic carbon (TOC), and microbial activity and overall soil fertility (Laudicina et al., 2009).

Tillage often has some short-term benefits such as favorable soil conditions for a good crop emergence, vigorous seedling growth, better nutrient availability, and more crop yield (Six et al., 1999). In long term, however, tillage causes soil crusting and compaction, accelerates soil erosion, increases mineralization of soil organic matter, degrades and breaks soil aggregates, and exacerbates the losses of plant nutrients and soil organic carbon (Triplett & Dick, 2008; de Moraes et al., 2015). The gradual decline in SOM through accelerated oxidation and in-field burning of crop residues aggravates emission of greenhouse gases and loss of essential plant available nutrients. Mechanized preparation of seedbed also reduces soil fertility and biodiversity (Das et al., 2013), degrades soil quality and jeopardizes sustainability (Fernandez et al., 2009).

Crop residues are good technique but the farmers burn it due to their impenetrability and difficulty in seed bed preparation for the following crop because the operation is time consuming and labor intensive (Mahmood et al., 2013). Additionally, in cooler temperate climates, retaining residues on the surface results in lower soil temperatures which can negatively affect crop production and practices such as strip tillage should be employed. In regions with high rainfall, excess soil moisture retention can present problems with waterlogging. Conversely in semi-arid regions with light rains residues may intercept rainfall and increase evaporation (Turmel et al., 2015). In cooler climates, however, reduced soil temperature from residue cover can be a disadvantage as it could slow down early crop growth and lower crop yields (Verhulst et al., 2010).

2.5 Phytoremediation and involved mechanism

Phytoremediation has several unique advantages over other salt remediation techniques. For instance, it can provide a more uniform removal of salts at higher depths than gypsum (Qadir et al., 2001), while also presenting the opportunity to treat salt and other pollutants simultaneously (Shelef et al., 2012). Various remediation techniques have been successfully developed and are being utilized (Srivastava, 2020), but still there is no proper technical method under different conditions. In different situations, phytoremediation.

technically as well as economically is the best available option.

Phytoremediation is a plant-assisted approach which can be utilized for ameliorating sodic/alkali and saline-sodic soils (Mishra et al. 2002; Qadir et al. 2002). The phytoremediation of sodic/alkali and saline-sodic soils can be done through the ability of plant roots to enhance the dissolution rate of native calcite and removal of Na^+ in plants (Oster et al. 1999). As it is an efficient approach, in salt affected soils, it can replace costly chemical amelioration (Qadir and Oster 2002). Many agriculturally important plant species effectively ameliorate calcareous and moderately sodic and saline-sodic soils. Generally, the species which produce more biomass and have more ability of tolerating soil salinity, sodicity or alkalinity and periodic inundation can ameliorate soil better (Qadir et al. 2001). Phytoremediation of salt affected soils can be done by cultivating those plant species that can tolerate ambient soil property. Several agriculturally important plant species are very effective in phytoremediation of these soils (Ghaly 2002; Qadir et al. 2002). Some forage species withstand sodicity which restricts the growth of conventional crops and are very easy to handle (Aydemir and Akıl 2012). Salt-tolerant varieties can be used for ameliorating calcareous soil, if sufficient amount of water for irrigation and drainage is available. These varieties dissolve more calcite by their roots which results in sufficient amount of calcium in soil solution to substitute exchangeable sodium. Phytoremediation can be done only through the salt removal by crop parts, if they are not supplied again to the same soil (Qadir and Oster 2002; Qadir et al. 2005). The mechanism of phytoremediation is driven by various methods like increase in the PCO_2 in the root zone, proton release in the rhizosphere of some of the legume crops and sodium and salt uptake by harvested shoots (Qadir et al. 2005). Phytoremediation increases the depth of remediation zone and also increases the strength of soil aggregates and soil hydraulic properties as it is a less expensive technology that increases plant nutrient availability (Qadir and Schubert 2002; Gharaibeh et al. 2011).

In phytoextraction, contaminants are extracted by plants and accumulated in their harvestable parts. It is the most widely used method to reclaim salt affected soils (US-EPA 2000). Halophytes and salt-tolerant non-halophytes have the ability to accumulate more salt especially Na^+ and Cl^- in their shoots and are ideal to fix salinity-affected landscapes (Manousaki and Kalogerakis 2011). Salt-tolerant *C. dactylon* stores sodium and chloride in glands of its leaves (Parthasarathy et al. 2015), while *T. pergranulata* stores salts in cell vacuoles (Flowers and Colmer 2008). If harvesting has to be done, then the less salt-tolerant plants which can store salts like *Brassica juncea* (Brassicaceae) can be used (Susarla et al., 2002).

Phytostabilization has two steps: the first one is salt immobilization (Gaskin 2008), and the second one is the storage of salts in roots instead of shoots (Huang and Chen, 2005). Plants selected for phytostabilization can withstand salt but do not transfer it to the aerial parts, like in *Populus alba* (Salicaceae) 90% of sodium are stored in its roots, while its level in shoots remains less (Imada et al., 2009). This is a very competent salt management mechanism as most of the absorbed salts are stored in roots (Tester and Davenport, 2003). Plant roots have significant role in changing the pH and soil moisture content around roots thus coagulating the salt ions and decreasing their ready availability to others.

In phytoremediation, plants are used to remove salts like NaCl, KCl, $CaCO_3$, $MgCl_2$, Na_2SO_4, etc. from soil by storing them in the tissues (Komives and Gullner, 2000; US-EPA 2000). In this method, either plants are intentionally grown into the salt contaminated area or those already present transport and accumulate salts in their tissues. Their roots also help in removing the salts from the area. This is known as phytoreclamation or biological reclamation (Qadir et al., 2007), biodesalination (Graifenberg et al., 2003) and soil desalinization using halophytes (Rabhi et al., 2009). Many halophytes were examined for reclaiming salt-affected soils in the past (Ravindran et al., 2007; Jithesh et al., 2006). Through various experiments many workers have shown that phytoremediation is a valuable amelioration method for calcium-rich saline-sodic and sodic soils. Their performance is similar to chemical remediation (Ahmad et al., 1990; Qadir et al., 1996a, b). Not only these halophytes have positive effect on salt-contaminated soils, but they can also be utilized like forage and oil seed crops (Glenn et al., 1999). Qadir et al., (2007) have shown that phytoremediation is useful in many aspects as there is no economic burden to buy chemicals. Not only there are monetary and other gains from crops used for remediation, but there is also an increase in soil aggregate strength, macropore formation for betterment of soil hydraulic features, root propagation, higher plant nutrient availability in soil following phytoremediation, more consistent and higher remediation in deeper zones of soil and environmental benefits like carbon sequestration in the post-amelioration soil (Hasanuzzaman et al., 2014).

Several plant species including food and non-food crops were examined for salinity reclamation (Table 2.3). Different grasses and legumes were cultivated in combination with success on a heavy black-alkali soil which gradually remediated. The mechanisms by which plants remove salt from the soil and the consequences of this process to soil properties are diverse (Jesus et al., 2015). Aside from increasing leaching conditions (Qadir et al., 2000; Qadir et al., 2005), there are two main mechanisms for the role of plants in salt affected soils remediation. The first one is pH reduction, which increases the dissolution of $CaCO_3$ and, therefore, the available Ca^{2+} for cation exchange with sodium (Rasouli et al., 2013; Walker et al., 2013). The second one is plant uptake of dissolved salts in general land/or sodium in particular (Shelef et al., 2012; Waler et al., 2013).

Yensen and Biel (2006) suggested a new classification system for halophytes, which partly accounts for their different behavior in salt remediation processes and divides them into three groups: excluder, accumulator, and conductor plants are well-known and applied classifications (Ammari et al., 2008; Gamalero et al., 2009; Shelef et al., 2012; Guittonny-Philippe et al., 2014). However, conductor plant is a relatively novel classification. Excluders prevent salts from entering their tissues as a salinity tolerance mechanism; accumulators uptake and accumulate salts in their tissues and third type, called conductor plants, absorb salts and excrete them by salt glands, conducting the salts from the soil into the air. This classification and the mechanisms behind phytoremediation actions are the main factors for plant species selection and associated remediation efficiency. Perennial plants would also allow for a more extended active period of remediation throughout the year (Jesus et al., 2015). Meanwhile, aromatic plants very promising and can be utilized effective tool for phytoremediation. In a long run, they aid in improvement of soil quality by ameliorating the salt affected soils while maintaining biological yield (https://doi.org/10.1201/9780429327643). One of the most promising and cost-effective approaches to increase the crop productivity is the use of salt-tolerant halophyte plants to remove toxic salt ions from the saline soils. Via this approach, pressure on generating salt tolerant crop plants would be reduced, and much heathier crop plants would be cultivated in less stressed saline soils (Karakas et al., 2020).

Table – 2.3 Relative salt tolerance of agricultural crops

Agricultural crops	Species	References
Fibre, Seed and Sugar Crops		
Barley	*Hordeum vulgare*	Kelley and Brown, 1935; Kelley, 1937
Cotton	*Gossypium hirsutum*	Kelley and Brown, 1935; Kelley, 1937
Jojoba	*Simmondsia chinensis*	Obaidi et al., 2017
Sugarbeet	*Beta vulgaris*	Hossain et al., 2017
Cowpea	*Vigna unguiculata*	Tavares et al., 2021
Oats	*Avena sativa*	Kumar et al., 2021
Rye	*Secale cereale*	Desheva et al., 2018
Safflower	*Carthamus tinctorius*	Shaki et al., 2018
Sorghum	*Sorghum bicolor*	Dehnavi et al., 2020
Soybean	*Glycine max*	Cao et al., 2017
Triticale	*X Triticosecale*	Deng et al., 2020
Bread Wheat	*Triticum aestivum*	Amirbahtiyar et al., 2019
Broadbean	*Vicia faba*	Tavakkoli et al., 2012
Castorbean	*Ricinus communis*	Zheng et al., 2021
Maize	*Zea mays*	Nawaz et al., 2020
Flax	*Linum usitatissimum*	Dubey et al., 2020
Millet, foxtail	*Setaria italica*	Yan et al., 2021
Groundnut/Peanut	*Arachis hypogaea*	Aram et al., 2018
Rice, paddy	*Oryza sativa L.*	Arif et al., 2018
Sugarcane	*Saccharum officinarum*	Niram et al., 2015
Sunflower	*Helianthus annuus*	Li et al., 2020
Wheat, Durum	*Triticum turgidum*	Borrelli et al., 2018
Grasses and Forage Crops		
Canary grass, reed	*Phalaris arundinacea*	Weston et al., 2021
White sweet clover	*Melilotus alba*	Zong et al., 2021
Clover, sweet	*Melilotus*	Dzyubenko et al., 2017
Fescue, tall	*Festuca arundinacea Schreb*	Shiade and Boelt 2020
Harding grass	*Phalaris aquatica*	Hayes et al., 2016
Paenchroic grass, blue	*Paenchroicum antidotale*	Eshghizadeh et al., 2012
Rape	*Brassica napus*	Wu et al., 2019

Rhodes grass	*Chloris gayana*	Daba et al., 2019
Ryegrass, Italian	*Lolium multiflorum*	Feng et al., 2021
Ryegrass, perennial	*Lolium perenne*	Wu et al., 2017
Sudan grass	*Sorghum sudanense*	Merwad, 2017
Trefoil, birdsfoot	*Lotus corniculatus*	Sun et al., 2014
Alkali grass, Nuttall	*Puccinellia airoides*	Riedell, 2016
Alkali sacaton	*Sporobolus airoides*	Pessarakli et al., 2017
seashore paspalum	*Paspalum vaginatum* Swartz	Pessarakli et al., 2017
Bermuda grass	*Cynodon dactylon*	Chen et al., 2019
Kallar grass	*Diplachne fusca*	Khodashenas et al., 2020
Saltgrass, desert	*Distichlis stricta*	
Wheatgrass, fairway crested	*Agropyron cristatum*	DeFalco et al., 2017
Alfalfa	*Medicago sativa*	Mohammad et al., 2020
Bentgrass	*Agrostis stolonifera palustris*	Ozdemir and Budak,2011
Bluestem, Angleton	*Dichanthium aristatum*	Bhat et al., 2011
Brome, smooth	*Bromus inermis*	Tavili et al., 2011
Buffelgrass	*Cenchrus ciliaris*	Al-Dakheel and Hussain, 2016
Burnet	*Poterium sanguisorba*	Shairiyat and Sharifabad, 2012
Clover, alsike	*Trifolium hydridum*	Mc Farland et al., 2014
Clover, Berseem	*Trifolium alexandrinum*	Mc Farland et al., 2014
Clover, ladino	*Trifolium repens*	Mc Farland et al., 2014
Clover, red	*Trifolium pratense*	Mc Farland et al., 2014
Clover, strawberry	*Trifolium fragiferum*	Mc Farland et al., 2014
Clover, white Dutch	*Trifolium repens*	Mc Farland et al., 2014
Corn (forage) (maize)	*Zea mays*	Shahbaz and Asraf, 2013
Cowpea (forage)	*Vigna unguiculata*	Rovelombola et al., 2018
Dallis grass	*Paspalum dilatatum*	Keshavarzi, 2020
Foxtail Barley	Hordeum jubatum	Israelsen et al., 2011
Grama, blue	*Bouteloua gracilis*	*Zhang et al., 2012*
Lovegrass	*Eragrostis sp.*	*Fontenot et al., 2015*
Milkvetch, Cicer	*Astragalus cicer*	*Wrobel et al., 2013*
Oatgrass, tall	*Arrhentherum elatius (L.) Beauvois ex*	*Grieve et al., 2012*
Orchard grass	*Dactylis glomerata*	*Yang et al., 2021*

Sesbania	*Sesbania grandiflora L.*	*Kusmiyati et al., 2019*
Siratro	*Macroptilium atropurpureum*	*Sevanayak et al., 2017*
Sphaerophysa	*Sphaerophysa salsula*	*Wang et al., 2021*
Timothy	*Phleum pratense*	*Soliman et al., 2018*
Trefoil, big	*Lotus uliginosus*	Granada et al., 2014
Vetch, common	*Vicia sativa L.*	*Aydinoglu et al., 2019*
Wheatgrass, tall	*Agropyron elongatum*	Riedell, 2016
Vegetable Crops		
Jerusalem Artichoke	*Helianthus tuberosus*	Zou et al., 2020
Beet, red	*Beta vulgaris*	Dadkhah, 2011
Squash, zucchini	*Cucurbita pepo L.*	Rouphael et al., 2017
Broccoli	*Brassica oleracea ittalica*	*Mohamed et al., 2016*
Brussels sprouts	*B. oleracea gemmifera*	*Mohamed et al., 2016*
Cabbage	*B. oleracea capitata*	*Mohamed et al., 2016*
Cauliflower	*B. oleracea botrytis*	*Mohamed et al., 2016*
Celery	*Apium graveolens*	*Duan et al., 2020*
Cucumber	*Cucumis sativus*	*Khan et al., 2013*
Eggplant	*Solanum melongena mill.*	*Wu et al., 2014*
Kale	*Brassica oleracea acephala*	*Mohamed et al., 2016*
Kohlrabi	*B. oleracea gongylode*	*Mohamed et al., 2016*
Lettuce	*Latuca sativa*	*Ahmed et al., 2019*
Muskmelon	*Cucumis melo L.*	*Akrami and Arzani 2019*
Pepper	*Capsicum annuum L.*	*Giorio et al., 2020*
Potato	*Solanum tuberosum L.*	*Jarsmaa et al., 2013*
Pumpkin	*Cucurbita*	*Kurum et al., 2013*
Radish	*Raphanus sativus L.*	*Sanoubar et al., 2020*
Spinach	*Spinacia oleracea L. cv Racoon*	*Ors et al., 2016*
Sweet potato	*Ipomoea batatas*	*Kitayama et al., 2020*
Tomato	*Lycopersicon lycopersicum*	*Kashyap et al., 2021*
Asparagus	*Asparagus officinalis*	Zhang et al., 2019
Fruit and Nut Crops		
Fig	*Ficus carica*	Sadder et al., 2021

Jujube	*Ziziphus jujuba*	Kumar et al., 2020
Olive	*Olea europaea L.*	Bazakos et al., 2015
Papaya	*Carica papaya*	Jiang and Paenchro, 2012
Pineapple	*Ananas comosus*	Samuel et al., 2018
Pomegranate	*Punica granatum*	Sun et al., 2018
Date palm	*Phoenix dactylifera*	Al Kharusi et al., 2019

2.6 Diverse cropping system and benefits

2.6.1 Above ground benefits

Agricultural intensification enhanced crop output while simplifying production by reducing crop diversity, increasing genetic uniformity, and homogenizing agricultural landscapes. Simplified agricultural are associated with loss of species diversity, soil degradation, loss of habitat and reductions in water quality (Tscharntke et al., 2012; Bommarco et al., 2013; McDaniel et al., 2014; Tiemann et al., 2015; Landis, 2017) and these negative impacts corrodes all the necessary ecosystem services requires to crop productivity like Pollination, pest management, water retention, and nutrient availability etc. (Swift et al., 2004; Zhang et al., 2007).

Crop diversification has the potential to sustain crop yield while also reducing negative environmental impacts and biodiversity loss (Hufnagel et al., 2020). Crop diversification, as opposed to specialized farming, can be defined as an attempt to promote crop diversity by diverse cropping / multiple cropping / intercropping, crop rotation, with the goal of improving productivity, stability, and supply of ecosystem services (Kremen et al. 2012; Wezel et al. 2014; Garbach et al. 2017). Higher crop diversity, more diverse crop rotations, mixed cropping, cultivation of grain legumes in some of the more cereal-dominated systems, perennial grassland, and variety mixtures are examples of crop diversification practices (Malézieux et al. 2009; Phelan et al., 2015; Bedoussac et al. 2015; Reckling et al. 2016; Haughey et al. 2018; Renard and Tilman 2019). Highly diverse cropping system increases the ability to control pest outbreaks and reduce pathogen transmission, as both are expected to be worsen under future weather conditions. In

agricultural landscape, diverse cropping has been demonstrated to be helpful in enhancing ecosystem services and sustainability (Tosti and Guiducci, 2010; Wood et al., 2015). Renard and Tilman (2019) discovered that at the national level, arable crop species variety is linked to greater year-to-year consistency in the overall national yield of all consumable crops. Extensive research has demonstrated that greater plant diversity leads to higher levels of productivity and other ecosystem services, and this enhanced diversity has been proposed as a means to improve agricultural yield and sustainability (Reiss and Drinkwater, 2018). Intercropping with increased crop diversity can be considered as an attempt to improve the delivery and stability of ecosystem services with enhanced crop productivity (Kremen et al. 2012; Wezel et al. 2014; Garbach et al. 2017). Generally, higher crop yield per unit of land and reduction in the risk of crop failure due to pests and diseases are noticed in cultivation of crops as combination crop rather than sole cropping (Horwith, 1985; Zhang and Li, 2003; Lithourgidis et al., 2011; Boudreau, 2013; Yu et al., 2015; Zhang et al., 2019). Wan et al., 2020 also explained the plant diversity effect on ecosystem functioning including diversity benefits on insects and plants. Studies suggested that corn yields under highest diversity treatment were recorded over 100 percent higher than in continuous monoculture, meanwhile, inorganic soil nitrogen availability was highly corelated with corn yield in highly diverse treatment (Smith et al., 2008). Plant diversity has an impact on a variety of ecosystem processes including as primary productivity, insect suppression, decomposition, nutrient cycling, and SOM dynamics (Hooper et al., 2000; Tilman et al., 2002, Zak et al., 2003; Orwin and wardle, 2005).

2.6.2 Below ground benefits

Diverse cropping has potential to counteract resource degradation (Lichtfouse, 2009; Feike et al., 2012). Increased crop diversity can assist agriculture's negative impacts, such as erosion losses, soil health, soil water availability, and ecosystem service, while still satisfying food production needs (Hendrickson and Colazo, 2019). Such kind of crop pattern can contribute to increases in soil carbon due to increased root biomass input, and can increase organic soil nitrogen as a result of better nitrogen cycling (Cong et al., 2015). As farmers attempt to be more sustainable and maintain soil health, diversified cropping is gaining significant global interest as an agricultural technique (Glaze-Corcoran et al., 2020). Huang et al., 2018 discovered that variety (a mix of 16 species) significantly boosted stand-level production, accumulating over twice the amount of carbon seen in average monoculture after 8 years of study. According to Genstch et al., 2020, diverse crop combinations boost carbon cycling efficiency in cropping systems and are a promising tool for long term soil management. Greater atmospheric C uptake (upto 2 times), higher transport rates towards the rhizosphere, higher microbial incorporation (3.4 times enhanced microbial carbon), and longer residence time in the soil environment over simplified cropping, all help to promote carbon cycling. The soil microbiome grew and became more active as rhizosphere carbon intake increased. McDaniel and Grandy (2014) reported that, adding one or more crops to a monoculture boosted total soil carbon by 3.6 percent and total nitrogen by 5.3 percent.

According to Tiemann et al., 2015, having a larger plant diversity improves soil microbial biodiversity and increases organic carbon and nitrogen concentrations in natural systems. Plant diversity have potential to increase long-term soil carbon storage, reduced N_2O emissions by 30–40%, and inhibit weed invasion, hence lowering pesticide use (Yang et al., 2019). Furthermore, increasing plant diversity can reduce the danger of nitrate leaching by reducing nitrate supply through nitrification and boosting nitrogen intake by plants, which reduces the risk of nitrate leaching (Dijkstra et a., 2007). Producers have recently been looking to crop diversification for the kinds of belowground, soil ecosystem service benefits seen in spatially diverse natural communities, such as increased SOM concentrations and microbial activity. The commonly observed favorable impacts of crop diversity on soil faunal and microbiological abundance and diversity may increase SOM (Liang et al., 2011a). Crop diversity is able to sustain soil fertility in terms of soil organic matter (SOM), total nitrogen (N), Olsen phosphorous (P), exchangeable potassium (K), and soil enzyme activities (Wang et al., 2014, 2015). Diverse cropping increases above-below ground interaction, affecting carbon allocation, rhizobiome growth and rhizodeposition. An ecological links between microbial and faunal network among roots, rhizosphere and bulk soil are strengthened by stronger above- below ground interactions (Zhang et al., 2016). Approximately, 115.5, 43.6 and 57.3 % increased soil fungus, bacteria and actinobacteria were quantified in diverse cropping of soybean/sugarcane and watermelon/pepper as compared to the matching monocultures (Sheng et al., 2012; Li et al., 2013a).

The most effective strategy to improve soil biological health and boost C stability is to incorporate perennial polycultures as a diversified crop. In comparison to annual and monoculture perpetual systems, perennial polycultures possessed 88 percent and 23 percent more mineralizable carbon, respectively (Sprunger et al., 2020). Several studies have shown that perennial cropping systems are more effective than annual farming systems at raising soil C (Sprunger et al., 2017; King & Blesh, 2018). Furthermore, plant diversity in combination with perenniality is particularly successful at increasing soil carbon sequestration, owing to the larger root systems formed by plant complementarity (Fornara et al., 2009; Weisser et al., 2017; Sprunger & Robertson, 2018). Perennial plants which persist for several years, help with carbon sequestration, soil quality and water purification as well as other ecosystem services. The potential for these new crops to provide ecosystem services and produce viable yields is attracting attention farmers, processors, retailers and consumer (DeHaan and Ismail, 2017).

The most well-known environmental benefits of perennial legumes in cropping systems include better soil nitrogen (N) availability, reduced soil erosion, and reduced weed and pest pressure in annual crops. Other benefits that are less well known and appreciated include soil organic matter accumulation, reduced greenhouse gas emissions, improved soil tilth, and generally better water quality (Russelle, M.P, 2010).

2.7 Incorporation of new cropping pattern

As per our data base *Abrus precatorius* L. have not been previously cultivated as an agricultural commercial crop on salt affected soil (alkali soil), although it can tolerate pH upto 8.3 (Bhatia and Siddiqui, 2014). Several crop species are cultivated on such kind of lands but their combination with other crops is not in practices except few grass species. Hence, requires new cropping combination which will suitable for cultivation in available agro-climatic condition and can generate high income for producer without compromising to productivity of food crops. Need of some commercialize medicinal crops with new above and below ground microbial and plant diversity can be design for profitable crops. Here we are approaching to develop a new ecology by intercropping with such kind of plant combinations like Indian Licorice, Stevia and Chilli.

India is one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world as it is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. About 17,000 species of Indian flora about 7500 species of higher plants are reported to possess medicinal value and in other countries it is projected about 7% and 13% (Patwardhan et al., 2004). In this regard India has a unique position in the world, where a number of recognized indigenous systems of medicine are available for the health care of people. No doubts that the herbal drugs are popular among rural and urban community of India. The demands for plant-based medicines are increasing very fast in India. From this, we can say that search is going on for more plants which can give therapeutic activity in treatment of various diseases.

Among the traditional system of medicine *Abrus precatorius* Linn is one of the important herbs commonly known as Indian licorice belonging to family Fabaceae(Leguminosae)(Bhatia & Siddiqui, 2014). High-climbing, twining, or trailing woody vine with slender herbaceous branches. Leaves alternate, petioled, 5-13 cm (2-5 in) long, even-pinnately compound with 5-15 pairs of leaflets, these oval to oblong, to 1.8 cm (< 1 in) long, with margins entire. Flowers shaped like pea flowers, white to pink or reddish, small, in short stalked dense clusters at leaf axils. Fruit a short, oblong pod, splitting before falling to reveal 3-8 shiny hard seeds, 6-7 mm (< 1 in) long, scarlet with black bases. Fruit 2 to 2.5 cm long pods containing 2 to 5 seeds. It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropica Africa and the West Indies. (Sheikh & Hedge, 2017). It is also cultivated in Punjab & Sub Himalayan tracts in India. Sandy loam fertile soils having pH of 6 to 8.2 have been found to promote better root development in India. The cuttings of the underground stem/root of 15-25 cm possessing 2-3 eye buds are planted directly in the field 6-8 cm deep in the soil at a distance of 90x45 cm. It is found that high yields are obtained from 2-3-year-old crop manual digging is performed for harvesting roots but is found very costly. The crop is harvested in winter season i.e., November or December months to obtain roots of high glycyrrhizic acid.

Abrus precatorius commonly known as Indian Licorice, has been used in folk remedies by Tribes for over many years, and is reported to have a broad range of therapeutic effects, like anti-bacterial, anti-fungal, anti-tumor, analgesic, anti-inflammatory, anti-spasmodic, anti-diabetic, anti-serotonergic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches and sores (Bhatia & Siddiqui, 2014; Sheikh & Hedge 2017). The roots, stems, and leaves also contain glycyrrhizin. The seeds were also used to treat diabetes and chronic nephritis. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties. This plant is known with various names such as Abrusseed, Jequirity, Imisimisi, Aivoeiro, Crab eye, Rotary pea, and Indian bead among others in Africa, India, China and Brazil (Sunday et al., 2016). Previous reports indicated that *A. precatorius* leaf, stem and root have human and veterinary uses as antimicrobial (including Mycobacteria tuberculosis), antiprotozoal, insecticidal and anti-snake venom remedies (Attal et al., 2010, Teklay et al., 2013).

The Indian licorice is a slow growing plant, it takes 3 years for a complete cycle hence other valuable crops can be cultivated with this in combination. As per our data base Indian licorice have never been cultivated in combinations with other crops. Few studies have been reported only for its soil reclamation property in which yield of wheat and cotton crop were 3 and 6 folds increased after its cultivation as rotating crop (Kushiev et al., 2005). Usually, it is grown as a sole crop for soil reclamation with wide row spacing at 50-60 cm, which makes it suitable to grow others as an intercrop. Hence, stevia and chilli were selected as other crops to grow in its combinations.

As a new crop Stevia is gaining much popularity among all types of sweeteners used for its synthesized product, stevioside as most ideal substitute of sugar. Stevia has several advantages, among other is having 200-300 times sweetness level compared to cane sugar with low-calorie level (Science, 2018). Stevioside is clinically tested and proved after frequently used by humans without any negative effect and an ideal, non-additive sweetener for children. Sedentary life style has been created several incidences of obesity and diabetic conditions has been increasing dramatically all over the world (Zaman et al., 2015). People uneasiness towards the use of synthetic sweeteners, with increasing cases of diseases due to excessive sugar consumption have enthused the search for low-calorie natural sweetener sources. Stevioside as a non-caloric sweetener present in the leaves of *Stevia* are reported to be non-nutritious, non-poisonous, high-potency sweeteners and being 300 times sweeter than sucrose might be good alternate as other synthetic sweeteners (Rather et al., 2019). Now it becomes a new avenue to do research for the researcher and is a good opportunity for the farmers to earn cash money through stevia.

Stevia (*Stevia rebaudiana* Bertoni), is a perennial herb belonging to the family Asteraceae, commonly known as "sweet weed", "sweet leaf", "sweet herb" and "honey leaf (Singh and Verma, 2015). Leaves of stevia contain around 10 sweetening glycosides, of which stevioside (3–10%), rebaudioside-A (13%), and rebaudioside-B, C, D are important (A. Singh et al., 2015). Stevia is also reported as an alternative source to the synthetic sweetening agents like saccharine, aspertame, asulfam-K that are available in the market to the diet conscious consumers and diabetics (Aladakatti *et al.*, 2012). From health perspective, consuming stevia have several benefits as reducing hypertension, will not causing caries, rich with vitamins that are not available in synthetic sweeteners, contain antioxidants, reducing diabetes, and curing stomach ache also (Donna et al.,2000).

In India, approximately 240 million tonnes of cane sugar are produced whereas beet sugar contributes about 19500 tones. Though these sugars have sweetening qualities, but are not advised for the consumption by diabetic patients due to highly caloric in nature. (Singh et al., 2005). Recently in many countries' stevia becomes a popular source of dietary supplement. Like India, Bangladesh is also an agro-based country, stevia cultivation has a vast scope for intensive agriculture and fits well for high return agriculture (Barathi, 2003). A limited number of studies have been published on stevia cultivation with other crops in combination. Ramesh et al.,2007 was studied through intercropping of stevia with Wheat, barley, lentil, gobhi and sarson and found intercropping reduced the production of *S. rebaudiana* up to 50% when compared to sole cropping.

In addition, stevia has other advantages, such as strong adventitious root for protecting land from erosion and strong ability to cope with drought (Science, 2018). Worldwide, 32000 hectares of land are covered under stevia cultivation, of which China has a major portion of 75% area. It is growing commercially in many parts of Brazil, Paraguay, Central America, Thailand, Korea and China. The Indian farmers have also started taking up stevia cultivation following the large demand for diabetic market here. The country's total annual production is currently nearly 600 tonnes of dry leaf. The climatic conditions in most parts of India are quite favorable for stevia cultivation. In India farmers have started growing stevia in some parts of Rajasthan, Punjab, Uttar Pradesh, West Bengal, Madhya Pradesh, Karnataka, Chhattisgarh and West Bengal etc.

Chilli (*Capsicum annum* L.) is one of the most widely used fruit vegetable, used as a universal spice in India, grown almost all parts of temperate, tropical and subtropical countries, belongs to the "Solanaceae" family. India has immense potential to grow and export different kinds of chillies as per requirement in various markets around the world. It is one of the major commercial crops of India with larger export potential approximately 1.9 million tons per year (FAOSTATS, 2021). Currently, India leads the world in production, consumption and export of chilli, contributing nearly 25% of the total chilli exports. As one of the most important spice and vegetable crop worldwide, it is produced and consumed both as fresh and in processed for. Chilli is rich in protein carbohydrate, fibers, lipids, mineral salts like Ca, P and Fe and vitamin C, hence it is an excellent component of a healthy diet. Moreover, it has antioxidant, hypocholestrolemic and immunosuppressive properties too (Sanati et al., 2018). In India chilli is grown over an area 774.9 thousand ha with total productivity of 1492.10 thousand tonnes (Tomar et al., 2020). The most important chilli growing states in India are Andhra Pradesh (49%), Karnataka (15%), Maharashtra (6%) and Tamil Nadu (3%) which constitutes nearly 75%

of the total area under chilli. India's major chilli exporters are Sri Lanka, USA, Nepal, Maxico and Bangladesh (Jagtap et al.,2012).

Chilli can be grown in range of soils but black soil, well drained, deltaic and sandy loamy soils are best suited to this. It can't tolerate acidic soil. Range of 20-26ºC is considered as ideal temperature for chilli growth. The experiment was consisted with five crop combinations viz., sole chilli, chilli + radish, chilli+ carrot, chilli + onion and chilli + garlic. Significantly the highest yield (green chilli) was obtained with sole chilli (10.26 t ha^{-1}). Reviews indicated that growth of chilli in intercrop is more beneficial than sole chilli (Aravazhi et al., 1997; Sadashiv 2004). Chilli has been intercropped with shallot, celery, coriander and palak (Rodge and Yadlod, 2009; Mulyono et al.,2019; Suharyan and Wahyudi, 2021) but intercropping of chilli with root crops are considered as more profitable than other vegetable crops in terms of maximum land availability and resources utilization. It has been intercropped with many root crops like Garlic, radish carrot, onion and groundnut (Begum et al., 2015; Kadir et al., 2021) but never been cultivated with Indian licorice. Therefore, the present study was undertaken to evaluate the performance of chilli with intercropping of Indian licorice and stevia.

2.8 Bioformulations as an alternative of chemical fertilizers to maintain sustainability of agro ecology

Plant requires few essential element (N, P, K, S, Ca, Mn etc.) for growth and survival which helps in creation of new cells. Fertilizers are essential components of modern agriculture because they provide essential plant nutrients. However, overuse of fertilizers can cause unanticipated environmental impacts. Chemical fertilizers can supply essential nutrients to crops increasing their yield, however, they can also cause serious environmental problems.

Chemical fertilizers often have low use efficiency, only a portion of the applied nutrients are taken up by plants. For example, P is precipitated after addition to soil, thus becoming less available to plants (Gyaneshwar et al., 2002). Chemical fertilization can also influence the enzyme activities of soil microbes, soil pH, and soil structure (Bohme and Bohme 2006).

Demanding cropping combined with increased chemical fertilizer application, as well as a decrease in the recycling of natural or other waste materials, has resulted in lower organic carbon levels in soils, deteriorated soil physical qualities, and reduced soil microbial biodiversity (Singh et al., 2021). Hence, bioformulations are an efficient technique to reduce the need of higher amount of mineral nitrogen fertilizer which also improve the soil biological properties with enhanced crop yields (Blanchet et al., 2016). Any biologically active chemicals formed from microbial biomass or a product comprising microorganisms and their metabolites that could be employed in plant growth promotion, nutrient acquisition, or disease management in an environmentally acceptable manner are referred to as bioformulation. Bioformulation is a process that involves combining an active element in a formed product with inert (inactive) ingredients (http://npic.orStaphylo.edu/factsheets/formulations.html). The bioformulation refers to the preparation(s) of microorganisms or their active gradients that could be used as pesticide/fertilizer alternatives (Amir et al., 2020). Furthermore, microbial-based bioformulations that improve biological performance, particularly those that have complimentary and synergistic effects with mineral fertilization, are desperately needed. Today, bioformulations sold under many trade names such as biostimulants, bioinoculants, biofertilizers, and biopesticides are regarded vital components of ecological sustainability, as they have increased agricultural productivity while remaining environmentally friendly (Singh et al., 2016). Peat, talc, vermiculite, carboxymethylcellulose, and polymers, particularly xantham gum and diatomaceous earth, are among the most frequent inert active substances.

Cow dung has long been recognized as perhaps the most desirable organic carrier because of its high nutrient and organic matter content. Addition of cow dung as a carrier material in bioformulation increases the organic carbon content of degraded soil which may lead to the increasing activity of beneficial soil microorganisms as well as the fertility status of soil by increasing the availability of nutrients for the plants from soil (Gudugi, 2013). The stability of the adduct, including antibiotics, siderophores, and phytohormones, volatile metabolites hydrolyzing enzymes in direct contact with the plant, are all advantages of such carrier-based bioformulations (Ardakani et al., 2010; Jorjani et al., 2011). Some additives, such as gum, silica gel, methyl cellulose, and starch along with carriers preserve bioformulated products from harsh environmental conditions while simultaneously improving nutritional characteristic (Schisler et al., 2004).

In general, microbial species used in bioformulated products comprise beneficial rhizospheric microorganisms that influence plant growth and development in their natural habitat, either directly or indirectly (Lugtenberg and Kamilova, 2009). The role of rhizospheric microorganisms in improving nutrient availability to plants is an important strategy and related to climate-smart agricultural practices (Pereg and McMillan, 2015; Hamilton et al., 2016). The bacterial genera Rhizobium, Bradyrhizobium, Mesorhizobium, Azotobacter, Bacillus, and Pseudomonas, as well as the most regularly utilised fungal genera belonging to Trichoderma spp., AMF are the most commonly used microbial species used in microbial bioformulations (Aamir et al. 2020).

When it comes to sustainable agriculture, plant growth promoting microorganisms (PGPMs) have proven to be quite important. PGPMs are defined as the microbes that have ability to colonize root or rhizosphere, competing with other microbiomes and promote plant growth (Yadav et al.,2017a). Plant-PGPMs interaction are an integral aspect of earthborn ecosystem. It helps in plant growth promotion by different mechanism. Azotobacter, Azospirillum, Pseudomonas, Acetobacter, Burkholderia, Bacillus, Paenibacillus, and representatives of the fungus Trichoderma, Gleocladium, and vascular arbuscular mycorrhiza are all included in the PGPMs group. PGPMs also aid in the protection of plants from phytopathogens and the encouragement of growth. In the field, PGPMs and green fertilizer have shown promise and could be a solution to the looming challenge of food scarcity. (Debasis et al., 2019). Because of their ancient, retained helpful abilities, plant growth-promoting bacteria are generally assumed to be beneficial to all plant genera with which they are associated.

In a sustainable agroecosystem, three types of interactions are particularly important. These include (i) PGPM collaboration to improve N2-fixation, phosphate solubilization, IAA production, siderophore production, and other plant growth-promoting traits; (ii) plant pathogen biocontrol provided by antagonistic activities of helpful host microbes; and (iii) interactions among various rhizosphere microbes to establish a beneficial mycorrhizosphere (Rana et al. 2019). PGPMs raise crop yield, improve soil fertility, encourage diversity and interaction with other beneficial microbes, prevent the growth and infective action of possible diseases, and maintain the systems' overall sustainability

(Koskey et al., 2021). The potential of these PGPMs to create phytohormones, organic acids, siderophores, atmospheric nitrogen fixation, phosphate solubilization, and antibiotics, as well as develop systemic resistance to control plant diseases, has been widely credited to their beneficial benefits (1995, Glick). Among PGPMs, Plant growth promoting rhizobacteria (PGPR) are rhizosphere competent bacteria that colonize plant roots aggressively and have the ability to reproduce and colonize throughout the ecological niches found on the roots at all phases of plant growth, even in the presence of competing microflora (Antoun and Kloepper, 2001). Whereas, plant growth-promoting bacteria (PGPB) are bacteria that enhance plant growth by fixing nitrogen, mobilising potassium, phosphate, and zinc, producing siderophores, mobilising other micronutrients, and secreting plant hormones (auxin, cytokinin, abscisic acid, gibberellins, ethylene, strigolactones) (Akhtar and Siddiqui 2010; Kumar 2016; Verma et al., 2017a, b; Yadav et al., 2017c). In some circumstances, PGPB aids plant growth in harsh environments such nutrient deprivation, aridity, salt, and drought (Vílchez and Manzanera 2011; Wang et al. 2012; Khan et al. 2017; Shinde et al. 2017).

PGPMs have several advantages as inoculants: (1) they are safer for the environment and human health, (2) they have much more specificity and effectiveness in small quantities, (3) they promote the growth of the host plant and its associated microbiota, (4) they degrade faster than conventional fertilisers and pesticides, (5) resistance development is minimal, and (6) they can be used multiple times. (Berg 2009; Kour et al. 2020b; Rana et al. 2020b). Fungi and bacteria that live in close proximity to plant roots in the soil are the most common PGPMs (Rastegari et al. 2020b; Singh and Yadav2020). The plant growth promoting microbes have been applied to enhance the productivity and yield of such crops which are considered as an ecological intervention for restoring soil carbon pool of agroecosystems and increase in the crop productivity as well as soil fertility (Shahmohammadi et al., 2014; Akhazari et al., 2015; Tahami et al., 2017; Gouda et al., 2018; Khan et al., 2019; Madhhesiya et al., 2021; Mishra et al., 2022).

2.7 Consortium: a way to enhance potential of bio formulations

Due to the environmental problems connected with the use of synthetic inorganic fertilizers in farming, biofertilizers have recently garnered a lot of acceptability and scientific attention, especially in industrialized countries. Development of microbial formulations/biofertilizer may be organism-specific or comprise a group of species (Odoh et al., 2020). A microbial consortium is a group of species of microorganisms that acts together as a community with huge potential of plant growth promotion. The beneficial soil microbes having multiple plant growth-promoting traits could be used as good biofertilizers as single or as in consortium for agricultural sustainability (Kour et al. 2020b, c; Rana et al. 2020b; Rastegari et al. 2020a; Soumare et al., 2021). A microbial consortium generally constitutes two or more compatible strains of different species in a synergistic or additive interaction. (Panwar and Tewari, 2014).

Consortium of PGPMs favors more plant growth promotion and secondary metabolites as compare to individual microbial strains (Trimurtulu et al., 2011; Schoebitz et al., 2016; Rajasekhar et al., 2016; Kalaiyararsi and Victoria 2016; Jayashree and Jagadeesh 2017; Mishra et al., 2021). Due to the presence of a varied collection of biological and plant growth promotion regulatory mechanisms, Consortium enhances more valuable features in crops than individual microbial strains, as well as a decrease in disease caused by pathogens (Ju et al., 2019; Sunita et al., 2020; Behera, B.; Das 2020, Bradacova et al., 2020). Many other studies revealed that consortium of PGPMs promote more seed germination, higher plant growth, productivity and nutrient concentration rather than single inoculants (Rastegari et al. 2020a; Kour et al. 2020b, c; Rana et al. 2020b; Mishra et al., 2021). Microbial consortia-based bioinoculants may contain bacteria of various types, while others may contain both beneficial bacteria and fungi.

The application of different PGPM species with diverse mechanisms of action may provide a wide spectrum of benefits for the plant, including direct stimulation of its growth and health, as well as increases in production. In addition, a decrease in diseases caused by pathogens would be expected (Bradácová and Kandele 2020). Due to the coverage of a varied collection of plant growth promotion and biological regulatory mechanisms, bacterial consortia have been shown to boost positive features in plants when compared to individual strains (Ju et al.,2019). There are two types of bacterial consortia known simple and complex. The distinctions are in the fermentation method or protocol (the generation of a large population of bacteria that will later be made into an inoculant), where strains are grown individually or in combination with other species/strains in a medium suited for all PGPB species. (Bashan and Prabhu, 2020). The organisms in a consortium collaborate in a complicated and synergistic way (Sudharani et al., 2014).

The source of the strain isolation determines which microbial strains are chosen for consortia creation, Because, consortium must thrive in the environmental conditions (soil types, climate and host) where they will be applied. It's also worth noting that when two or more strains form a bacterial consortium, each strain not only competes functionally for plant growth promotion, but also complements the others in terms of soil and/or plant establishment (Ney et al., 2018). Several studies are reported on advantages of microbial consortium over individual microbial strains, Jain et al., 2015 found that plants treated with microbial consortia have increased plant height, number of leaves, total biomass, nodules, and secondary roots, carotenoid and total chlorophyll content, and yield. Although single microorganisms have been demonstrated to have beneficial impacts on plants in numerous studies, it is becoming increasingly clear that when a microbial consortium two or more interacting microorganisms is involved, additive or synergistic consequences can be predicted (Santoyo et al., 2020).

Several studies have been conducted on microbial consortium to assess its efficacy on different plant species as well as improved soil properties. A microbial consortium of *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus megaterium* var. *phosphaticum* and *Trichoderma viride*, was assessed on growth promotion of sunflower (*Helianthus annuus* L.) by Alamraj et al., 2015. Jha and Saraf (2012) depicted the growth effect of consortium of *Bacillus licheniformis* and *Micrococcus sp*., *Acinetobacter calcoaceticus*, *Brevibacillus* on *Jatropha curcas* L brevis. Meanwhile, Kalaiyararsi and Victoria (2016) showed the collective effect of *Trichoderma harzianum*, *Lactobacillus casei* and *Saccharomyces cerevisiae* on plant growth and nodulation of *Vigna unguiculata* L.). The effectivity of mixed culture of *Trichoderma harzianum*, *Rhizobium*, *Bacillus subtilis* and *Pseudomonas fluorescens* was evaluated on pigeon pea (*Cajanus cajan* L.) by Rajasekhar et al. (2016). Trimurtulu et al. (2011) also conducted a study on effect of consortia of phosphate solubilizer, PGPR, AMF and *Azospirillum* on plant growth.

Jayashree and Jagadeesh (2017) showed beneficial effect of potassium solubilizer, *Leuconostoc mesenteroides*, *Pseudomonas sp*. B15, *Azospirillum* and *Pseudomonas striata* on seedlings of chilli, tomato and brinjal. A combined effect of *Pseudomonas putida*, *B.*

subtilis, *B. polymyxa*, *B. megaterium*, *Nocardia corallina*, *B. macerans*, *Trichoderma viride*, *Bacillus licheniformis* and *P. fluorescens* recorded on growth of blueberry seedlings (Schoebitz et al., 2016). Raja et al. (2006) demonstrated the effect of *Azospirillum lipoferum*-Az 204, *Bacillus megaterium* var. *phosphaticum* and *Pseudomonas fluorescence* Pf-1 as a consortium on root exudates of rice plant under hydroponic condition. Romero et al. (2017) investigated the impact of cold tolerant microbes (*Sphingomonas* sp. OF178, *Pseudomonas putida* KT2440, *Azospirillum brasilense* Sp7 and *Acinetobacter sp*. EMM02) in a consortium on maize growth. Likewise in current study we have evaluated the effect of consortium of *Bacillus pseudomycoides* RS6B, *Bacillus firmus* RS7B, *Aspergillus luchuensis* RS6F, *Aspergillus tamarii* RS8F on designed plant community as well as on soil fertility.

Materials and Methods

3.1 Sample collection

Twelve samples of stone pieces and base soil were collected using sterile tool son kit in the month of February 24, 2017 from different locations of Ralegan-Siddhi, district Ahmednagar, Maharashtra, India and stored in sterile bags. A composite of all twelve samples were used for biochemical and microbial analysis. At that day temperature was around 23°C and site was almost barren (Figure 3.1). Collected samples were kept at -20°C during storage until isolation of microbes and studies of physio-chemical properties. The site of sample collection is located on 18.9156º N, 74.4145º E Latitude which is considered as a model semi-arid site with average annual rainfall between 450-650 mm, temperature varies with season and ranges between 12 and 44° C, and average wind NE at 6Km/h with a very low average humidity of about 34%.

3.2 Isolation of microbial isolates

For isolation of the microorganisms, 2 g soil was suspended in 10 ml sterile 0.9% (w/v) NaCl solution, whereas stone pieces were put in autoclaved petri plates and washed with solution. The supernatants were serially diluted (10^{-1} to 10^{-6}) and spread on nutrient agar – NA (Hi-media) and potato dextrose agar-PDA (Hi-media). The plates were incubated at 28°C for up to 48 h for appearance of bacterial and fungal colonies. The microbes from different colonies were re-cultured based on the morphological characteristics of the colonies on NA (for bacteria) or PDA (for fungus) media to obtain pure isolates. The bacteria and fungi were streaked on plates and their stocks were prepared with 30% v/v glycerol and stored at -80°C for further studies.

3.3 Characterization of plant growth promotion and biochemical activities

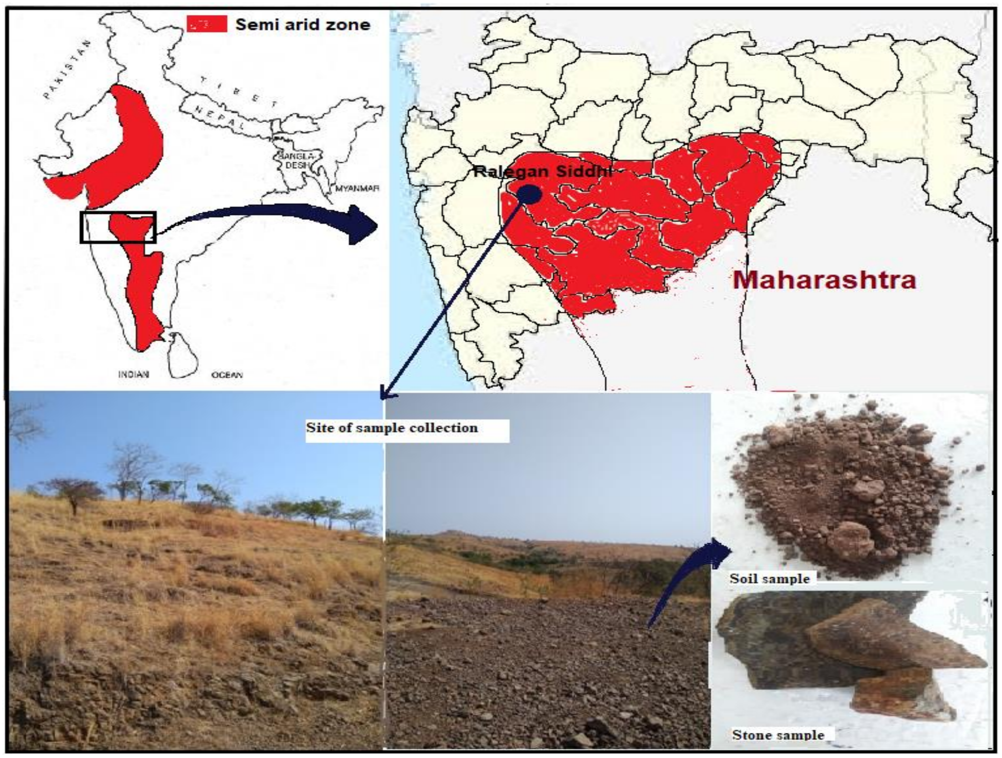


Figure -3.1 Sampling site and Samples (Ralegarh Siddhi, Ahmadnagar, Maharashtra, India)

3.3.1 Plant growth-promoting traits

3.3.1.1 Ammonium production

Microbial isolates tested for the production of ammonia in peptone water by following modified method of Islam et al, 2009. Freshly grown cultures were inoculated in 10ml peptone water in each tube and incubated for 48–72 h at 28^{0}C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonium production which was further quantified by spectrophotometrically at 450 nm using a standard curve prepared with 0.1-10 µmol ammonium sulfate.

3.3.1.2 Nitrogen fixation

Nitrogenase activity was detected by acetylene reduction/ethylene production assay as described earlier (Mirza et al., 2001). Pure bacterial and fungal colonies were inoculated into NFM (Nitrogen Free Malate) semisolid medium vials and incubated at 28 ± 2˚C for 48 h.

3.3.1.3 Indole Acetic Acid production

IAA production was detected by the modified method as described by Brick et al. (1991). Microbial cultures were grown on their respective media (NB or PDB) at 28^0C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production. Optical density was taken at 530 nm with the help of spectrophotometer. Concentration of IAA produced by cultures was measured with the help of standard graph of IAA (Hi-media) obtained in the range of 10–100 mg/ml. Salkowski method has been used for the measurement of IAA production (Glickmann and Dessaux, 1995).

3.3.1.4 Phosphate solubilization

Plant growth promotion (PGP) activities (in vitro) of the bacterial isolates were determined by employing standard protocols for phosphate solubilization test following Pikovskaya's method (Sylvester-Bradley et al., 1982), Bacterial and fungal isolates, cultured on Pikovskaya media. These cultures are stored upside down in an incubator at a temperature of about 30°C for 24 hours. After incubation for 24 hours and then observing the ability to dissolve tricalcium phosphate marked with widest clear zone (Halozone) on media Pikovskaya plate. The formula used to measure the width of clear zone and the ratio of clear zones.

The width of the clear zone = diameter of clear zones - the diameter of the colony

3.3.1.5 Siderophore production

Microbial strains were checked for siderophore producing ability by universal CAS assay. The CAS reagent was prepared as per Schwyn and Neilands 1987. Briefly, 121 mg CAS was dissolved in 100 ml distilled water and 20 ml of 10 mM ferric chloride ($FeCl_3.6H_2O$) solution prepared in 10 mM HCl. This solution was added to 20 ml hexadecyl trimethyl ammonium brome (HDTMA) solution under stirring. HDTMA solution was prepared by mixing 729 mg HDTMA in 400 ml distilled water. The CAS – HDTMA solution was sterilized before further use. This assay was performed according to modified method given by Hu and Hu 2011. CAS agar plates were prepared by mixing 100 ml CAS reagent in 900 ml sterilized nutrient agar medium for bacteria and potato dextrose agar medium for fungal strains. The bacterial and fungal strains were spot inoculated on each plate. The plates were incubated at growth temperature (28 ± 2ºC) for 7 days in dark and observed for the formation of orange zone around the microbial colonies (Louden et al., 2011). An uninoculated plate was taken as control.

3.3.1.6 HCN production

All the isolates were screened for the production of HCN by adopting the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine/liter and bacteria and fungi were streaked on modified agar plate. A whatman filter number 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed in the top of the plate. Plates were sealed with parafilm & incubated at 28±2°C for 4 days. Development of orange to red color indicates HCN production.

3.3.1.7 Zinc and Calcium Solubilization

To check the Zn solubilization ability of selected strains, microbes were subjected to grow on Bunt & Rovira media containing 0.1% insoluble zinc compound (ZnO) as described by (Bunt and Rovira, 1955). Experiments were done in triplicate. Cultures were spot inoculated on the media using a sterile loop full of bacterial culture and a disc of fungal culture. The Petri plates were incubated at 28±1°C for 5 days in dark to observe clear halozone formation around the colonies. The halo diameters of colonies were measured. Zinc solublization area was calculated according to Saravanan *et al*. (2003).

$$\textbf{Area} = \pi r^2$$

3.3.2 Screening of isolates for abiotic stress

3.2.2.1 Drought tolerance

To check the growth potential under drought stress, the isolated microbial strains were grown on nutrient and potato dextrose broth medium containing 5-30% Polyethylene glycol (PEG, 6000), a dehydrating agent following Michel and Kaufmann, 1973.

3.2.2.2 Salinity tolerance

The bacterial strains were tested for salinity stress upto 15% while fungal strains were tested upto 25% salinity stress following Bharti et al., 2013. The microbial strains were grown on freshly prepared nutrient and potato dextrose agar plate with added NaCl salt at 5,10,15, 20 and 25 % levels and incubated 28-32ºC for 48 h. The microbial growth was showing positive result against salt stress. A non-salt added agar plate was used as a control (Bharti et al., 2014)

3.2.2.3 Heat stress

The heat stress tolerance of bacterial and fungal strains was determined by maintaining the microbial cultures at various temperatures i.e., 4°C, 10°C, 28°C and 45°C for up to 48 h. The colonies showing visible growth of varied magnitude measuring the colony size. The same was also confirmed by colony forming unit (cfu) count in the bacterial cultures.

3.3.2.4 Pesticide tolerance

The microbes were also tested for tolerance to organophosphate pesticide (dichlorvos). The different concentration of pesticide i.e., 100 mgl^{-1} to 1000 mgl^{-1} were supplemented in the respective media of bacteria and fungi. The pure strains of bacteria and fungi were cultured on pesticide containing media and incubated for 48 h. Based on the microbial colony size the pesticide tolerance limit of microbes was estimated.

3.3.3 Gram Staining

The bacterial cultures were gram stained by the following the Gram's method. Smeared log phase cultures of isolated bacterial strains were air dried and heat glued on glass slides. Then the smear was treated with few drops of crystal violet and left for 1 minute before being rinsed with distilled water (DW) and few drops of iodine solution added and left for 30 seconds. The smear with iodine solution was washed with DW again, then washed with 95 percent ethyl alcohol and rinsed with DW again. After washing, the smear was inundated for 1 minute with safranin and then washed with DW. Finally, the smear was air dried before being examined under a light microscope at 100x (oil immersion lens) to determine the form and gramme response.

3.4 Molecular identification of microbial isolates

Genomic DNA was isolated from the bacterial and fungal cultures by using genomic

DNA isolation kit (Sigma, India). Its quality was evaluated on 1.0% Agarose Gel. Fragments of 16S rDNA and Fragment of ITS gene were amplified for sequencing of bacterial and fungal isolates. The PCR amplicon was purified by column purification to remove contaminants. The DNA sequencing reaction of PCR amplicon were carried out with 8F and 1492R primers for bacteria and with ITS primer for fungus using BDT v3.1 Cycle sequencing kit on ABI 3500xl Genetic Analyzer. The 16S rDNA sequence and ITS sequence were used to carry out BLAST with the database of NCBI genebank database. Based on the maximum identity score first ten sequences were selected and aligned using multiple alignment software programs.

3.5 Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. This analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pair wise deletion option). There were a total of 1573 positions in the final dataset. Evolutionary analysis was conducted in MEGA X 10.0.5 (Kumar et al., 2018).

3.6 Selection of PGPMs and their compatibility test

On the basis of higher production of plant growth promotion activity and highly stress tolerance 9 microbial strains i.e., *Bacillus pseudomycoides* RS6B, *Bacillus firmus* RS7B, *B. subtilis* RS8B, *Staphylococcus hominis* RS9B, *Alcaligens faecalis* RS10B, *Aspergillus luchuensis* RS6F, *Epicoccum sorghinum* RS7F, Aspergillus *tamarii* RS8F and *Penicillium polonicum* RS9F were selected for further study. All the selected microbial strains were tested for their compatibility with each other in all possible combinations. The compatibility with each other were tested by the formation of inhibition zone or overlapping growth between the paired cultures. The compatibility between bacterial strains and between fungal strains were tested on nutrient agar (NA) and potato dextrose agar (PDA) medium plate by spot inoculation and disc placement (James and Mathew, 2017) respectively. While compatibility between Bacteria - Fungi were tested on modified agar media containing nutrient agar, potato dextrose agar (1:1) and beef extract (0.5%) at 30±2ºC and incubated for 48-72 h.

3.7 Selection and preparation of planting material

Two medicinal viz; *Abrus precatorius* L. (Indian licorice) and *Stevia rebaudiana* Bertoni (Mithi patti) and one commercial vegetable crop viz; *Capsicum annum* L. (Chilli) were selected for this experimental set up. Indian licorice and stevia are important commercial perennial crop for cosmetic, pharmaceutical and other industry due to presence of several group of secondary metabolites like alkaloids, flavonoids triterpenoids, phenolic compounds, amino acids etc. (Karwasara et al., 2010). These crops can promote better root development in sandy loam soils (Hossain et al., 2017). The seeds of Indian licorice (*A. precatorius L)* were purchased from Mahadeva Enterprises, Delhi, India with a brand name herbveda. The hard seed coat was broken by soaking seeds in sulphuric acid (99.9%) for 30-34 min, rinsed thrice with distilled water, and sowed in the nursery at room temperature for germination. One-month- old seedlings were used for transplantation in pots. The plantlets of stevia were purchased from CIMAP (Central institute of medicinal and aromatic plant), CSIR, India and Chilli from plant nursery of local market, Bangla Bazaar, Lucknow, UP, India.

3.8 Seed germination assay

On the basis of available literature *Staphylo hominis* RS9B, *Alca faecalis* RS10B, *Ep. sorghinum* RS7F and *Pen. polonicum* RS9F are considered as pathogens so only *B. pseudomycoides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F and *A. tamarrii* RS8F as individual and in their all-possible compatible combinations were assessed for their effectivity on seed germination and seedling growth. The chilli seeds were used for the study and purchased from S. D. agrochemicals, Ayodhya. The seeds were surface sterilized with 0.1% $HgCl_2$ solution for 3 min and rinsed 5 times with distilled water. Then 6 decontaminated seeds were transferred into the germination tray filled with sterilized cowdung (5G). Total 15 (T_1 – T_{15}) treatments were prepared using 4 microbes and their compatible combinations (Figure 3.2). The freshly prepared cultures were grown in their respective broth medium (Bacteria - NB, Fungi - PDB and consortia - NA+PBD (1:1) + 0.5% Beef extract) for 48 h at 28-32ºC temp. The study was conducted in replicates (n = 3) and each set was treated with 1 ml of broth medium of respective culture maintaining cell population 10^8 with the help of broth medium. The nontreated seeds were used as control (C). After 15 of germination the plantlets were transferred to plastic pots having 1 kg soil mixed with cowdung to assess efficacy of treatments on plant growth (1 month).

3.9 Formulation of bioinoculants

On the basis of findings from seed germination assay and seedlings growth, T_1 (*B. pseudomycoides* RS6B), T_2 (*B. firmus* RS7B), T_3 (*A. luchuensis* RS6F), T_4 (*A. tamarrii* RS8F) and T_{15} (consortium of all 4) were selected for further pot and field study. For the preparation of bioformulation, the freshly grown cultures individually as well as in

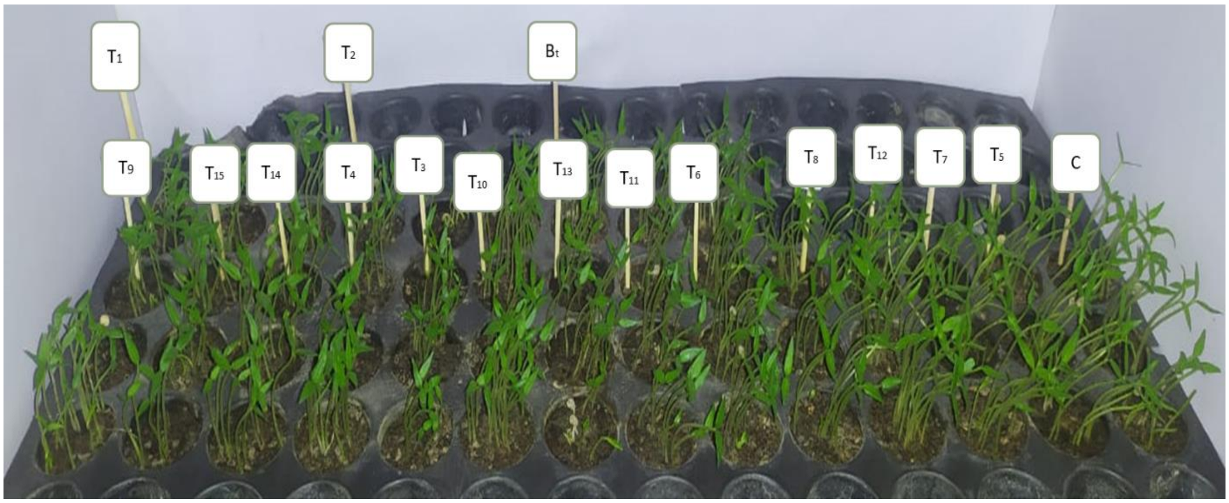


Figure – 3.2) In vitro seed germination assay

consortium were centrifuged at 5000 rpm for 10 min at 4ºC. The filtrate was separated and 40 mL of each culture was mixed with 100 g of organic material (90 g cow dung + 10 g jaggery) following the method given by Vidhyasekaran and Muthuamilan (1995). Cowdung and jaggery were grounded, air-dried and double sterilized in an autoclave (121ºC, 15 PSI for 20 min) before mixing and curing. The different inoculants placed within the cow dung and jaggery were kept at room temperature with 15% moisture content in sterilized polybags for 15 days to assess the effect of this organic material on the multiplication of microbial colony (figure 3.3). The microbial density in the organic material was monitored using colony forming units (cfu) and fungal spores count (Boukouvalas et al., 2018).

3.10 Pot experiment for selection of a bioformulation for field trial

3.10.1 Experimental design

A. precatorius plants were grown in pots with the application of different microbial inoculants in the greenhouse of the Environmental Science Research Station, Babasaheb Bhimrao Ambedkar University, Lucknow, India between July to October 2018. The soil was collected from non-agricultural land, sterilized at 121ºC, 15 PSI for 1 h. After sterilization 8 kg of soil was filled in each pot having a diameter of 30 cm and depth of 10 cm. The soil used in the pot was sandy-loam in nature with pH 8.1, EC 0.53 dS/ m^{-1}, and 1.36 g/cc bulk density. Subsequently, the seedlings of *A. precatorius* were surface sterilized with 0.1% $HgCl_2$ solution for 3 min and rinsed 5 times with distilled water. The decontaminated seedlings were transplanted into the pots and the bioformulations were added to the rhizospheric soil. The microbial density was maintained at 10^8 counts/g of soil following the modified method of Prakash et al. (2020). Approximately 50 G of powdered products of each bioinoculant were added to each pot. The bioinoculants were added twice into the soil (at the time of transplantation and after one month of transplantation of seedlings). The same amount of water was used weekly for irrigation in all the sets.

3.10.2 Measurements of plant growth and Glycyrrhizic acid content

The crops were harvested after four months of transplantation and their roots and shoots length were measured using meter scale (cm). Freshly harvested plants were washed by running tap water to remove soil and dust particles then roots, stems and leaves were separated and their fresh weight was taken (g). The roots, shoots, and leaves isolated from the plants after harvesting were made in small pieces of 5 mm, dried separately in the hot air oven (temp-70º C, 72 h) and taken dry weight. Glycyrrhizic acid was extracted by dipping method using water as solvent (Tian et al., 2009). The dried plant parts were crushed into a fine powder and 1.0 g powder of each sample was dissolved in 50 mL of water and kept for 90 min with continuous shaking at room temperature. After 3 h, samples were tickled through Whatman filter paper no. 42. The filtrate obtained was re-filtered through 0.22 Millipore membrane filters for determination of GA content by High-performance liquid chromatography (HPLC) with M 930 solvent delivery pump (Young Lin Co. Korea), an UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea), an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea), and Reodyne injection valve with 25 µL sample loops. The glycyrrhizic acid standard (99.9 %, Sigma Aldrich) was used as reference material. The sample running time was 15 min with a 1.0 mL/min flow rate and UV wavelengths were set at 252 nm. All the solvents were HPLC grade and filtered by Disposable Syringe Filter Unit (0.2 µm) for HPLC analysis. Glycyrrhizic acid was analyzed by a column (C18, 5µm, 150×4.6 mm, RStech Corporation, Korea) with a mobile phase consisting of methanol-water (70:30, v/v, containing 1% acetic acid). These results were confirmed by comparing peaks of chromatograph obtained through HPLC analysis of standard GA (sigma, 99.9%).

3.11 Field experiment

3.11.1 Site Selection

A slightly alkaline soil (pH - 8.1, Electrical conductivity - 0.95 ds m^{-1}, Bulk density - 1.52 g cc^{-1}, Total organic carbon - 0.7 %, Available nitrogen - 98 kg ha^{-1}, Sand, silt and clay ratio - 66:18:15) of university premises (26º46’5” N, 80 º 55’39” E) of Babasaheb Bhimrao

Ambedkar university was selected for assessment of effect of beneficial soil inoculants (PGPMs) and crop diversity levels of Indian licorice, stevia and chilli. Category of alkaline soil comes under these criteria (pH 7.8-8.5, ESP 19 percent and EC- 1.06 ds m^{-1}) have been reviewed as marginal soil (Singh et al., 2012). The soil in these pH ranges and little organic carbons are not appropriate for traditional cropping so it can be considered as marginal soil.

3.11.2 Experimental methodology

The experiment was arranged in randomized completely bock design (RCBD) comprising of seven plant groupings with three replications (Figure 3.4). The boundary of each plot was blocked by 20-25 cm thick soil cover to prevent surface run off of microbial treatment during the rainy season and a gap of 1 ft was maintained between the two blocks. Three crop species i.e., Indian licorice (L), Stevia (S) and Chilli (C) were transplanted in all probable groupings at one (L, S and C), two (LC, LS, SC) and three (LSC) Species diversity levels on selected field. Total 7 crop groupings with microbial treatment ($PGPMs^{+}$) and without microbial treatment ($PGPMs^{-}$) were used. Thus, the experimental design was constructed with two key factors (Species diversity levels and PGPMs consortium). Each experimental plots were treated with 1.5 kg of cattle dung (compost) before plantation (Mahimairaja et al., 2008).

One month old seedlings were rinsed with tap water and roots were surface sterilized by 0.1 % hypochlorite solution for 3 min and rinsed 5 times with distilled water (Maiyapan et al., 2010). Then sterilized plants in respective groupings were transplanted in to 3 m^2 (1.5m x 2m) area each in the end of January 2019. Indian licorice was transplanted first with amendments to acclimatize the soil for support of other crops before 20 days of experimental set up. In this way 6 plants were transplanted in each plot at the inter – spacing of 0.70 meters for all groupings and in case of death of plantlets, plants were replaced by new plant to maintain availability for further sampling. Total 42 plots (7 groupings X 2 treatments X 3 replications) were used in experiment. Light irrigations were given after transplanting to facilitate the establishment of seedlings. Hand weeding was done after one and two months of transplantation and hoeing was done after each weeding. The experimental plots were maintained properly and kept free of weeds. Approximately 50 G of microbial treatment (10^8 cell density) was applied trice in cycle; after transplantation, 2nd and 3rd month of transplantation in each plot. Water irrigations were maintained as per crops requirement. Harvesting of stevia and chilli was done in month of May (late autumn) and December (late spring) for a year while Indian licorice was harvested once in a year (December).

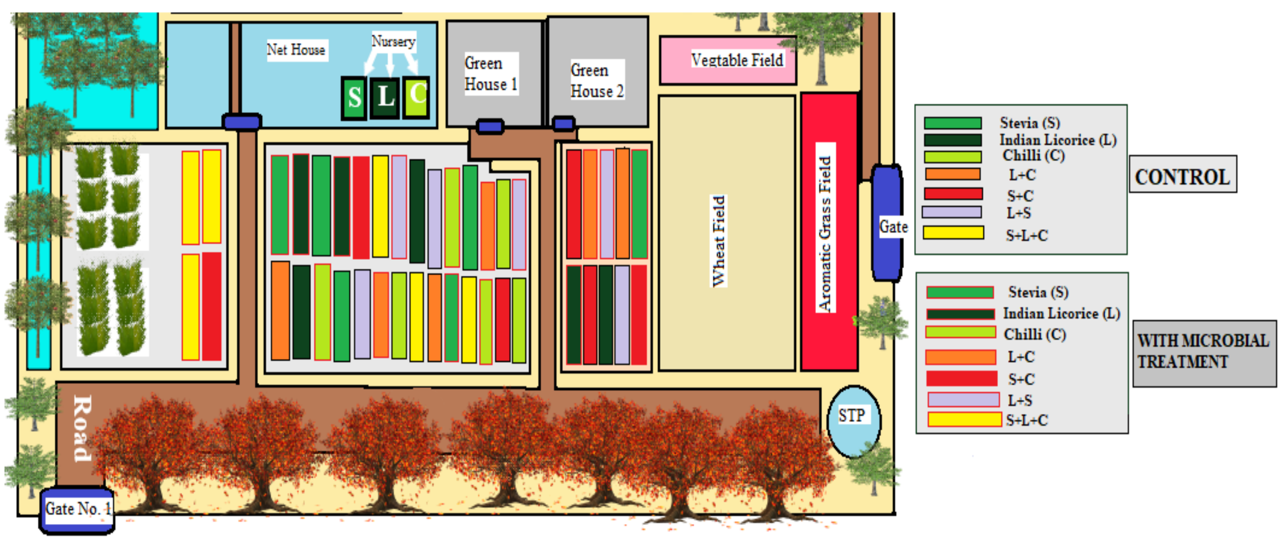


Figure- 3.3 Field layout of experiment. *Blocks with black boundary is showing plant combinations without microbial treatment (control) and red boundary representing plant combinations with microbial treatment*

3.11.3 Estimation of Plant growth, productivity and biomass generation

The plants grown in each plot were harvested after a complete cycle by a simple digging method in replicas of three. The plants (root and shoot) height was measured by using a meter scale (cm) just after harvesting. Fresh plants were washed with tap water to remove soil particles then their fresh weight was taken (G) afterward plant was kept in a hot air oven (70°C) to get its constant dry weight. The average yield of each crop was calculated by the weight of the quality product of that plant i.e., stevia leaf dry weight, Indian licorice root dry weight and green chilli fresh fruit weight, generated in a year. The dry weight of stevia leaf and licorice root were subtracted from total plant dry weight of plant and green chilli fruit was plucked from the plant before its harvesting. In the case of stevia and chilli, total yield in a year is the sum of yield calculated in two seasons. On the basis of number of plants in each plot we calculated a minimum of 20000 plants can be cultivated in a hectare of land. Therefore, the average yield was multiplied by the total number of plants in a hectare of land to get per hectare yield in a year.

The above ground biomass production was calculated by the amount of dry weight generated in two years by all possible crop groupings. The dry weight of plants in each plot was multiplied by 20000 to get per hectare biomass generation value. Stevia and chilli were harvested twice in a year (May and December) and Indian licorice were harvested once in once years (December). So, in case of stevia and chilli dry biomass generated in two year was the sum of biomass generated in four harvesting and in case of Indian licorice sum of two harvesting.

3.11.3.1 Intercropping Indices for designed crop system

3.11.3.1.1 Land Equivalent Ratio (LER)

Land equivalent ratio is highly effective and widely used intercropping index to compare intercropping systems where various crop species are growing on the same unit area (Yayeh et al., 2014b). It explains the effectiveness of intercropping in comparison to mono cropping for using the same environmental resources. Its values were calculated using the formula given by Takim (2012).

$$LER = \frac{Ysc}{Yss} + \frac{Ycs}{Ycc} + \frac{Yscl}{Ylll}$$

Where, Ysc = Intercrop yield of crop ‘s’ Ycs = Intercrop yield of crop ‘c’ Yss = Pure stand crop yield of ‘s’ Ycc = Pure stand crop yield of ‘c’, Yscl = Intercrop yield of crop “l”, Ylll= Pure stand crop yield of “l”.

3.11.3.1.2 Crop Equivalent yield Value (CEV)

Crop equivalent yield refers to required relative land for the sole crop to produce the equal yield produced under mixed or intercropping with the same management level. The value of crop equivalent yield of anyone is the yields of different intercrops based on the price of the sole crop, which can be calculated by following Mishra et al., 2018.

$$CEV = \frac{Yield\ of\ intercrop\ (kg\ /ha) X\ Price\ of\ intercrop\ (INR/kg)}{Price\ of\ sole\ crop\ (INR\ /kg)}$$

3.11.3.1.3 Monetary Advantage Index (MAI)

Cost benefit ratio is the most important part of any recommended cropping system because growers are highly concerned about the monetary return of the system. The economic returns in terms of monetary value (MAI) of the different intercropping systems were calculated by equation 2 proposed by Mahapatra, (2011).

$$MAI = (Psc + Pcs + Pscl) \times \frac{(LER-1)}{LER}$$

Where, Psc = Ps ×Ysc; Pcs =Pc ×Ycs; Ps = Price of species 's' and Pc = Price of species 'c'; Pscl = Pl* Yscl, Pl= Price of crop "l". The cropping system with higher MAI value is more profitable and recommendable (Ghosh, 2004).

3.11.4 Assessment of Microbial Dependency (MD)

Microbial dependency was estimated by the modified formula proposed by Menge et al., 1978.

$$MD = \frac{Plant\ growth\ with\ Mt - Plant\ growth\ without\ Mt}{Plant\ growth\ with\ Mt} * 100$$

Where plant growth is the dry weight of plant at different intercropping levels, Mt is microbial treatment.

3.11.5 Estimation of above ground carbon sequestration and net profit income

The C-sequestration in plant biomass was calculated by formula given by Schlesinger and Andrews (2000). To calculate average income generated from established plant groupings, the total dry weight of quality product of each crop was multiplied by their respective market price conquer at ending of experiment and then cost generated by each plant was multiplied by total number of possible plants in a hectare land to obtain total cost generated for per hectare area in two years. Bio economics of system was analyzed by calculating the cost of cultivation, gross and net returns per hectare in two years (Net profit income = total generated cost – capital cost). The capital cost includes cost of plant material, irrigation and biofertilizer.

3.12 Soil sampling and analysis

For pot study the samples were collected from each earthen pot at the depth of 0-10 cm before the plantation and after four-months of the transplantation of seedlings while for field study the soil samples of each plot were sampled from 0-15 cm depth at the time span of 0 month (prior to plantation), 4, 8, 12, 16, 20 and 24 months. These time spans were selected on the basis of plants growth. At the 4 month of transplantation plants grow faster therefore knowing the changes in soil properties along with plant growth was the aim of time selection. Three random samples were collected from each plot and mixed to make a composite. The samples were mixed thoroughly and sieved to pass a 2 mm screen to get homogenized sample. For physicochemical soil analysis samples were dried in hot air oven (Temp – 65ºC, 48 h) and for microbial assay the fresh samples were stored at -20ºC just after sampling.

The soil analysis for pot and field study was done by following standard protocols.

3.12.1 Physicochemical analysis

3.12.1.1 pH and EC

1.0 g of dried soil was dissolved in 10 ml of deionized water (1:10) to estimate pH and Electrical conductivity (EC) following glass electrode method.

3.12.1.2 Soil texture

The soil texture was determined by using hydrometer, followed by Bouyoucous, G.J. (1927). 50 g of soil was taken into 500 ml flask and treated with H_2O_2. 200 ml of distilled water and 100 ml of sodium hexametaphosphate (5%) was added to the soil, mix well and the suspension was kept overnight. After 24 h the suspension was transfer into 1 L. measuring cylinder and maintained the volume upto 1 L, shaked vigorously for a min, hydrometer was placed safely and reading was recorded just after and 2 h later to calculate the % sand, % silt and % clay value.

The percentage value to sand, silt and clay was putted into soil textural triangle to determine to soil texture (Figure 3.5).

% Silt + Clay = Vol of 1st reading X 2 (A)

% Clay = Vol of 2nd reading X 2 (B)

% Silt = (A-B)

% Sand = 100 – (A)

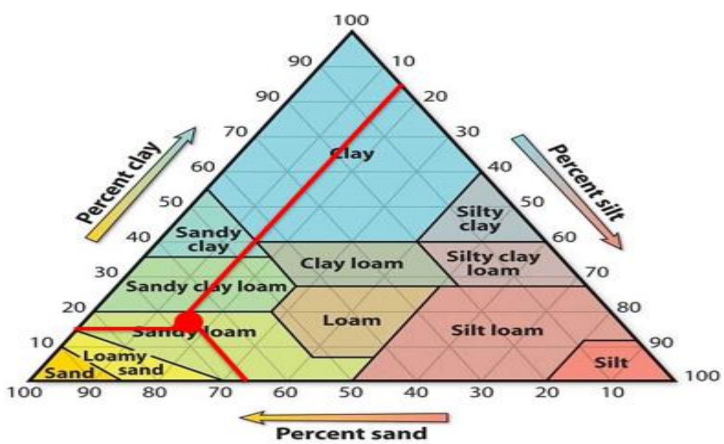


Figure – 3.4 Soil texture triangle

3.12.1.3 Bulk Density

The bulk density was measured by placing the volume of water, empty and soil filled weight of pycnometer into the formula proposed by Blake, G.R. (1965).

Bulk density (g/cm^3) = mass of dry soil / total volume of soil

3.12.1.4 Total Organic Carbon

The determination of soil organic carbon is based on the Walkley-Black (1937) chromic acid wet oxidation method. Oxidizable matter in the soil is oxidized by 1 N $K_2Cr_2O_7$ solution. The reaction is assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume (10 ml) of the dichromate. The remaining dichromate is titrated with 0.4 N ferrous ammonium sulphate in the presence of ferroin indicator. The titre is inversely related to the amount of C present in the soil sample. The percentage carbon is determined from the following:

$$\text{Organic carbon (\%)} = \frac{\left[0.003 \text{ X N X } 10 \text{ ml X } \left(1-\frac{T}{S}\right) \text{X } 100\right]}{Volume\ of\ soil\ (G)}$$

N = Normality of $K_2Cr_2O_7$ solution, T = Vol. of titrant for sample, S = Vol. of titrant for blank

3.12.1.5 Available Nitrogen

The determination of Nitrogen availability (N_{avl}) in soil involves three successive phases i.e., digestion of organic material, distillation and volumetric analysis of ammonia formed during the digestion process. The determination of nitrogen was done by Kjeldahl method of Bermner, J. (1960). The digestion of 50 g of soil was carried out by 30 ml conc. H_2SO_4 in the presence of 1 g $CuSO_4$. H_2O as a catalyst and 10 g K_2SO_4. The liberated ammonia was distilled by excess 40% NaOH and evolved ammonia was absorbed in 0.1N HCl. The excess of standard HCl was titrated against standard 0.1N NaOH using methyl red as an indicator. Acid-base titration determines the decrease in multi equivalence of acid, which offers a measure of the sample's N content. A change in colour from pink to yellow indicates the conclusion point.

$$\% \text{ of N in sample} = \frac{W \times 100}{5}$$

Where, W is weight of nitrogen in 5 g of sample.

Vol. of HCl consumed by liberated NH_3 present in blank (V_1) ml = A-B

Where, A is vol of HCl and B is Vol of NaOH used for blank. Meanwhile, the vol of HCl and NaOH used for sample is C and D. So, the vol consumed by liberated NH_3 present in sample (V_2) ml = C-D.

Now, the vol of HCl consumed for NH_3 liberated by sample only (V) ml = $V_2 - V_1$

1000 ml 1 N HCl = 1000 ml 1 N NH_3 = 17 g NH_3 = 14 g N
1 ml 1 N HCl = 1 ml 1 N NH_3 = 0.014 g N
1 ml 0.1 N HCl = 1 ml 0.1 N NH_3 = 0.0014 g N
So, the weight of nitrogen in 5 g of soil (W) = V X 0.0014 g N

3.12.1.6 Available Potassium

Flame photometric analysis was done to measure available potassium (K_{avl}) proposed by Panda and Patra (2018). Available potassium from dried air soil sample was extracted by using 0.5 M ammonium acetate solution after 30 minutes of continuous shaking. As a result, the potentially available K^+ ions were successfully relocated which was further determined in flame photometric analysis.

3.12.1.7 Available Phosphorus

The available phosphorus was extracted using Bray no. 1 extractant solution (Bray, 1945). The extracted phosphorus is measured calorimetrically based on the reaction with ammonium molybedate and colored developed as molybdenum blue. The absorbance of the compound was measured at 882 nm in a spectrophotometer and is directly proportional to the amount of phosphorus extracted from the soil. The Bray extracting solution was prepared by using ammonium fluoride and conc. HCl. 1 g of oven dried soil sample was treated with 7 ml of extracting solution, Shaked vigorously for a minute then centrifuged at 6000 rpm for 5 min. 0.5 ml of supernatant was added to 2 ml of reagent C, mixed and stand for 30 min to develop molybedate blue color. Reagent C was prepared by dissolving 0.53 g of L- ascorbic acid in to deionized water and added 70 ml of solution A (17.14 g ammonium molybdate in 200 ml D.W + 0.39 g potassium antimony tartrate in to 150 ml D.W + 200 ml conc. H_2SO_4 into 500 ml D.W). A set of reference standard was prepared from 2.50 mg/L phosphorus solution, the absorbance was recorded for both standard and sample at 882 nm wavelength. A graph was prepared from standard data to plot phosphorus conc. against absorbance and this graph was used to determine the phosphorus conc. in sample.

$$\text{Available phosphorus } (mg/kg) = \frac{\text{C X 14}}{\text{weight of dried soil sample}}$$

Where, C = Phosphorus concentration and 14 = Dilution factor

3.12.2 Enzymatic analysis

3.12.2.1 Dehydrogenase

Specific dyes that can identify electron flow, such as triphenyl tetrazolium chloride (TTC), are helpful indicators of electron transport system (ETS) activity. Dehydrogenases in the soil environment reduce a colourless, water soluble substrate (TTC) to produce an insoluble product with a red colour (triphenylformazan-TPF) which can be easily measured calorimetrically in the visible light range (485 nm). Dehydrogenase enzyme was quantified by the standard protocol of Pepper et al., (1995), with the addition of 1 ml of 3% (w/v) solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) and 2.5 ml distilled water in known amount of soil (6 g), obtained pink color density of 1, 3, 5 triphenyl formazan (TPF) was recorded at 485 nm wave length by spectrophotometer. The sample with added chemicals was incubated at 37°C for 24 h to develop pink color.

3.12.2.2 Alkaline Phosphatase

The alkaline phosphatase enzyme activity was measured according to published method of Tabatabai (1994). Modified universal buffer (MUB) stock solution was made by dissolving 12.1 g tris-hydrochloric aminomethane, 11.6 g maleic acid, 14.0 g citric acid, and 6.3 g boric acid in distilled water in 488 ml of 0.1 M NaOH and adjusting the final volume to 1,000 ml. MUB stock solution (200 ml) was diluted to 1,000 ml with distilled water after being adjusted to pH 11 with 0.1 M NaOH. In continuation MUB substrate solution was prepared by dissolving 0.928 g of p- nitrophenyl phosphate in to 100 ml of prepared MUB stock solution. 0.25 g of soil sample was incubated with 50 µl of toluene (Merck, grade), 1 ml of MUB stock solution and 250 µl MUB substrate solution for an h at 37ºC in a water bath. With the addition of 250 µl of 0.5 M $CaCl_2$ and 1 ml of 0.5 M NaOH, the process was stopped. The absorbance of filterate was measured at 440 nm wavelength with UV-VIS spectrophotometer. The amount of alkaline phosphatase was calculated as

$$Z\ (\mu mol/min) = \frac{\Delta C\ X\ V\ /1000}{\Delta t}$$

Where, ΔC = increase in PNP concentration (µmol), V = total volume of substrate solution (ml), Δt = time of incubation (min)

3.12.2.3 Microbial Biomass Carbon

The soil microbial biomass carbon (SMBC) was calculated by using fumigation extraction method given by Vance et al., (1987). The difference in extractable fractions between fumigated and unfumigated soil was used to determine microbial C, which was reported in mg kg–1 oven-dry soil. Approximately 25 g of soil was taken for fumigation and non-fumigation process each. Unfumigated control soils were extracted immediately with 140 ml of 0.5 M K_2SO_4 solution (about 1:4 w/v) and filtered through a Whatman no. 42 filter paper using an end-over-end shaker at 35 rev min^{-1}. Soil fumigation process was carried out in sieve boxes, base was covered with filter paper and 25 g of soil was spread uniformly to mix the soil with chloroform and to expose maximum surface area. After incubation for 24 h in dark at 25ºC, the chloroform was allowed to evaporate in fume hood at 25ºC for 30 min then soil was extracted as described above. 5 ml soil extracts were pipetted into 75-ml digestion tubes and treated with 5 ml 0.07 N $K_2Cr_2O_7$, 10 ml 98 percent H_2SO_4, and 5 ml 88 percent H_3PO_4. The samples were thoroughly combined and digested for 30 minutes at 150°C. After cooling, samples were titrated against 0.01 N Fe $(NH_4)_2(SO_4)_2$ $6H_2O$ solution using N – phenylanthranilic acid as an indicator.

3.12.2.4 Colony Forming Units

The CFU count was measured by serial dilution of 1 g of soil sample with sterilized distilled water upto level. 1 ml sample of 10^{-8} times diluted suspension was spread on NA (nutrient agar) plate and kept for incubation at 28ºC for 48 h. The obtained colonies were numbered by colony counter (Boukouvalas et al., 2018).

3.13 Soil Carbon Sequestration Potential (CSP)

The C- sequestration potential of each crop combination level was calculated by proposed equation of Manrique and Jones, 1991 i.e., CSP (Mg ha-1) = MSOC (final) – MSOC (control), where the MSOC is mass of soil organic carbon and is calculated by multiplication of soil organic carbon (SOC), bulk density (BD) and depth of soil (0-15cm).

3.14 Statistical Analysis

All experiments were replicated for three times with two independent determinants (n = 6). Statistical analysis was performed by using IBM SPSS statistics version 20.0. changes in soil properties, plant growth, biomass and yield without and with microbial application was tested for significance using one- way analysis of variance (One-way ANOVA) followed by the Duncan's test at significance level of 5% ($p<0.05$). The effect of Species diversity levels (Factor A), microbial consortium (Factor B) and their interaction (factor A X factor B) on soil properties, biomass production and economic gain in two year per hectare land were performed by two-way analysis of variance (two-way ANOVA). The magnitude of correlation among plant combinations and soil fertility indices with and without treatment were determined by Pearson's correlation coefficient and correlation heatmap was performed between microbial formulation and glycyrrhizic acid content using metaboanalyst version 4.0 online software. All the values are placed as arithmetic mean of replicates with their standard error and the level of significance was at $p<0.05$.

Results

Section A

Climate resilient plant growth promoting microorganism isolated from semi-arid plateau of Ralegarh Siddhi

Beneficial soil microorganisms derived from various ecological niches have been treated exogenously to boost plant output in agricultural fields. Due to rhizospheric pressures and variations in certain agro-climatic variables, pH, salinity, C/N ratio, and abiotic stresses connected to climate change and global warming, these exogenously applied microbial inoculants are often ineffective. Furthermore, pesticide residues and heavy metals in agroecosystems, temperature stresses, drought and flooding, and other factors influence the biological functions of these microbial inoculants (Backer et al., 2018). Though it is usually considered that the native microbes can be better inoculants due to their suitability and stability for the habitat, the significant loss in their natural population in changing agro climatic conditions, habitat fragmentation and contamination of soil (with toxic chemicals in the intensive agricultural agro-ecosystems) is a frequent phenomenon (Rashid et al., 2016). In light of these facts, we hypothesized that the niches from which we isolate microbes play an important role in microbial properties and their beneficial utility in sustainable agriculture. To verify this, we have isolated certain bacteria and fungi from stone and base soil taken from a semi-arid drought prone plateau of Ralegan Siddhi, Maharashtra, India.

4.1 Isolation, identification and characterization of microbial isolates

4.1.1 Microbial isolates

Total 10 microbial strains were isolated from stones and soil samples of Ralegarh siddhi, Maharashtra in the month of February, 2017 using sterile tool son kit. Among them 5 (2 bacteria and 3 fungi) microbes were isolated from soil sample while rest 5 (3 bacteria and 2 fungi) were from stone samples (Table 4.1).

Table 4.1 Microbial isolates isolated from stone and soil samples of semi-arid zone of Ralegan siddhi

Sample collected	Number of isolates	
	Bacterial strains	Fungal strains
Soil	2	3
Stone	3	2
Total	5	5

The microbes were isolated on culture plates having respective media i.e., Bacteria – NA plate and fungi- PDA plate

4.1.2 Morphological identification of microbial isolates

Bacterial and fungal strains were identified first on the basis of morphological characters i.e., colony appearance color of conidia and bacterial cells on culture media (Figure 4.1 and 4.2). The spores of RS6F and RS7F culture was strongly embedded on media in concentric rings while RS8F, RS9F and RS10F were recorded to have spores spreaded on full plates as powdery texture. The growth of RS6F and RS7F culture was recorded very slow over other fungal strains after 72 h of incubation. Among all isolated bacterial strains RS6B was found to have very different colony looks like white opaque hairy while others were as appears as normal bacterial colonies in different colors. Except RS9B, all the bacterial strains were recorded in rod shaped in microscopic study (Table 4.2). The gram staining was performed for all the bacterial strains following Gram's method explained in chapter -3. RS6B, RS7B and RS8B was showing positive test with purple color appearance while RS9B and RS10 showed negative test with red color cell wall.

4.1.3 Microbial identification at genetic level

4.1.3.1 Identification and phylogenetic analysis of the isolated microbes

In total 10 microbes (5 bacteria and 5 fungi) were isolated (Table 4.3). The species level confirmation of isolated microbial strains was carried out based on 16S r RNA and ITS4 region gene sequence analysis. The sequences were deposited in NCBI for access to the number of each strain. A nBLAST search and analysis were done for the 1446 bp 16S

Table- 4.2 Morphological features of microbial isolates after 24/72 hrs. of incubation

	Microbial isolates	Conidia	The reverse side of the colony	Radial growth
Fungi	*Aspergillus luchuensis* RS6F	Black spores arranged in concentric rings	Black, outer ring white	31mm
	Epicoccum sorghinum RS7F	White spores arranged in concentric rings	Centre black, outer ring broad white	28mm
	Aspergillus tamarii RS8F	Back spores grown over full plates	Greenish	45mm
	Penicillium Polonicum RS9F	Green spores arranged in different small rings	Greenish, outer ring white	45mm
	Aspergillus terreus RS10F	Pinkish spore spread on full plate	Centre dark pink color, outer light	45mm
		Gram staining	**Cell shape**	**Cell color**
Bacteria	*Bacillus pseudomycoides* RS6B	+	Rod	White opaque
	Bacillus Firmus RS7B	+	Rod	White
	Bacillus subtlilis RS8B	+	Rod	Off white
	Staphylococcus hominis RS9B	-	Spherical	Slightly yellowish
	Alcaligens faecalis RS10B	-	Rod	Slightly pinkish

+; positive, -; negative

rRNA nucleotide sequence for bacteria and ITS4 for fungus isolates, which revealed that RS6B was phylogenetically 87% similar to *Bacillus pseudomycoides* (NR_113991.1), RS7B 92% similar to *Bacillus firmus* (HQ397582.1), RS8B 84% similar to *Bacillus subtilis* (NR_112116.2), RS9B 94% similar to *Staphylococcus hominis* (NR_041323.1) and RS10B 90% similar to *Alcaligenes faecalis* (NR_113606.1). RS1F 99% similar to

Fusarium chlamydosporum (AY213655.1), RS6F 99% similar to *Aspergillus luchuensis* (MF185986.1), RS7F 99% similar to *Epicoccum sorghinum* (MH497563.1), RS8F 100% similar to *Aspergillus tamarii* (NG_069608.1), RS9F 100% similar to *Penicillium polonicum* (MF186068.1) and RS10F 92% similar to *Aspergillus terreus* (MH236153.1). The evolutionary relationship between isolated microbes can be seen in the phylogenetic tree (Figure 4.3).

Table 4.3. Identified microbial species

No. of isolates		Identified as	
		Genus	**Species**
Bacterial strains	3	*Bacillus*	*B. firmus* *B. pseudomycoides* *B. subtilis*
	1	*Staphlococcus*	*Staphylo. hominis*
	1	*Alcaligens*	*Ala. faecalis*
Fungal strains	3	*Aspergillus*	*A. luchuensis* *A. tamarii* *A. terreus*
	1	*Penicillium*	*Pen. polonicum*
	1	*Epicoccum*	*Ep. sorghinum*

4.1.4 Characterization of microbial isolates

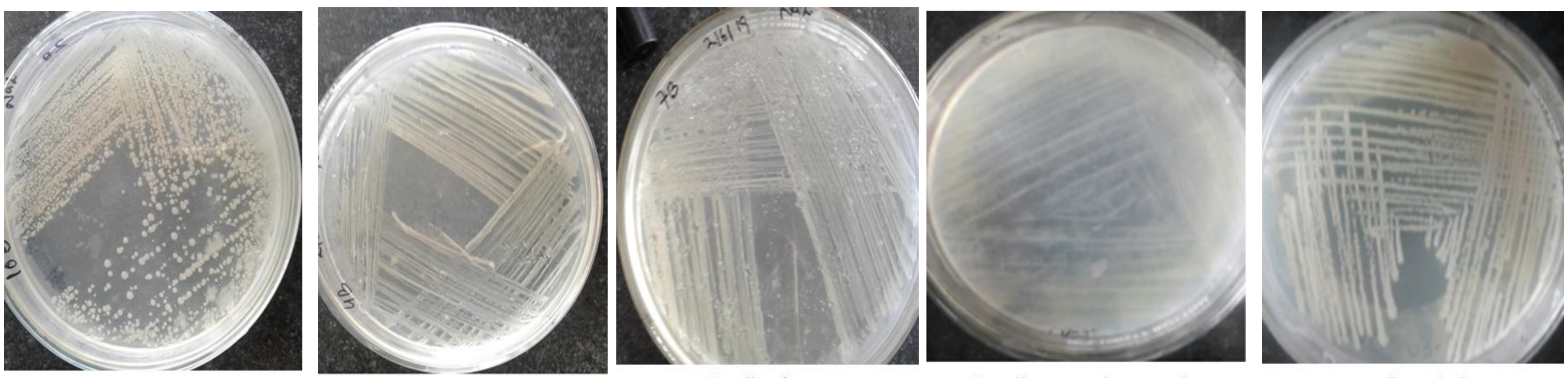
4.1.4.1 Plant growth promotion traits

4.1.4.1.1 Qualitative test

A total of 10 microbial strains were isolated from the stone and soil samples collected from the rocky site, out of which 5 isolates were bacteria (RS6B, RS7B, RS8B, RS9B and RS10B) and 5 were fungi (RS6F, RS7F, RS8F, RS9F and RS10F), initially differentiated based on their colony morphology. The isolates were characterized for their ability to produce IAA, growth on nitrogen free media, ammonia production, siderophore

Aspergillus tamarii *Penicillium polonicum* *Aspergillus luchuensis* *Epicoccum sorghinum* *Aspergillus terreus*

Figure-4.1) Different isolates of Fungi isolated from stones and soil samples of Ralegan Siddhi



Alcaligens faecalis *Staphylococcus hominis* *Bacillus firmus* *Bacillus pseudomycoides* *Bacillus subtilis*

Figure - 4.2) Different isolates of Bacteria isolated from stones and soil samples of Ralegan Siddhi

production, HCN production as well as phosphate, zinc and calcium solubilizing properties. All the bacterial and fungal strains isolated from the stone and soil samples were found to produce IAA in detectable amount. Siderophore production was found in *Pen. polonicum* RS9F and *A. luchuensis* RS6F whereas bacterial strains of *Staphylo. hominis* RS9B, *B. subtilis* RS8B and *B. pseudomycoides* RS6B were detected as phosphate solubilizers than *B. subtilis* RS8B (Table 4.4, Figure 4.4 and 4.5). All other bacterial strains either showed properties of growth on nitrogen free media or ammonia production. On the other hand, all the isolated fungal strains showed ammonia production, growth on nitrogen free media and phosphate solubilizing properties in addition to IAA production except *A. terreus* RS10F (Table- 4.4). The isolated strain of *A. luchuensis* RS6F, *Ep. sorghinum* RS7F, *A. tamarrii* RS8F and *Pen. polonicum* RS9F were observed as good Zn solubilizers (Figure 4.6) and *Ep. sorghinum* RS7F, *A. tamarrii* RS8F and *Paenchro. polonicum* RS9F was good calcium solubilizer (Figure 4.7). Among 10 microbial strains only *B. pseudomycoides* RS6B and *A. terreus* RS10F was found to show Zn tolerance and only *A. tamarrii* RS8F showed positive test for HCN production.

4.1.4.1.2 Quantitative test

The isolates that showed positive plant growth promotion activities were assayed for quantitative estimations of indole acetic acid and ammonium production. The inorganic mineral solubilization index (phosphate, calcium and zinc) was also calculated (Table 4.5). *B. pseudomycoides* RS6B showed highest ammonium production (3.62±0.01 µg ml^{-1}) and *Alc. faecalis* RS10B recorded for highest indole acetic acid production (11.06±0.04 µg ml^{-1}) over other bacterial strains. The fungal strains found significantly higher plant growth promoter as compare to bacterial strains. Isolate *Pen. polonicum* RS9F and *A. luchuensis*

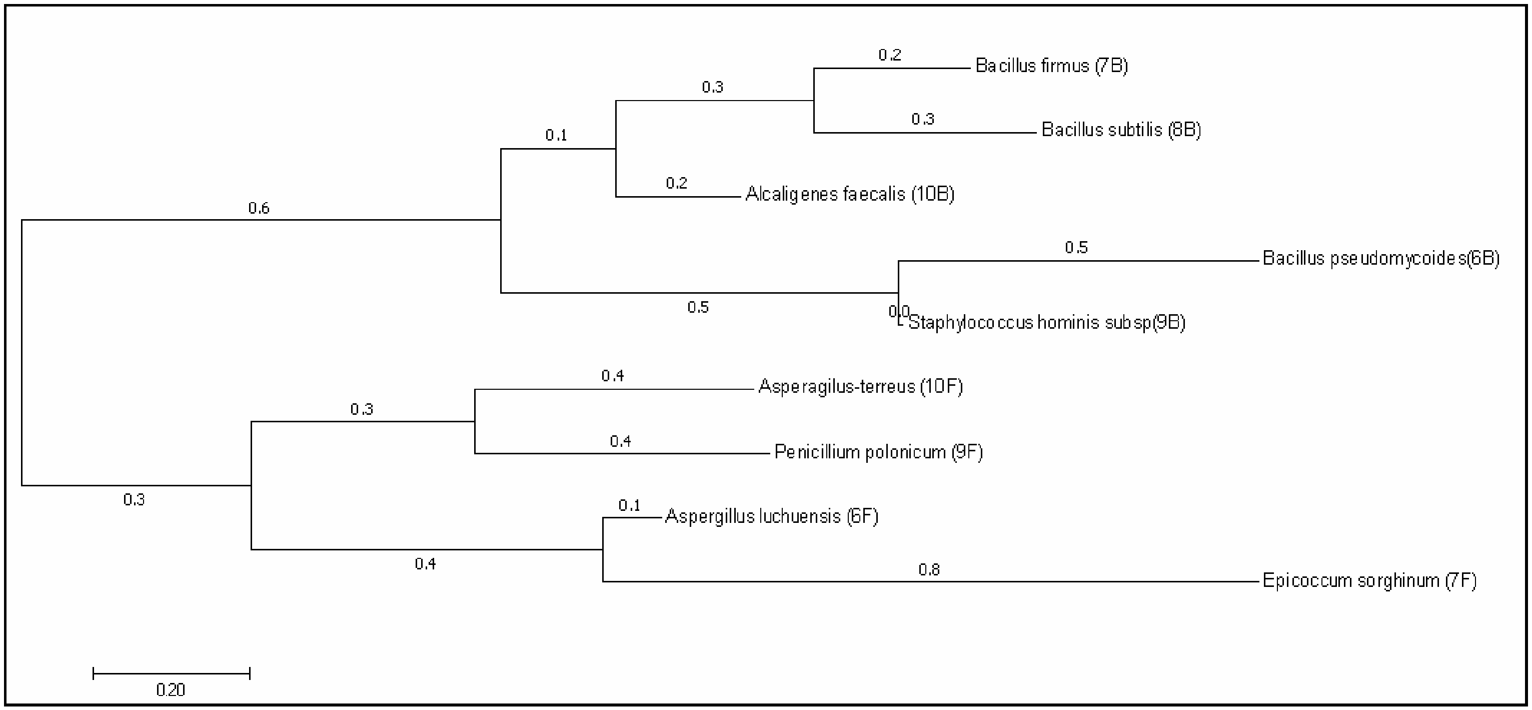


Figure-4.3) Evolutionary relationships between isolated microbes: *The phylogenetic tree was constructed by using neighborhood method.*

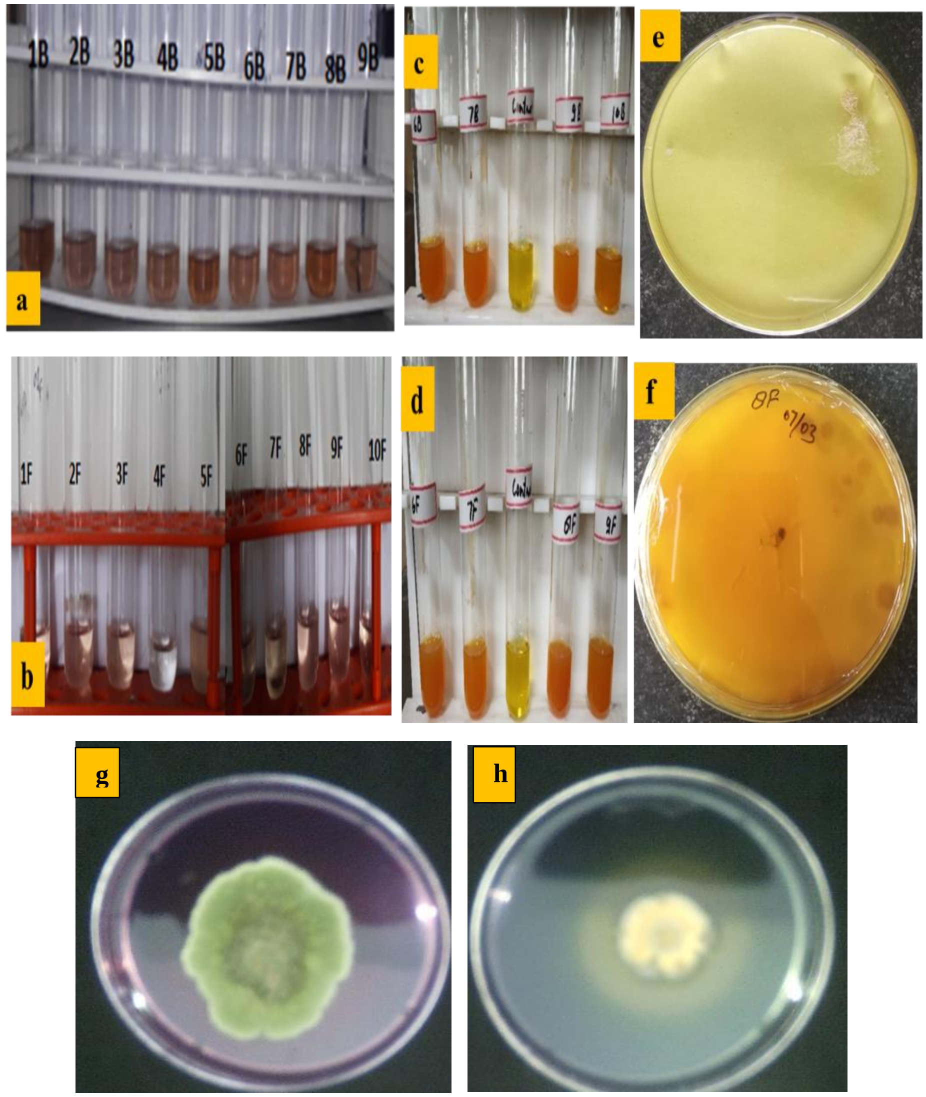


Figure – 4.4) PGP traits by microbial isolates. a-b) Indole acetic acid production by Bacteria and Fungi; c-d) Ammonia production by Bacteria and Fungi (centre: control; left and right: strong positive); e-f) HCN production (e: control; f: positive) f) by A. tamarrii RS8F**;** g-h) Siderophore production test (g: control, no color zone formation by A. tamarrii RS8F; h-clear orange zone around the colony is showing positive test by A. terreus RS10F)

Table – 4.4 Plant growth promoting attributes of bacterial and fungal isolates

	Sample code	Identified as	Plant Growth Promoting Activity							
			IAA production	Ammonia production	Nitrogen solubilization	Phosphate solubilization	Siderophore production	HCN production	Zinc tolerance/ solubilization	Calcium solubilization
Bacterial strains	RS6B	*Bacillus pseudomycoides*	+	+	-	+	-	-	+	-
	RS7B	*Bacillus firmus*	+	+	+	-	-	-	-	-
	RS8B	*Bacillus subtilis*	+	+	-	+	-	-	-	-
	RS9B	*Staphylococcus hominis*	+	+	+	+		-	-	-
	RS10B	*Alcaligens faecalis*	+	+	+	-	-	-	-	-
Fungal strains	RS6F	*Aspergillus luchuensis*	+	+	+	+	+	-	+	-
	RS7F	*Epicoccum sorghinum*	+	+	+	+		-	+	+
	RS8F	*Aspergillus tamarii*	+	+	-	+	-	+	+	+
	RS9F	*Penicillium polonicum*	+	+	+	+	+	-	+	+

- =Negative; + = positive

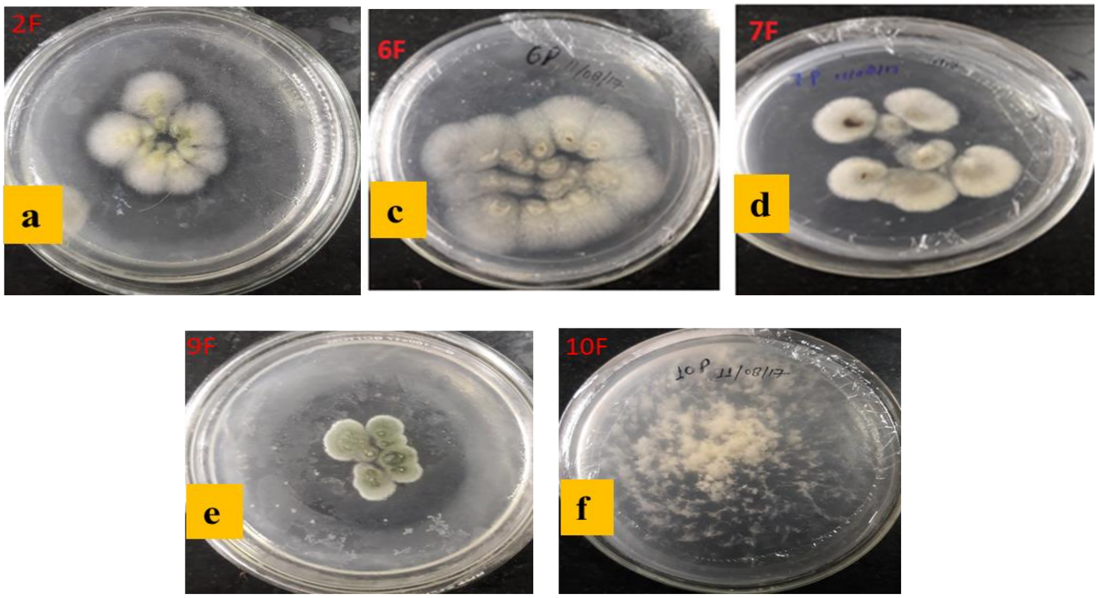


Figure – 4.5) Insoluble tricalcium phosphate [$Ca_3(PO_4)_2$] solublization by isolated fungal strains. a, c and e) clear halo zone showing phosphate solubilization by *A. tamarrii* RS2F, *A. luchuensis* RS6F and *Pen. polonicum* RS9F; d-f) showing non-phosphate solubilizing property of *Ep. sorghinum* RS7F and *A. terreus* RS10F

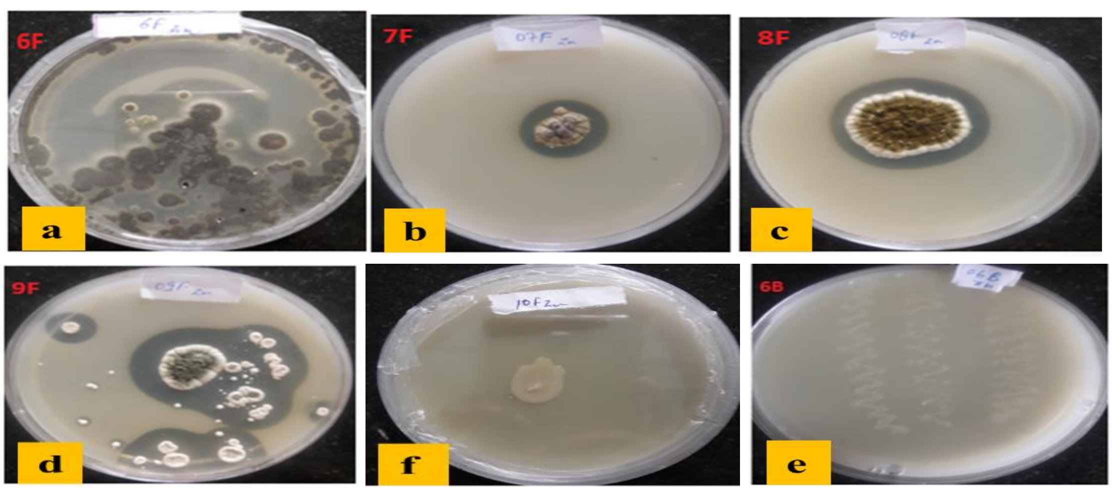


Figure – 4.6) Zinc solublization and tolerance by microbial isolates. a, b, c and d) clear halo zone showing mineral ($ZnCO_3$) solubilization by *A. luchuensis* RS6F, *Ep. sorghinum* RS7F, *A. luchuensis* RS8F and *Pen. polonicum* RS9F; e) showing Zinc tolerance by *B. pseudomycoides* RS6B; f) showing non-zinc solubilization property of *A. terreus* RS10F

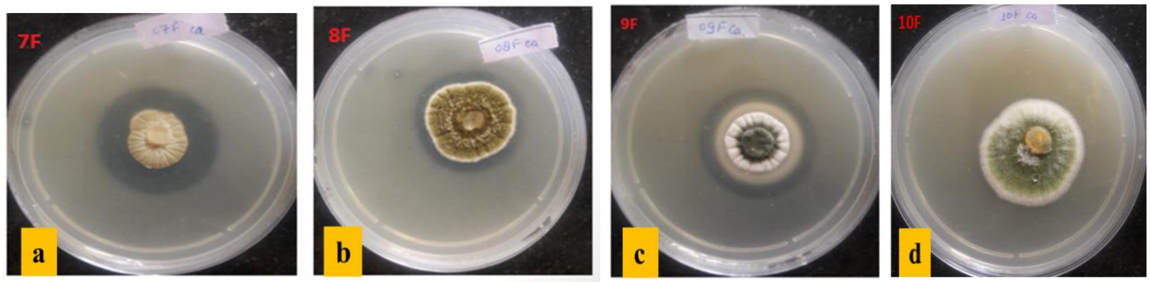


Figure – 4.7) Calcium solublization and tolerance by fungal isolates. a, b and c) clear halo zone showing mineral ($CaCO_3$) solubilization by *Ep. Sorghinum* RS7F, *A. tamarrii* RS8F and *Pen. polonicum* RS9F; d) showing non-zinc solubilization property of *Aspergillus* RS10F

RS6F was observed to produce highest ammonium (5.41±0.01 µg ml^{-1}) highest indole acetic acid (21.56±0.05 µg ml^{-1}) followed by *A. tamarrii* RS8F (5.33±0.01 µg ml^{-1}) as compare to other plant growth promoters. The maximum and significant Zn and Ca solubilization index values were recorded for *A. luchuensis* RS6F (3.07±0.12) and *Ep. sorghinum* RS7F (2.03±0.08) followed by *Pen. polonicum* RS9F (Zn - 2.34±0.14 and Ca - 1.47±0.05). *A. tamarrii* RS8F possesses highest phosphate solubilization index value (1.20±0.03) following *Pen. polonicum* RS9F (1.75±0.10) after 8 days of incubation.

4.1.4.2 Abiotic stress tolerance of microbial isolates

All the isolated strains were able to tolerate NaCl up to 5%, except *B. pseudomycoides* RS6B strain, however, *B. firmus* RS7B showed maximum salinity tolerance up to 25%. Most of the bacterial and fungal strains showed salt tolerance up to 15%. (Table 4.6). The water stress test was performed by adding polyethylene glycol (PEG) to NB and PDB growth media. All the fungal isolates and bacteria like *B. pseudomycoides* RS6B, *B. firmus* RS7B, and *Alc. faecalis* RS10B exhibited significant growth in 30 % PEG. The microbes were plated on respective NA or PDA plates and incubated at different temparature

Table – 4.5. Quantitative estimation of IAA, NH_3, solubilization index value for mineral salts (phosphate, zinc and Calcium)

	Isolates	NH_3 (µg ml^{-1})	IAA (µg ml^{-1})	Phosphate solubilization Index (PSI value)	Zinc solubilization Index (ZSI value)	Calcium solubilization Index (CSI value)
Bacterial	*B. pseudomycoides* RS6B	3.62 ± 0.01^{bc}	6.10 ± 0.06^{bc}	-	-	-
	B. firmus RS7B	3.57 ± 0.01^{bc}	6.34 ± 0.61^{bc}	-	-	-
	B. subtilis RS8B	1.95 ± 0.01^{de}	7.69 ± 0.01^{bc}	-	-	-
	Staphylo. hominis RS9B	3.52 ± 0.61^{bc}	7.24 ± 0.07^{bc}	-	-	-
	Alc. faecalis RS10B	3.58 ± 0.01^{bc}	11.06 ± 0.04^{ab}	-	-	-
Fungal	*A. luchuensis* RS6F	5.10 ± 0.01^{b}	21.56 ± 0.05^{a}	1.08 ± 0.04^{b}	3.07 ± 0.12^{a}	-
	Ep. sorghinum RS7F	4.55 ± 0.01^{ab}	11.95 ± 0.05^{ab}	-	1.55 ± 0.03^{c}	2.03 ± 0.08^{a}
	A. tamarrii RS8F	5.33 ± 0.01^{a}	8.28 ± 0.08^{bc}	1.20 ± 0.03^{b}	1.44 ± 0.14^{c}	1.30 ± 0.04^{b}
	Pen. polonicum RS9F	5.41 ± 0.01^{a}	7.83 ± 0.02^{bc}	1.75 ± 0.10^{a}	2.34 ± 0.14^{b}	1.47 ± 0.05^{b}
	A. terreus RS10F	2.74 ± 0.00^{cd}	11.08 ± 0.04^{ab}	-	-	-

to assess their temperature tolerance. *B. subtilis* RS8B, *Staphylo. hominis* RS9B and *Alc. faecalis* RS10B were able to grow at 28°C and 45°C. The fungal strains *Pen. polonicum* RS9F and *A. terreus* RS10F have shown growth at 12°C to 28°C, on the other hand, *A. luchuensis* RS6F, *Ep. sorghinum* RS7F and *Pen. polonicum* RS9F were able to grow at 28°C (Table 4.6). In general, all the fungal isolates were more tolerant to organophosphate pesticide i.e., dichlorvos (600-1000 mgl^{-1}) than the isolated bacterial strains except *Ep. sorghinum* RS7F. The bacterial strains, on the other hand, were able to tolerate up to 400 mgl^{-1} of pesticides except *Alc. faecalis* RS10B which was tolerant to the pesticide upto 500 $mgl^{-1.}$ The fungal strain *A. luchuensis* RS6F and *Pen. polonicum* RS9F have shown pesticide tolerance up to 1000 mgl^{-1}.

4.2 Compatibility of selected plant growth promoting microbes

All the selected plant growth promoters from microbial isolates were tested for compatibility test with each other. Total 9 isolates (5 bacteria; *B. pseudomycoides* RS6B, *B. firmus* RS7B, *B. subtilis* RS8B, *Staphylo. hominis* RS9B, *Alc. faecalis* RS10B and 4

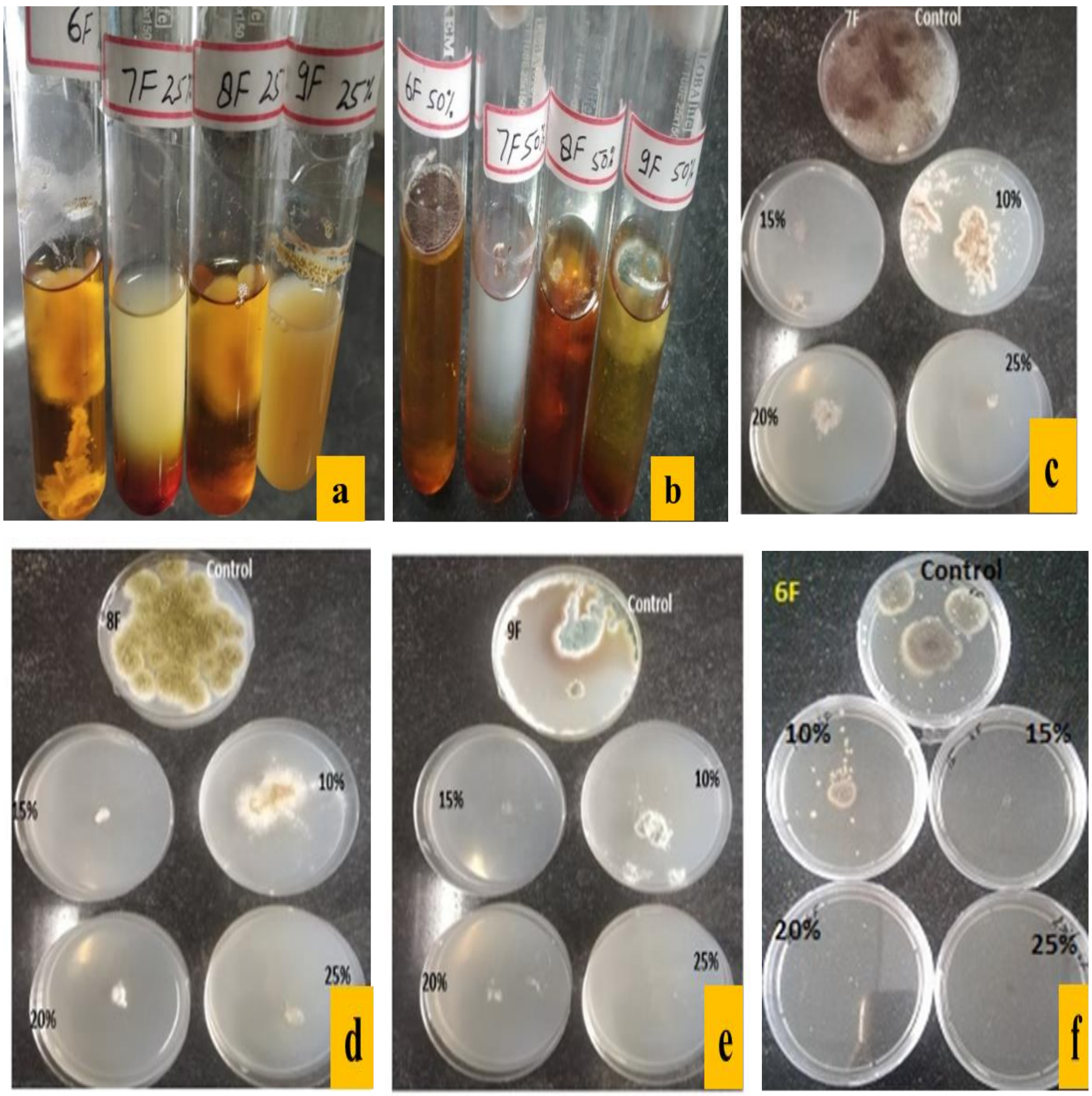


Figure – 4.9) Fungal growth against salinity and water stress. a-b) showing growth against 50% added PEG (Polyethylene glycol) in media by tolerance by *B. pseudomycoides* RS6B and *B. firmus* RS7B; c-d) upto 7.5% tolerance by *Staphylo. hominis* RS9B and *Alc. Faecalis* RS10B

Table – 4.6 Stress attributes of bacterial and fungal isolates

Bacterial isolates

Isolated microbes	**Microbes Identity**	**Salinity tolerance (% NaCl)**					**Drought tolerance (% PEG)**				**Temperature tolerance (°C)**			**Pesticide tolerance (organo phoaphate pesticide in ppm)**				
		2.5	**5**	**7.5**	**10**	**15**	**5**	**10**	**15**	**30**	**12**	**28**	**45**	**100**	**200**	**300**	**400**	**500**
RS6B	*Bacillus pseudomycoides*	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	-
RS7B	*Bacillus firmus*	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	-
RS8B	*Bacillus subtilis*	+	+	+	+		+	-	-	-	-	+	+	+	+	+	-	-
RS9B	*Staphylococcus hominis*	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-
RS10B	*Alcaligens faecalis*	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+

Fungal isolates

Isolated microbes	**Microbes Identity**	**Salinity tolerance (% NaCl)**				**Drought tolerance (% PEG)**			**Temperature tolerance (°C)**			**Pesticide tolerance (organo phoaphate pesticide in ppm)**					
		10	**15**	**20**	**25**	**25**	**30**	**50**	**12**	**28**	**45**	**500**	**600**	**700**	**800**	**900**	**1000**
RS6F	*Aspergillus luchuensis*	+	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+
RS7F	*Epicoccum sorghinum*	+	-	-	-	+	+	+	-	+	-	ND	ND	ND	ND	ND	ND
RS8F	*Aspergillus tamarii*	+	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+
RS9F	*Penicillium polonicum*	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
RS10F	*Aspergillus terreus*	+	+	-	-	+	+	-	+	+	-	+	+	+			

- =Negative; + = positive; PEG = Polyethylene glycol

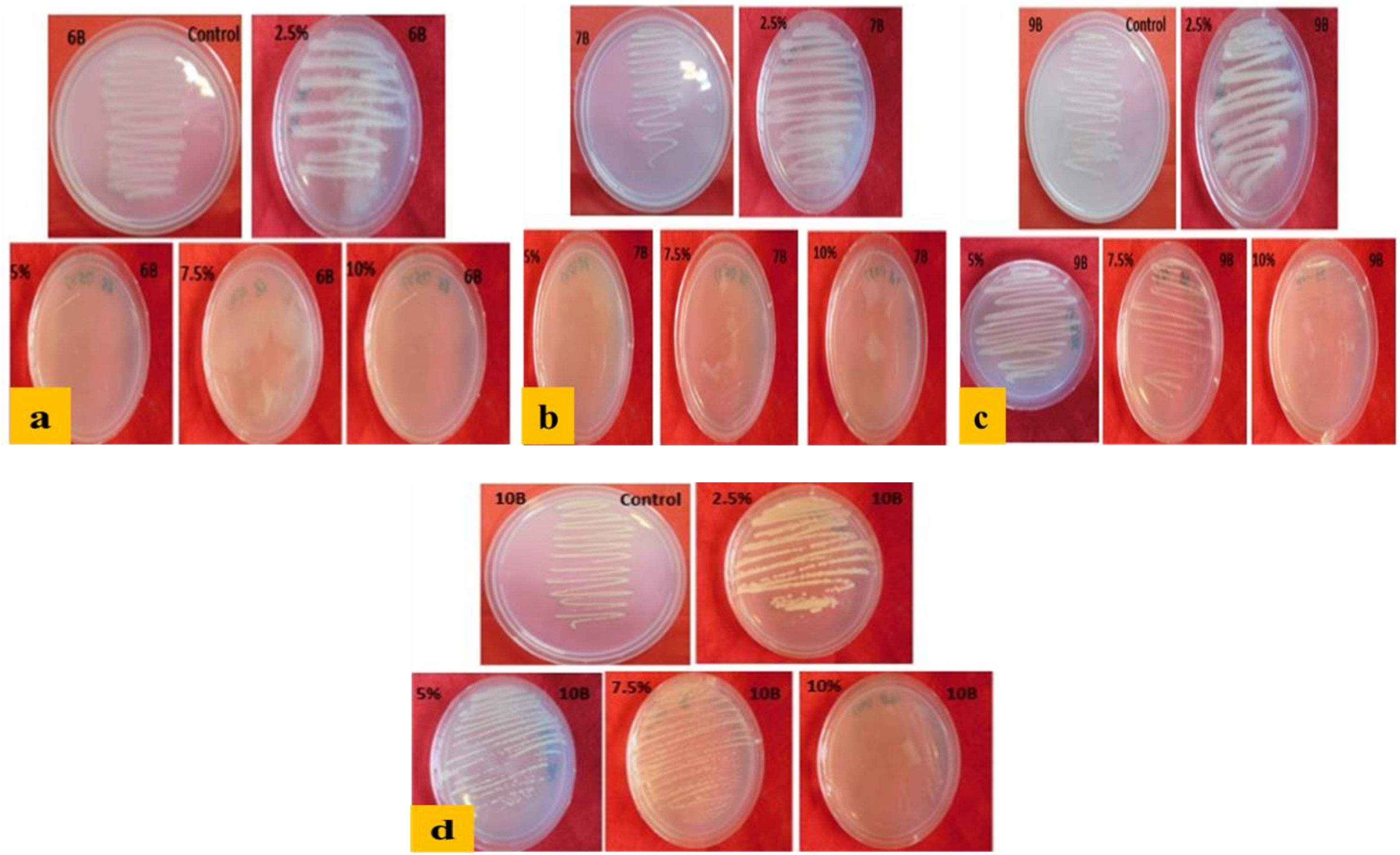


Figure – 4.8) Salinity tolerance by bacterial isolates. a-b) upto 2.5% tolerance by *B. pseudomycoides* RS6B and *B. firmus* RS7B; c-d) upto 7.5% tolerance by *Staphylo. hominis* RS9B and *Alc. faecalis* RS10B

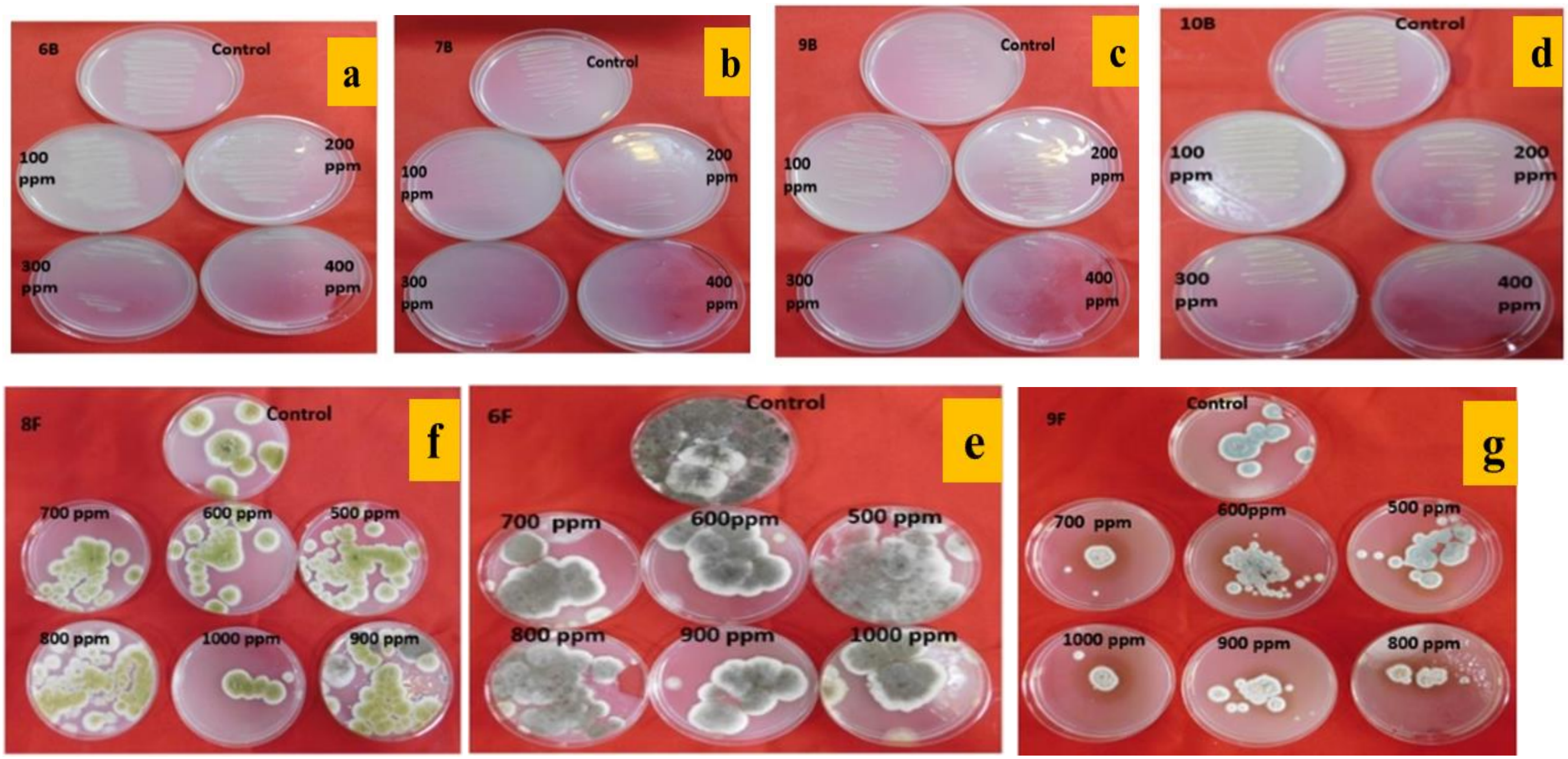


Figure – 4.10) Microbial growth against organic pesticide (Dichlorovas 76%) at different ppm concentration. a, b, c-) showing tolerance upto 200 ppm by *B. pseudomycoides* RS6B, *B. firmus* RS7B and *Staphylo. hominis* RS9B; d) upto 300 ppm by *Alc. faecalis* RS10B; e, f, g-) tolerance upto 1000 ppm by *A. luchuensis* RS6F, *A. tamarrii* RS8F and *Pen. polonicum* RS9F fungi; *A. luchuensis* RS6F, *Ep. sorghinum* RS7F, *A. tamarrii* RS8F, *Pen. polonicum* RS9F) were tested for compatibility based on zone of interaction and inhibition (Figure 4.11). To select highly compatible microbes as a single consortium of bacteria and fungi for further pot and field experiment was the aim of study hence, these microbes were tested with each other in all possible combinations as group of 2, 3 and 4. The results obtained (Table – 4.7) shows that 27 microbial groups are compatible with each other (forming zone of interaction) while 6 groups were found not compatible (forming zone of inhibition). The test was performed in three levels i.e., compatibility between bacterial strains only, between fungal strains only and between bacterial and fungal strains. Among bacterial compatibility test *B. pseudomycoides* RS6B, *B. firmus* RS7B and *B. subtilis* RS8B were compatible to grow with all isolates except *Staphylo. hominis* RS9B. All selected fungal strains showed positive interaction with fungal strains but for bacterial- fungal interaction, not a single fungal strain showed positive interaction with *Staphylo. hominis* RS9B. The fungal strain *A. luchuensis* RS6F, *Ep. sorghinum* RS7F and *A. tamarrii* RS8F was found to grow well with *P. pseudomycoides* RS6B, *B. firmus* RS7B and *Alc. faecalis* RS10B in all possible combinations. At the end of the result two bacterial – fungal interaction groups i.e., *P. pseudomycoides* RS6B + *B. firmus* RS7B + *A. luchuensis* RS6F + *A. tamarrii* RS8F and *P. pseudomycoides* RS6B + *B. firmus* RS7B + *A. tamarrii* RS8F + *Pen. polonicum* RS9F were selected on the basis of maximum compatible strains for further study but after extensive review of literature *Pen. polonicum* RS9F was found as an opportunistic pathogen so it was excluded for study and group 1 (*B. pseudomycoides* RS B + *B. firmus* RS7B + *A. luchuensis* RS6F + *A. tamarrii* RS8F) was selected as a consortium of maximum strain.

Table – 4.7 Microbial compatibility of selected plant growth promoting microbes

	Positive interaction	Negative interaction
Bacteria-Bacteria	*B. pseudomycoides* RS6B + *B. firmus* RS7B	*B. subtilis* RS8B + *Staphylo. hominis* RS9B
	B. pseudomycoides RS6B + *Alc. faecalis* RS10B	*B. firmus* RS7B + *Staphylo. hominis* RS9B
	B. pseudomycoides RS6B + *Staphylococcus* RS9B	-
	B. firmus RS7B + *B. subtilis* RS8B	-
	B. firmus RS7B + *Alc. faecalis* RS10B	-
	B. subtilis RS8B + *Alc. faecalis* RS10B	-
	Staphylococcus RS9B + *Alc. faecalis* RS10B	-
	Pseudomonas RS2B + *B. firmus* RS7B + *B. subtilis* RS8B	-
	Pseudomonas RS2B + *B. firmus* RS7B + *Staphylococcus* RS9B	-
	Pseudomonas RS2B + *B. subtilis* RS8B + *Staphylococcus* RS9B	-
	Pseudomonas RS2B + *B. firmus* RS7B + *B. subtilis* RS8B + *Staphylococcus* RS9B	-
Fungi-fungi	*A. luchuensis* RS6F + *Ep. sorghinum* RS7F	-
	A. tamarrii RS8F + *Pen. polonicum* RS9F	-
	B. luchuensis RS6F + *Ep. sorghinum* RS7F + *Pen. polonicum* RS9F	-
	A. luchuensis RS6F + *Ep. sorghinum* RS7F + *A. tamarrii* RS8F	-
Bacteria-Fungi	*B. firmus RS7B* + *A. luchuensis* RS6F	-
	B. firmus RS7B + *Ep. sorghinum* RS7F	-
	B. pseudomycoides RS6B + *A. luchuensis* RS6F	-
	Alc. faecalis RS10B + *A. luchuensis* RS6F	*Staphylo. hominis* RS9B + *Ep. sorghinum* RS7F
	Alc. faecalis RS10B + *Ep. sorghinum* RS7F	*Staphylo. hominis* RS9B + *A. pseudomycoides* RS6F
	Alc. faecalis RS10B + *Pen. polonicum* RS9F	*Staphylo. hominis* RS9B + *Pen. polonicum* RS9F
	Alc. faecalis RS10B + *A. tamarrii* RS8F	*Staphylo. hominis* RS9B + *A. tamarrii* RS8F
	Alc. faecalis RS10B + *Pen. polonicum* RS9F + *A. tamarrii* RS8F	-
	B. firmus RS7B + *A. tamarrii* RS8F + *Pen. polonicum* RS9F	-
	B. pseudomycoides RS6B + *A. tamarrii* RS8F + *Pen. polonicum* RS9F	-
	B. firmus RS7B + *B. pseudomycoides RS6B* + *A. tamarrii* RS8F + *Pen. polonicum* RS9F	-
	B. pseudomycoides RS6B + *B. firmus RS7B* + *A. pseudomycoides* RS6F + *A. tamarrii* RS8F	-

Positive interaction= both microbes are compatible with each other; Negative interaction = not compatible

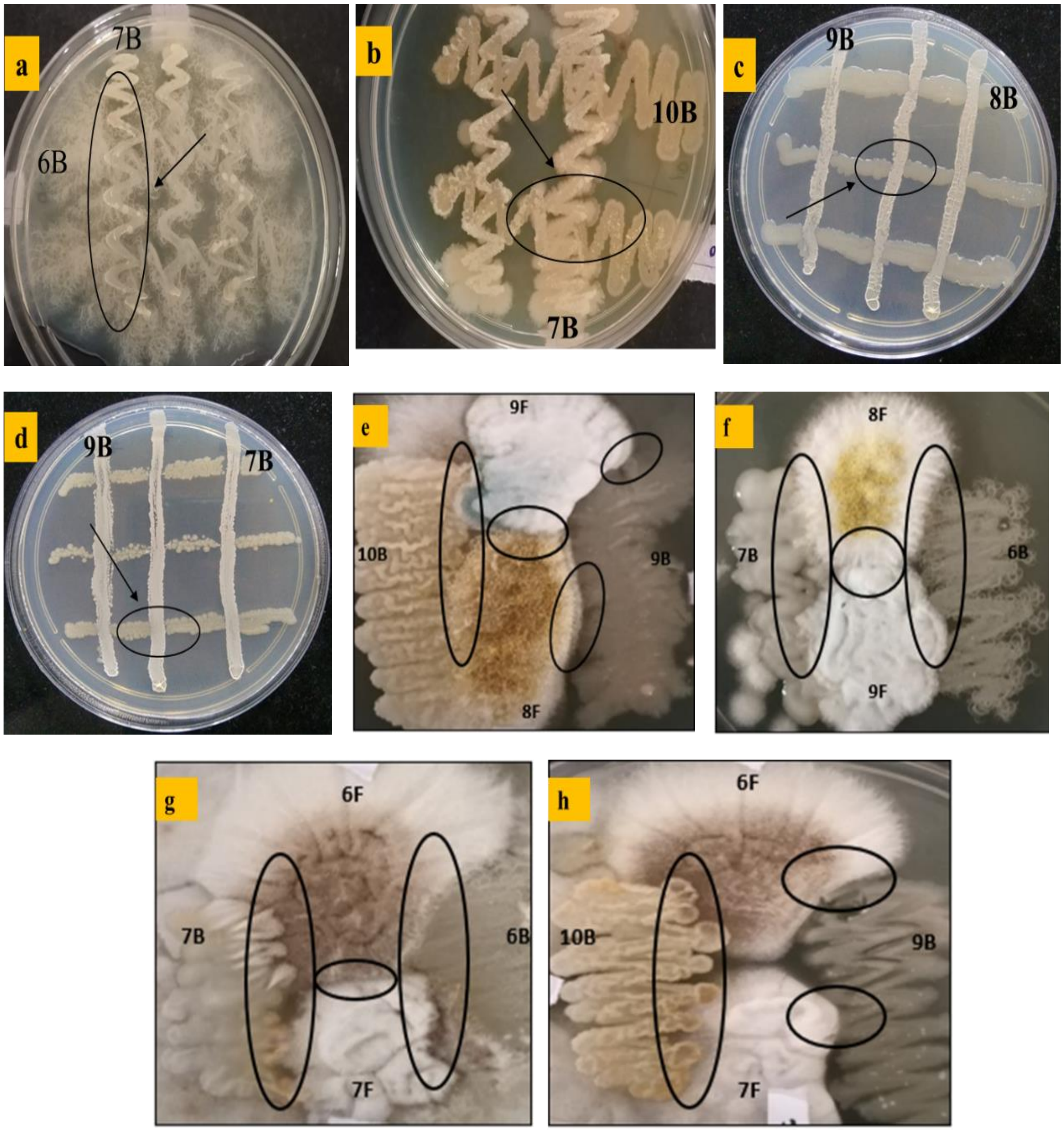


Figure – 4.11) Dual culture assay for compatibility test. a-b) circled area is showing positive interaction between *B. pseudomycoides RS6B*, *B. firmus RS7B* and *B. firmus RS7B* and *Alc. faecalis RS10B*; c-d) showing Inhibition zone by *B. firmus RS7B*, *B. subtilis RS8B* and *Staphylo. hominis* RS9B; f, g) strong positive correlation between *B. pseudomycoides RS6B*, *B. firmus RS7B*, *A. luchuensis* RS6F and *A. tamarrii* RS 8F; e-h) showing compatibility of *Alc. faecalis RS10B* with *A. tamarrii* RS8F and *Pen. polonicum* RS9F and inhibition by *Staphylo. hominis* RS9B for same fungus

4.3 Seed germination assay

Effect of selected plant growth promoting microbes in all possible combinations (15; named as T_1 to T_{15}) were assessed for seed germination and seedling length under in vitro condition and one month pot trail (Table 4.8 and Figure 4.12). Inoculation of chilli seeds with treatment T_1, T_2, T_3, T_4, T_{10}, T_{11}, T_{15} was recoded for 100% germination rate over all other treatments and control. The highest increased seedling length was calculated by inoculation of consortium treatment T_{15} (70.31%) followed by T_{14} (60.79%), T_{13} (57.61%) and T_{12} (56.66%) over control. After consortium, treatment $T_{1,}$ $T_{2,}$ T_3 and T_4 as a single microbial strain application showed 39.20%, 37.61%, 39.20% and 42.38% higher seedling length over control and other multistrain treatments, respectively. Hence on the basis of higher survivability and seedling length treatment $T_{1,}$ $T_{2,}$ $T_{3,}$ $T_{4,}$ and T_{15} were selected for further application.

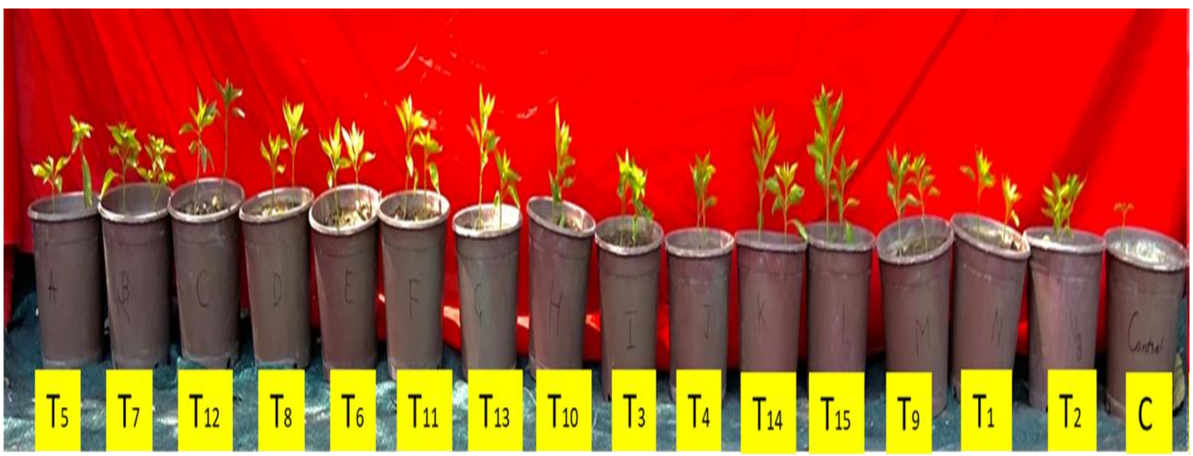


Figure – 4.12) Pot experiment to screen microbial consortium of selected isolated microbes. C- control; T_1- T_4 - single microbial strains; T_5- T_{10} – consortia of two microbial strains; T_{11}- T_{14} - Consortia of three strains; T_{15} – consortium of four microbial strains; T_1-T_{15} are mixed in cow dung and jaggery as a nutrient source

Table – 4.8. Effect of microbial treatments on seed germination

Treatments	Microbial strains	Germination percentage (%)	Mean seedling length
T_1	*B. pseudomycoides RS6B*	100	8.77±0.48[ab]
T_2	*B. firmus RS7B*	100	8.67±1.23 [ab]
T_3	*A. luchuensis* RS6F	100	8.77±0.87 [ab]
T_4	*A. Tamarrii* RS8F	100	8.97±2.25 [ab]
T_5	*B. pseudomycoides RS6B+B. firmus RS7B*	66.66	8.33±0.09 [ab]
T_6	*B. pseudomycoides RS6B+A. luchuensis* RS6F	66.66	8.57±1.42 [ab]
T_7	*B. pseudomycoides RS6B+A. tamarrii* RS8F	66.66	8.40±0.15 [ab]
T_8	*B. firmus RS7B+A. luchuensis* RS6F	50	8.63±0.22 [ab]
T_9	*B. firmus RS7B+A. tamarrii* RS8F	66.66	7.77±0.62 [ab]
T_{10}	*A. luchuensis* RS6F+*A. tamarrii* RS8F	100	7.30±1.64 [ab]
T_{11}	*B. pseudomycoides RS6B+B. firmus RS7B+A. luchuensis* RS6F	100	8.57±0.87 [ab]
T_{12}	*B. pseudomycoides RS6B+B. firmus RS7B+A. tamarrii* RS8F	66.66	9.87±0.71 [ab]
T_{13}	*B. pseudomycoides RS6B+A. luchuensis* RS6F+*A. tamarrii* RS8F	66.66	9.93±1.19 [ab]
T_{14}	*B. firmus RS7B+A. luchuensis* RS6F+*A. tamarrii* RS8F	66.66	10.13±2.62 [ab]
T_{15}	*B. pseudomycoides RS6B+B. firmus RS7B+A. luchuensis* RS 6F+ *A. tamarrii* RS8F	100	10.73±1.15 [a]
Control	No microbial amendments	50	6.30±0.12 [b]

Control = treated only with distilled water; Values are mean of the number of seeds germinated; ± = Standard error; values followed by different letters are significantly different according to DUNCAN test at 5% significance level

Section B

Assessing the efficacy of climate resilient microbial inoculants for enhanced phytochemical production from Indian licorice

With huge demand for pharmaceutical, cosmetics, and other industries due to the presence of many important secondary metabolites like alkaloids, tannin, protein, flavonoids, phenolic compound etc., Indian licorice are still away from commercial cultivation. The quality and production of medicinal plants are largely affected by biotic and abiotic factors. The application of microbes as rhizospheric amendments in the soil as biofertilizers has not been attempted for the cultivation of Indian licorice in soil. Hence, the present study was focused on the assessment of the effect of microbial stimulants single as well as in consortium on plant growth and metabolite production from the plant. Among all the isolated microbes, *B. pseudomycoides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F and *A. tamarrii* RS8F were selected for the further pot and field experiment on the basis of its PGP activity, seed germination assay and positive correlation among each other.

4.4 Retention of plant growth promoting traits of microbes and viability of bioinoculants

The efficacy of one-year-old microbial isolates (*B. pseudomycides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F and *A.tamarrii* RS8F) was reassessed after 12 monthly recultures for their PGP activity. All the isolates used for this study showed significant enhancement in ammonium and IAA production with various magnitudes (Table 4.9). In the qualitative analysis, the phosphate solubilization ability and growth on nitrogen-free media were also found retained completely. The production of ammonium and IAA by these microbes was recorded 25-50% and 31-131% increase over the freshly isolated culture. However, the microbial consortium of these selected microbes was found to produce 7-9 times higher

IAA and ammonium as compare to the individual strains (Table – 4.9). Further mixing of microbes individually or in consortium with dry powder of cow dung and jaggery in the ratio of 9:1 increased cell/spore density up to 260 % in 15 days (Table 4.10).

Table – 4.9. Production of Ammonium and IAA by one year old cultures recultured monthly on media to revive its viability

Microbial isolates	Ammonium production (μg ml^{-1})		IAA production (μg ml^{-1})	
	March 2017	**March 2018**	**March 2017**	**March 2018**
B. pseudomycoides RS6B	2.89±0.06	3.62±0.01	3.63±0.35	6.10±0.06
B. firmus RS7B	2.49±0.13	3.57±0.01	3.06±0.31	6.34±0.61
***A. luchuensis* RS6F**	*3.51±0.01*	*5.10*±0.01	9.34±0.83	21.56±0.05
***A. tamarrii* RS8F**	3.54±0.01	5.33±0.01	6.33±0.34	8.28±0.08
***Consortium* (RSC4)**	NA	21.14±0.15	NA	55.52±0.02

Fresh microbial cultures were used after 12 revivals from month of isolation,

Data are mean of three replicas, in duplicate determination (n=6) ± standard error of means.

4.5 Pot experiment for screening best performing bioinoculant

4.5.1 Effect of bioinoculants on plant growth

Application of microbial inoculants mixed with the organic carrier in the pots increased growth and biomass of *Abrus precatorius* by different magnitude (Figure 4.13 and 4.14). The single microbial inoculum of *B. pesudomycoides* RS6B, *B. firmus* RS7B, *A.luchuensis* RS6F, and *A. tamarii* RS8F increased root length by 2.32 - 55.56 %, the diameter of the root base adjoining to shoot by 1.61 - 16.19 %, shoot length by 11.79 - 30.31 %, fresh and dry weight of root by 11.12 - 96.29 %, stem by 12.5 - 86.75 %, and leaves by 25.28 - 125.25 %. The bacteria, *B. firmus* RS7B was found best performing for the increase in growth of Indian licorice as an individual inoculant, whereas the consortium of all four PGPMs (RSC4) showed the highest increase by 72.99 % for root length, 80.97 % for root diameter, 31.57 % for shoot length and 148, 142 and 141 % for a fresh and dry weight of root, stem and leaves (Table 4.11.)

Table – 4.10 Increase in colony forming unit (cfu) in bacterial inoculants and spore density in fungal bioinoculants in presence of cow dung and jaggery powder used as carrier for its rhizospheric inoculation

Microbial culture	Microbial Density	
	Cell/ spore density (0 d)	Cell/ spore density in the carriers (after 15 d)
B. pseudomycoides RS6B	2.43×10^4 cell/g	6.1×10^4 cell/g
B. firmus RS7B	2.36×10^4 cell/g	4.80×10^4 cell/g
***A. luchuensis* RS6F**	7.95×10^6 spore/g	1.28×10^7 spore/g
***A. tamarrii* RS8F**	2.4×10^6 spore/g	4.8×10^6 spore/g
RSC4 (Consortium)	2×10^6 cell/g (Bacteria)	7.2×10^6 cell/g (Bacteria)
	2.8×10^7 spore/g (fungi)	8.4×10^7 spore/g (fungi)

The same amount of microbial biomass and carrier was used for each bioinoculum (0.54g of microbial culture + 90g of cowdung + jagger (8:1)). The microbial density was measured by cfu and spore count before mixing with carrier and after 15 days placing.

4.5.2 Effect of bioinoculants on glycyrrhizic acid content in different parts of plants

The glycyrrhizic acid (GA) content in different part i.e., root, stem and leaves were determined by HPLC chromatogram (Figure – 4.16). The highest increase in GA content by the application of microbial inoculants followed the same trend as plant growth and showed 383.33% more GA in the roots, 363.75% more in the stems and 161.53% more in the leaves of the plant raised with the microbial consortium (RSC4) over non-inoculated controls. As a single strain *B. firmus* RS7B was found to be highly effective for the increase in GA content i.e., 245.83% in the roots, 235% in the stems, and 147.59% in leaves over the controls. Another single microbial inoculant *B. pseudomycoides* RS6B *A. luchuensis* RS6F, and *A. tamarii* RS8F also increased GA in the root by 37.5 – 64.58%, in the stem by 4.80 – 50.48% and in leaves by 53.75 – 125% as compared to control sets (Figure 4.15).

Figure – 4.13) Photographs of Indian Licorice cultivated for 120days in presence of different Bioinoculants. Control = nontreated plant; RS6B = *B. pseudomycoides* RS6B, RS7B = *B. firmus* RS7B, RS6F = *A. luchuensis* RS6F, RS8F = *A. tamarrii* RS8F and RSC4 = Consortium of all four microbes

Figure – 4.14) Pot experiment to screen Plant growth promoting attributes of isolated microbes as single strains and in consortium

Table – 4.11 Growth of *A. precatorius* as affected by various bioinoculants after 120 days of plantation

Treatment	Root length(cm)	Root Diameter(cm)	Root (g)		Shoot length(cm)	Stem (g)		Leaf (g)	
			Fresh wt.	Dry wt.		Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Control	10.33±0.66^{c}	2.47±0.01^{c}	1.43±0.05^{d}	0.27±0.01^{e}	41.80±1.79^{d}	9.48±0.26^{e}	5.36±0.35^{e}	2.93±0.36^{c}	1.74±0.04^{d}
B. pseudomycoides RS6B	10.57±0.75^{c}	2.51±0.04^{c}	3.64±0.32^{c}	0.36±0.02^{d}	46.73±1.43^{bc}	11.66±0.03^{d}	6.03±0.61^{de}	3.13±0.06^{bc}	2.18±0.03^{cd}
B. firmus RS7B	15.83±0.82^{b}	2.87±0.03^{b}	5.26±0.18^{b}	0.53±0.03^{b}	54.47±1.48^{a}	18.26±1.11^{b}	10.01±1.01^{b}	4.81±0.01^{a}	3.92±0.03^{a}
***A. luchuensis* RS6F**	12.00±0.21^{c}	2.48±0.04^{c}	1.54±0.04^{d}	0.30±0.01^{de}	44.16±0.31^{cd}	12.04±0.04^{d}	7.39±0.20^{cd}	3.49±0.32^{bc}	2.68±0.25^{bc}
***A. tamarrii* RS8F**	16.07±0.60^{ab}	2.60±0.04^{c}	4.28±0.65^{c}	0.42±0.01^{c}	49.00±0.70^{b}	13.48±0.33^{c}	8.25±0.42^{c}	3.92±0.10^{b}	2.98±0.37^{b}
RSC4 (Consortium)	17.87±0.20^{a}	4.47±0.07^{a}	7.79±0.08^{a}	0.67±0.01^{a}	55.00±0.15^{a}	21.14±0.64^{a}	12.95±0.17^{a}	5.21±0.40^{a}	4.20±0.21^{a}

Data are mean of three replicas of two sets of experiments ± standard error of means (n=6), Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)

4.5.3 Correlation Heatmap

A correlation heatmap study was performed to assess the effect of bioinoculants on GA content. Correlation heatmap uses colored cells, typically in a monochromatic scale, to show a 2D correlation matrix table, the values of the first dimension appear as rows of the table while the values of the second dimension are represented by the column of the table. Each square shows the correlation between the variables on each axis. The high correlation (near +2 or -2) means a good relation between two variables, and its concentration around zero means no relationship between them. In the below correlation heatmap red means positive and blue means negative. The strongest the color, the larger the correlation magnitude so it can be observed that consortium (RSC4) and *B. firmus* RS7B treatment are strongly positively correlated with Glycyrrhizic acid content in leaf, root and stem while *B. pseudomycoides* RS6B, *A. luchuensis* RS6F and *A. tamarrii* RS8F are strongly negatively correlated with GA content in stem and leaf of plant as compare to other treatment and control (Figure 4.18).

4.5.4 Effect of bioinoculants on changes in soil physicochemical and enzymatic properties

The result showed in Table 4.12 indicates that the soil pH and EC varied from 7.87-7.40 and 0.66-1.11 dSm^{-1} among the different treatments. The pH of the soil which was slightly alkaline before plantation decreased after 4 months of cultivation of Indian licorice significantly which lowered further by the bioinoculation with various magnitude. The maximum decrease in pH and EC of soil with the microbial application may be due to the higher solubility of the salt in presence of these inoculated microbes. The average soil total organic carbon (TOC) level increased significantly with the application of PGPMs. It ranged from 5.76 - 42.23% in presence of the single microbial strains as well as a consortium of all. The increased available phosphorus (P_{avl}) content was recorded in a range of 163.79 - 204.49% with the different microbial applications. It appears that during this study, the phosphate solubilizing fungal strain i.e., *A. tamarii* showed maximum increase in alkaline phosphatase individually as well as in consortium. Whereas, available nitrogen (N_{avl}) and available potassium (K_{avl}) was increased by 34.83- 45.16 % and 21.06 – 23.05 % in soil treated with different microbial strains i.e., *B. pseudomycoides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F, *A. tamarrii* RS8F, and the consortium of four i.e., RSC4.

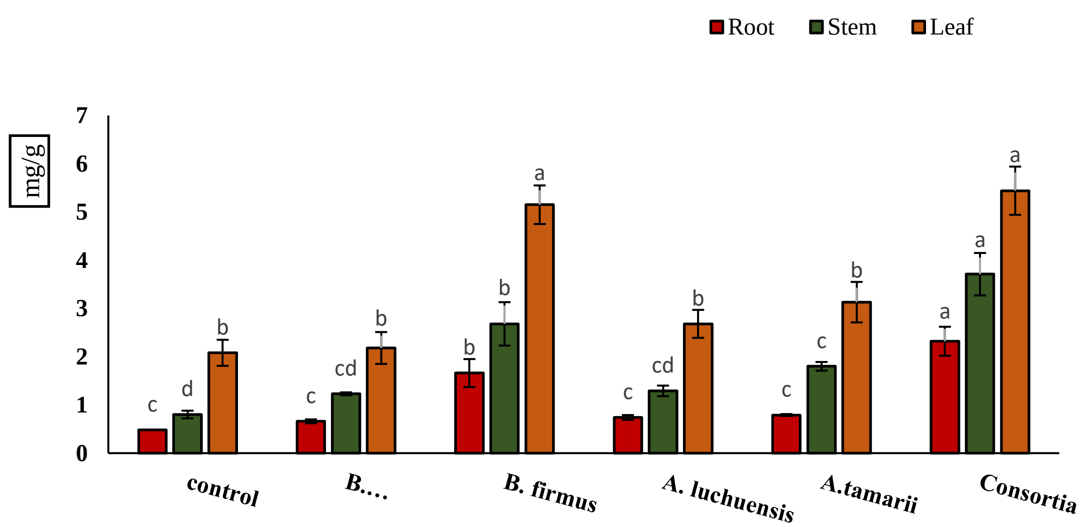


Figure – 4.15) Effect of microbial treatments on Glycyrrhizic acid content of *A. precatorius* (120Days). These results were confirmed by comparing peaks of chromatograph obtained through HPLC analysis of standard GA (sigma, 99.9%) and extracted glycyrrhizic acid (from treated and untreated plants).

The soil enzyme dehydrogenase (DHA), microbial biomass carbon (MBC), and CFU of the soil-applied with bioinoculants increased very significantly with a maximum enhancement in the pot applied with microbial consortium i.e., 70.58, 215.18, and 140.26% (Table 4.12).

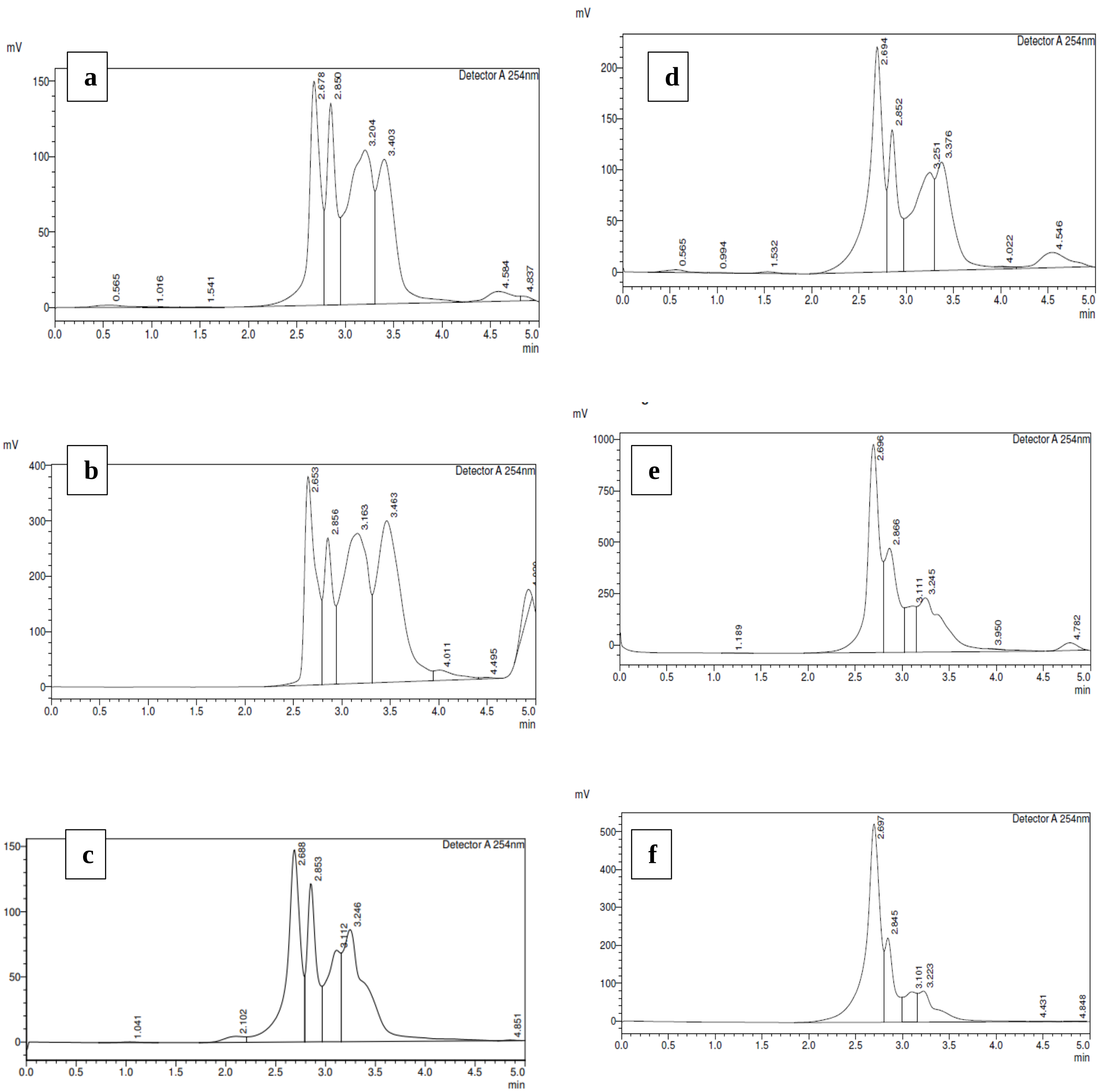


Figure – 4.16) HPLC chromatogram- a (Root), b (Leaf), c (Stem) showing glycyrrhizic acid content in control of *A. precatorius*, d (Root), e (Leaf), f (Stem) is treated with microbial consortium

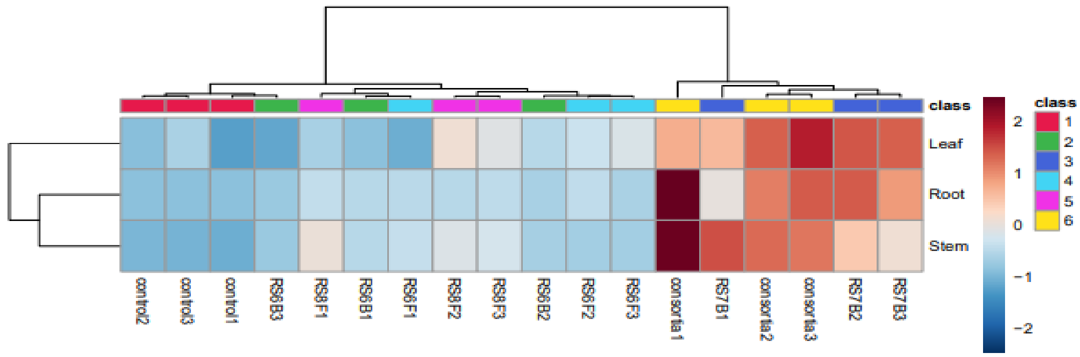


Figure – 4.17) Heatmap (correlation) study between Glycyrrhizic acid content in different part of plant with effect of microbial formulations where RS6B represents *B. pseudomycoides*, RS7B is *B. firmus*, RS6F is *A. luchuensis*, RS8F is *A. tamarii* and consortium is microbial consortium of all four strains

4.5.5 Principal component analysis (PCA)

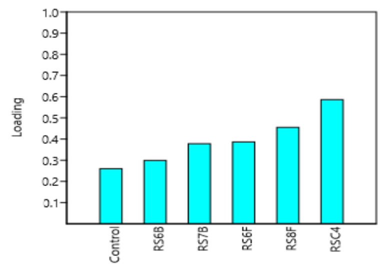
The principal component analysis was applied using PAST software version 9.0. PCA of soil parameters showed total variability of 96.32% (Eigen value 287811) for PC1 and 3.65% (Eigen value 10891.70) for PC2 (Figure 4.18). These PCs seemed irregular and didn't put into any logical explanation of spatial patterns. Therefore, the biplot graph was made for PC1 or PC2. The soil parameters; N_{avl}, K_{avl} and MBC showed positive loading value while pH, EC, TOC, P_{avl}, DHA, Alkaline phosphatase and CFU showed negative loading value for PC1 and PC2. The loading value for all the treatments; *B. pseudomycoides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F, *A. tamarrii* RS8F and RSC4 were found positive for PC1 while for PC2, RS8F and RSC4 showed negative score and rest showed positive score. PCs score value of soil parameters with application of bioinoculants in biplot of this study were closer to centre of plot recommended maximum correlation. Our study suggested that the application of bioinoculants positively affected soil properties.

Table – 4.12 Changes in soil properties after application of microbial formulations along with *A. precatorius* (120 DAS)

Parameters/ Treatments	0 day	Control	*B. pseudomycoides* RS6B	*B. firmus* RS7B	*A. luchuensis* RS6F	*A. tamarrii* RS8F	RSC4
pH	8.13±0.03	7.87±0.03^{a}	7.50±0.00^{b}	7.50±0.06^{b}	7.57±0.03^{b}	7.77±0.09^{a}	7.40±0.06^{b}
EC (dS/m-1)	0.53±0.00	0.66±0.02^{d}	0.55±0.00^{e}	0.71±0.00^{c}	0.83±0.00^{b}	0.57±0.00^{e}	1.11±0.02^{a}
TOC (%)	0.23±0.02	0.52±0.01^{e}	0.70±0.01^{b}	0.63±0.01^{c}	0.56±0.01^{d}	0.55±0.02^{de}	0.75±0.02^{a}
N_{avl} (kg ha-^{1})	46.12±0.28	116.25±1.50^{e}	156.75±1.50^{b}	142.50±3.27^{c}	126.00±1.30^{d}	123.75±3.44^{de}	168.75±3.90^{a}
P_{avl} (kg ha-^{1})	8.90±0.06	16.24±1.42^{e}	42.84±1.11^{b}	38.65±0.93^{c}	28.04±0.80^{d}	28.05±0.79^{d}	49.45±0.47^{a}
K_{avl} (kg ha-^{1})	346.33±1.20	419.33±1.45^{d}	507.67±1.86^{b}	443.67±2.19^{d}	437.33±2.91^{d}	456.33±1.76^{c}	516.00±3.79^{a}
DHA (µg g-^{1})	0.62±0.02	0.68±0.03^{d}	3.54±0.01^{a}	2.28±0.19^{b}	1.37±0.03^{c}	1.33±0.04^{c}	1.16±0.04^{c}
Alkaline Phosphatase (µg g-^{1})	3.47±0.27	64.83±0.31^{d}	72.24±0.37^{b}	69.44±0.27^{c}	65.79±0.17^{d}	72.95±0.43^{b}	77.05±1.94^{a}
MBC (µg g-^{1})	142.19±33.05	306.57±31.37^{d}	341.15±33.44^{cd}	554.18±57.32^{bc}	574.61±101.56^{bc}	713.87±56.96^{b}	966.25±119.01^{a}
CFUx10^{8} g-^{1}	0.95±0.05	2.98±0.43^{d}	5.15±0.63^{bc}	4.91±0.08^{c}	6.18±0.03^{ab}	5.22±0.04^{bc}	7.16±0.36^{a}

RS6B; *B. pseudomycoides*), RS7B; (*B. firmus*), RS6F; (*A. luchuensis*), RS8F; (*A. tamarii*), RSC4; (Microbial consortium), EC; Electrical conductivity, TOC; Total organic carbon, AN; Available nitrogen, AP; Available phosphorus, AK; available potassium, DHA; Dehydrogenase, MBC; Microbial biomass carbon, CFU; colony forming units, µgg-1; Microgram per gram, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)

PC	Eigenvalue	% variance
1	287811.00	96.32
2	10891.70	3.65
3	84.09	0.03
4	4.90	0.00
5	1.75	0.00
6	0.08	0.00



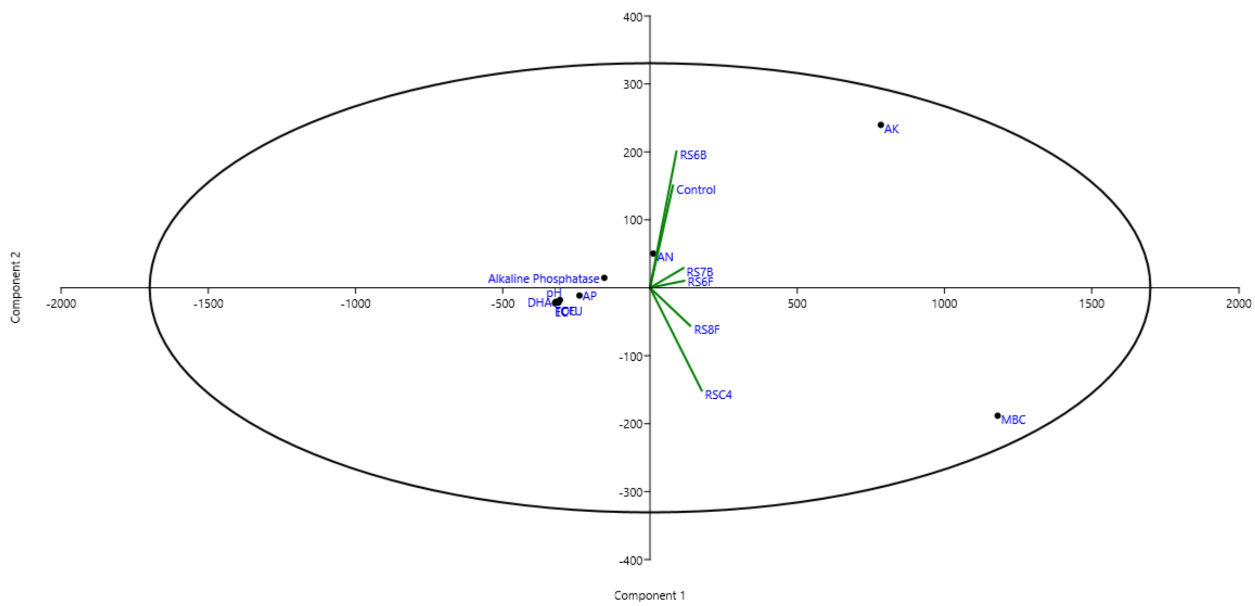


Figure – 4.18) Principal Component Analysis (PCA) of soil properties affected by different microbial inoculants where C represents control, BP1 is *B. pseudomycoides*, BF2 is *B. firmus*, AL3 is *A. luchuensis*, AC4 is *A. tamarii* and BF5 is microbial consortium

Section C

Effect of designed cropping pattern with microbial application on plant growth, productivity, biomass generation, net economic gain, carbon sequestration and soil fertility of degraded land (Field experiment)

4.6 Assessment of designed cropping system and microbial consortium on plant production

A total of seven crop groups were finalized comprising sole and intercrops i.e., L, S, C, LC, LS, SC and LSC were transplanted into a 3 m^2 (1.5m x 2m) area each in early February and late august 2019. The experiment was carried out in a randomized completely block design with three replications. All groups were treated with the microbial consortium.

4.6.1 Effect of crop combinations and PGPMs on plants growth

4.6.1.1 Stevia

The planting of combination of stevia and Indian licorice as an intercrop (SL) was recorded for the highest increased shoot (7.53%) and root (20.48%) length while other combinations i.e., SC and LSC showed 6.36 and 1.73% decreased shoot length over sole stevia crop (Figure 4.19 C-D). Stevia leaves were harvested 4 times in two years (1^{st} and 3^{rd} harvesting in late spring and 2^{nd} and 4^{th} harvesting in late autumn). The highest leaf weight was calculated for SL combination at every harvesting. It was 16.24% and 23.24 % higher in 1^{st} and 3^{rd} harvesting and 46.69% and 23.24% higher in 2^{nd} and 4^{th} harvesting with microbial amendments over sole stevia cultivation. Other crop combinations showed huge fluctuation in different harvestings like CS showed 55.36% and 24.13% decreased leaf weight on 1^{st} and 3^{rd} harvesting while 13.43% and 18.38% increased weight on 2^{nd} and 4^{th} harvesting with PGPMs. Similarly, LSC showed 36.62% and 20.66% decreased leaf weight on 1^{st} and 3^{rd} harvesting while 18.80% and 21.54% increased leaf weight during 2^{nd} and 4^{th} harvesting with microbial amendments over sole stevia cultivation (Table – 4.13 A

& B). The microbial consortium showed significant changes for plant growth as compared to control crops. It ranged approx. 4.91-5.54 % increased plant height for S, SL, SC and LSC over uninoculated controls and benefited 98.06% more leaf weight to sole stevia, 37.76% more to SL, 52.05% more to SC and 2.73% more leaf to LSC combination on 1st harvesting, 145.03% higher to sole stevia, 55.66% higher to SL, 116.25% to SC and 57.18% higher to LSC combination on 2nd harvesting, 2.79% higher to sole stevia, 27.69% more to SL, 30.12% to SC, 1.93% to SC combination on 3rd harvesting, and 9.20% higher to sole stevia, 39.62% more to SL, 67.37% higher to SC and 37.41% more stevia leaf to LSC combination when treated with microbial amendments over non-treated control.

4.6.1.2 Chilli

The highest chilli shoot length was also recorded for crop combinations rather than sole crops. Combinations of CL, CS and LSC showed 22.30%, 8.95% and 34.07% increased shoot length than sole chilli crop. The root length was also higher in similar way for combinations rather than sole crops i.e., 15.82% higher for CL, 29.67% higher for CS and 16.61% higher for cultivation of LSC combination over sole chilli cultivation (Figure 4.19 A-B). The microbial amendment showed 12.07-5.60% higher shoot length for all combinations against the nontreated controls. The microbial amendment was significantly effective for all the cropping patterns but showed maximum increase in sole chilli crop i.e., approx. 20.03 % at every harvesting. CL combination produced the maximum amount of chilli fruit at every harvesting which was recorded to be 64.85 and 35.83% higher on 1st harvesting, 74.96 and 54.89% higher on 2nd harvesting, 53.78 and 32.26% higher on 3rd harvesting, 60.63 and 46.58% higher on 4th harvesting with and without PGPMs as

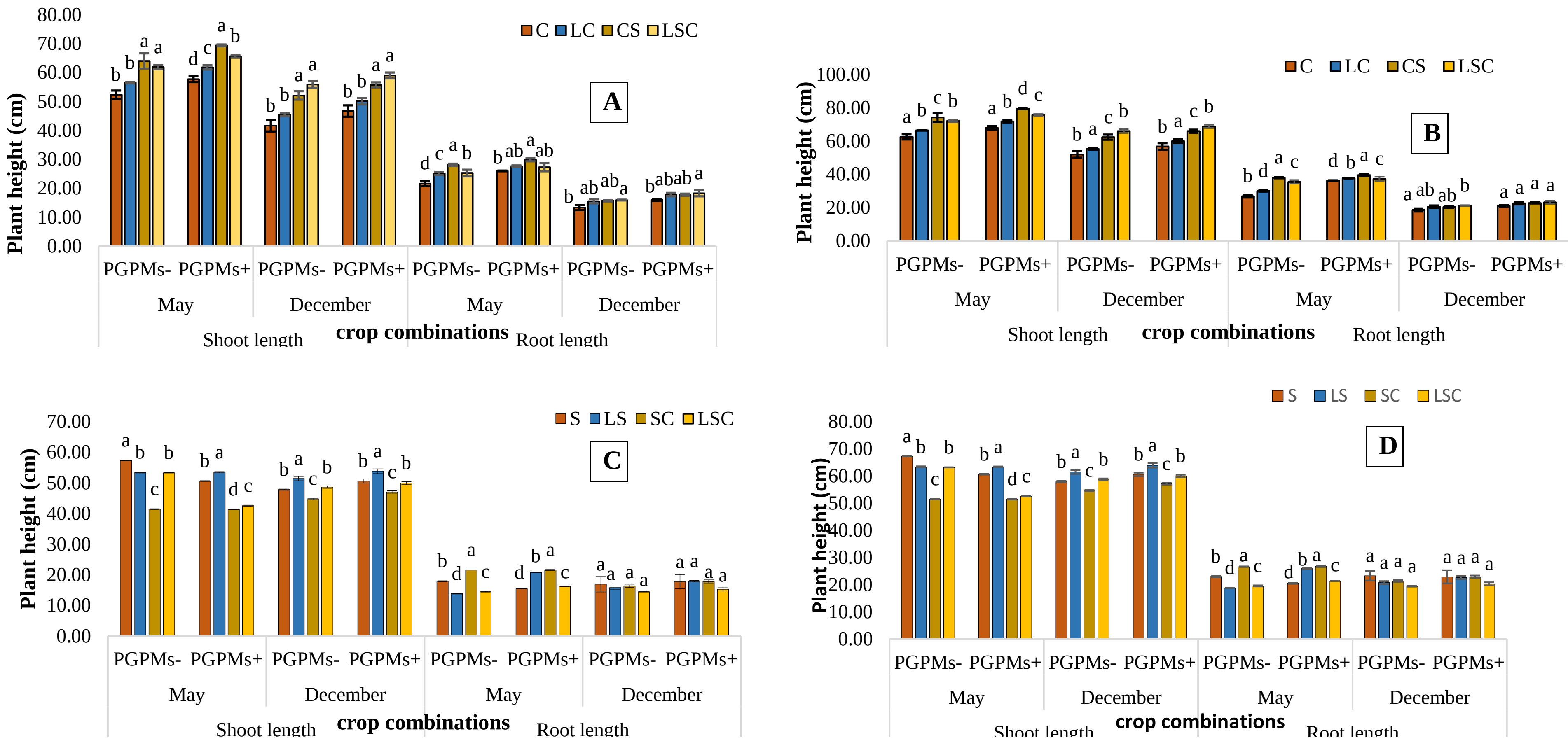


Figure – 4.19) Effect of crop combinations and PGPMs application on plant height in different seasons for two years of study. A-B) showing stevia growth; C-D) showing chilli growth for 1st and 2nd year *S* Stevia, C*Chilli, PGPMs⁻*without microbial treatment, PGPMs⁺*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

Table – 4.13 A-B). Stevia leaves weight(G) at the time of harvesting in different seasons for two years

A) 1st Year

Sample Code	Late Spring 2019				Late Autumn 2019			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+
S	42.32±4.14[bc]	81.82±1.47[a]	14.48±2.45[b]	28.68±0.41[b]	34.18±3.19[b]	69.48±5.97[b]	10.48±0.84[c]	25.68±3.64[b]
LS	66.58±4.08[a]	84.11±0.60[a]	24.20±1.77[a]	33.34±1.07[a]	65.91±3.12[a]	104.11±6.19[a]	24.20±2.21[a]	37.67±0.78[a]
CS	36.93±2.68[c]	47.45±0.35[b]	12.14±1.897[b]	18.46±0.05[c]	40.59±1.10[b]	73.78±0.97[b]	13.47±0.44[c]	29.13±0.3[b]
LCS	53.49±6.09[ab]	5.19±6.23[b]	17.71±2.56[ab]	18.18±2.56[c]	60.49±1.75[c]	77.87±1.46[b]	19.41±1.54[b]	30.51±0.45[b]

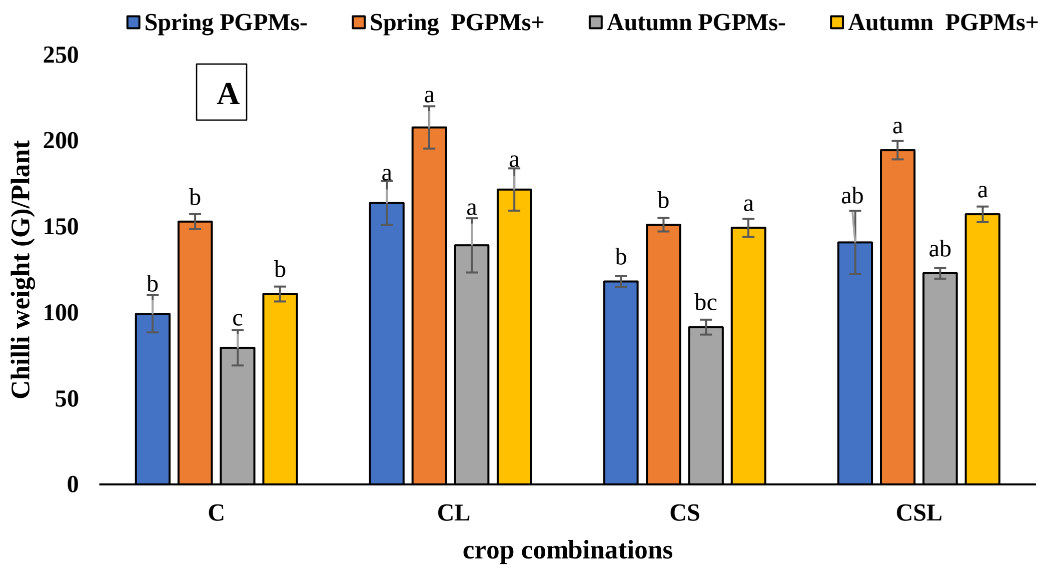
B) 2nd Year

Sample Code	Late Spring 2020				Late Autumn 2020			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+
S	62.29±4.19[a]	85.19±2.37[b]	34.32±2.53[a]	35.28±1.29[b]	53.99±3.26[b]	69.49±5.98[c]	30.44±0.85[ab]	33.24±1.21[c]
LS	76.64±4.20[a]	94.11±0.65[a]	34.05±1.76[a]	43.48±0.69[a]	75.88±3.07[a]	114.05±6.30[a]	34.40±2.29[a]	47.73±0.68[a]
CS	46.99±2.67[b]	57.58±0.44[d]	21.84±1.77[b]	28.42±0.17[c]	50.57±1.06[b]	83.90±1.17[bc]	23.51±0.43[c]	39.35±0.42[b]
LCS	63.56±6.03[a]	71.85±3.94[c]	27.46±2.68[ab]	27.99±2.41[c]	70.44±1.75[a]	87.88±1.18[b]	29.40±1.54[b]	40.40±0.42[b]

*PGPMs-*without microbial treatment, PGPMs+*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)* compare to sole chilli (Figure 4.20). Other combinations i.e., CS and LSC were also recorded for higher fruit biomass i.e., CS produced 18.79% and 2.28% higher chilli on 1^{st} harvesting, 15.14% and 34.78% higher on 2^{nd} harvesting, 15.48 and 1.83% higher on 3^{rd} harvesting, 5.76 and 29.56% higher chilli on 4^{th} harvesting and LSC produced 41.82 and 27.16% higher fruit on 1^{st} harvesting, 54.54 and 39.14% higher on 2^{nd} harvesting, 34.62 and 24.13% higher on 3^{rd} harvesting and 43.99 and 35.53% higher chilli fruit on 4^{th} harvesting with and without treatment over control sole crop (Figure 4.20).

4.6.1.3 Indian licorice

The highest shoot and root length were recorded for LS combination which was 18.93% and 14.32% higher than sole crop while other combinations i.e., LC and LSC also showed 3.21 and 9.28% increased shoot length and 2.45 and 6.85% increased root length than sole crop on 1^{st} harvesting Microbial treatment promoted higher plant height in the range of 83.92-41.99% (shoot) and 3.84-5.58% (root) for all the cropping patterns i.e., L, LC, LS and LSC over the nontreated controls (Figure 4.21). For the 2^{nd} harvesting LS combination again showed highest shoot and root length i.e., 12.15 and 13.39% with PGPMs than sole crop while LC and LSC were found with 7.04% higher shoot & 7.20% higher root and 0.48% & 0.50% higher shoot and root than sole Indian licorice with effect of microbial amendments (Figure- 4.21). The gain root weight followed the same pattern of root length as LS and obtained 4.65% and 8.78% higher dry root on 1^{st} harvesting and 2.27 and 2.51% higher dry root on 2^{nd} harvesting with and without PGPMs as compared to sole crop (Table 4.14). Other combinations i.e., LC showed 3.35 and 1.66% increased dry root wt. with PGPMs on 1^{st} and 2^{nd} harvesting while LSC were recorded with 1.41 and 1.01% decreased dry root wt. on both harvesting as compare to sole crop cultivation.



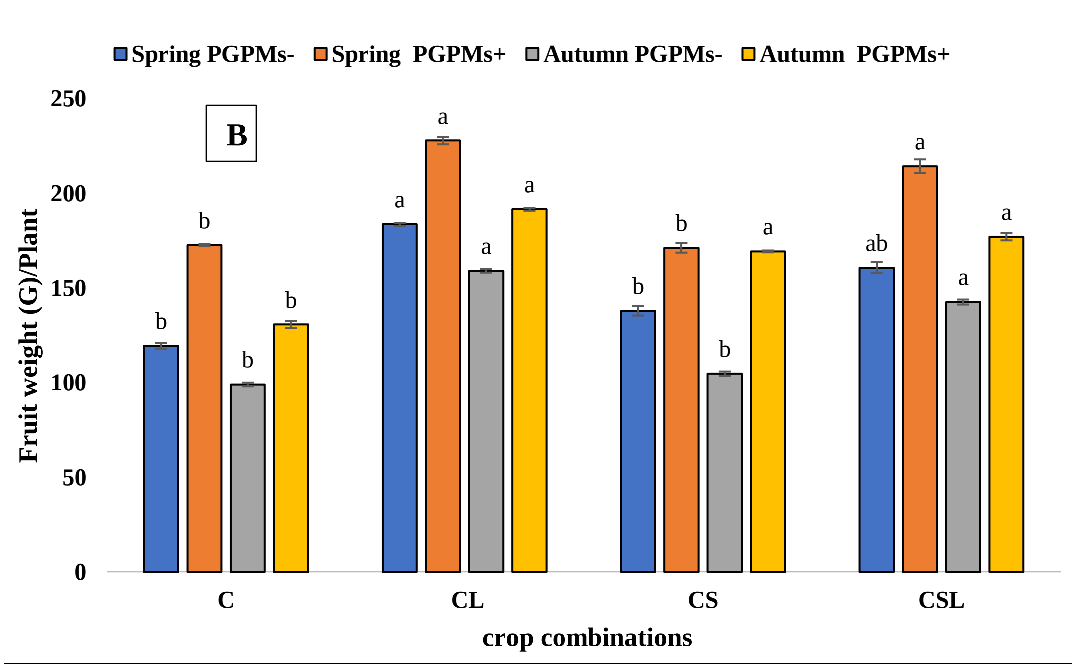


Figure – 4.20) Chilli fruit wt. in different seasons for two consecutive years A) 2019; B) 2020 *C*Chilli, CL* chilli-Indian licorice, CS* chilli- stevia, CSL* chilli-stevia-Indian licorice, PGPMs^{-}*without microbial treatment, PGPMs^{+}*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

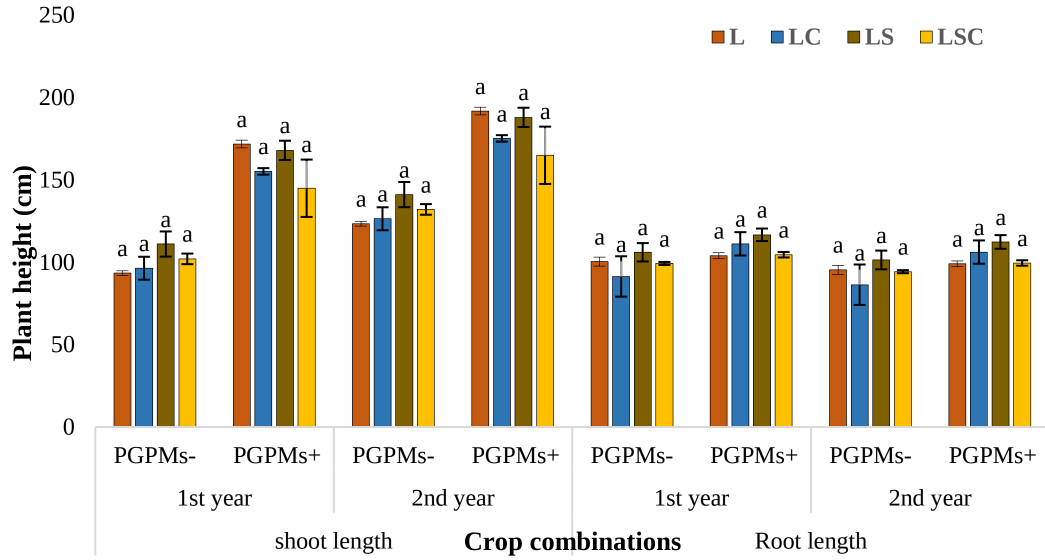


Figure - 4.21) Effect of crop combinations and PGPMs application on plant height of Indian licorice in two consecutive years, *L* Indian licorice, PGPMs⁻*without microbial treatment, PGPMs⁺*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

Table – 4.14) Licorice root weight (g) in different crop combinations with and without microbial amendments

Sample Code	1st year				2nd year			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+	PGPMs-	PGPMs+
L	35.10 ±1.54a	37.31 ±0.92b	19.13 ±0.40a	19.70 ±0.24b	55.10 ±1.54a	57.31 ±0.92b	39.13 ±0.40a	39.70 ±0.24b
LC	31.60 ±5.22a	39.84 ±2.33ab	18.22 ±1.36a	20.36 ±0.61ab	51.60 ±5.22a	59.84 ±2.33ab	38.22 ±1.36a	40.36 ±0.61ab
LS	38.53 ±2.82a	43.97 ±1.28a	20.02 ±0.73a	21.43 ±0.33a	58.53 ±2.82a	63.97 ±1.28a	40.02 ±0.73a	41.43 ±0.33a
LSC	34.09 ±0.59a	36.97 ±1.15b	18.86 ±0.15a	19.60 ±0.30b	54.09 ±0.59a	56.97 ±1.15b	38.86 ±0.15a	39.60 ±0.30b

*L*Indian licorice, CL* chilli-Indian licorice, CS* chilli- stevia, CSL* chilli-stevia-Indian licorice, PGPMs⁻*without microbial treatment, PGPMs⁺*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT*

4.6.2 Effect of crop combinations and PGPMs on productivity

The productivity of different crops was calculated by dry weight of quality product of that crop. In case of stevia and chilli productivity in a year is the sum of productivity of two harvesting (Figure 4. 22). The highest annual yield (7.59 Mg ha^{-1} y^{-1}) was generated for the chilli when grown with Indian licorice (LC) over other sole crops and in combinations with effect of microbial amendments. Other combinations i.e., CS and LSC generated 13.85% and 33.39 % more chilli yield with PGPMs over sole chilli crop. In case of stevia and Indian licorice higher yield was generated by again two crop combinations rather than sole crops. For stevia, SL combination produced 30.27 % higher stevia yield while CS and LSC produced 12.84% and 11% less yield with and without PGPMs over sole stevia. Similarly, for Indian licorice, SL combination again produced highest crop yield which was 9.32% higher than sole crop. Meanwhile, LC and LSC combinations also produced 3.38 % higher crop yield with PGPMs over sole Indian licorice. The microbial input always showed higher growth and yield of the crop as compared to uninoculated controls (Figure 4.22).

4.6.3 Assessment of intercropping indices

4.6.3.1 Land equivalent ratio (LER)

The highest LER value was recorded for LSC (3.94) intercrop, while other intercrops also showed higher LER value than sole crop i.e., LC (2.71), LS (2.98) and SC (2.19). The microbial consortium was not very effective for higher LER value (Figure 4.23). Results indicate that 3.94, 2.71, 2.98 and 2.19% more land area in the monocropping system would produce the same amount of yield as in intercropping of LC, LS, SC and LSC.

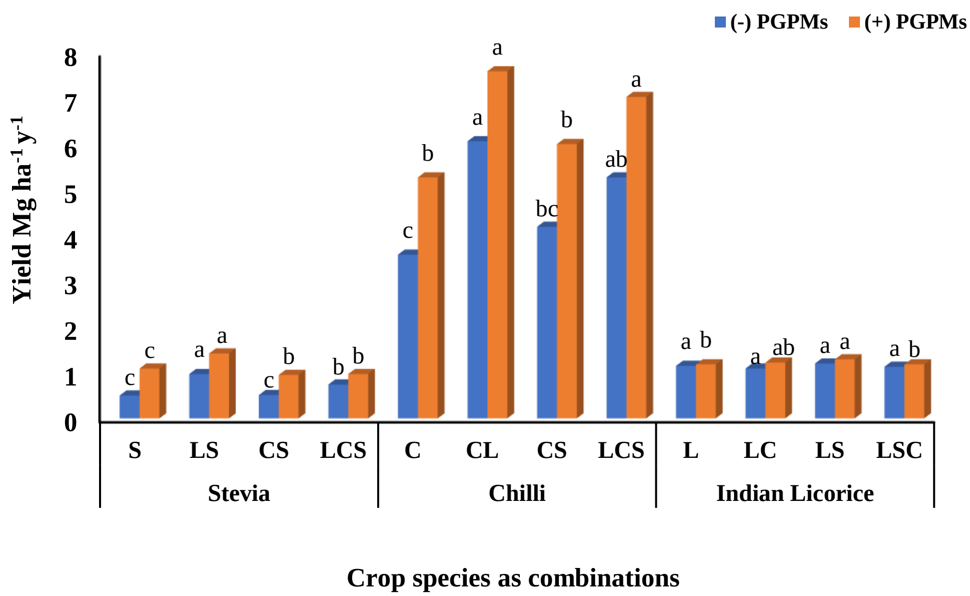


Figure – 4.22) Productivity of crop species in combinations in a year. *S*stevia, C*chilli, L*Indian licorice, CL* chilli-Indian licorice, CS* chilli- stevia, CSL* chilli-stevia-Indian licorice, PGPMs^{-}*without microbial treatment, PGPMs^{+}*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

4.6.3.2 Crop equivalent yield (CEV)

In multiple cropping sequences, it is very difficult to compare the economic productivity of one crop to another. For example, the yield of stevia cannot be compared with the yield of fruit, cereals or pulse crops and so on. In such situations, comparisons can be made based on economic returns. Crop equivalent yield is the yields of different intercrops/crops, converted into an equivalent yield of any one crop based on the price of the produce. The highest CEY value was recorded for chilli crop intercropped as LSC and LC i.e., 17765 and 13663 while Indian licorice was calculated for the lowest CEY value in intercropping with stevia (LS) i.e., 377.46 and chilli (LC) i.e., 484.61. Stevia showed moderate CEY value and was highest in three crops intercropping of LSC i.e., 3983.9 (Figure 4.23). Microbial treatment was always found to produce a higher CEY value for all crops in all intercropping as compared to uninoculated controls.

4.6.3.3 Monetary Advantages Index (MAI)

The highest MAI value was recorded for Indian licorice - stevia (LS) intercropping, i.e., $9.7x10^{12}$, afterwards LSC ($5.1x10^{9}$), LC ($3.8x10^{9}$) and SC ($7.8x10^{8}$) intercrop showed higher MAI value which was further increased with the microbial amendment.

4.6.4 Assessment of microbial dependency for designed plant community

The microbial dependency was highest for stevia monocrop (50.81%) and lowest for three crops intercropping of LSC (23.60%). The MD at the level of two crops intercropping was recorded highest for LS (37.79%) followed by LC (32.81%) and SC (29.23%) over uninoculated controls (Figure 4.24).

4.7 Effects of crop combinations and microbial consortium on biomass production

Effect of crop combinations in terms of species diversity levels, microbial application and their interaction were highly significant ($p<0.001$) on biomass production per hectare area in two years (Table- 4.16). The highest total plant (Shoot + root) dry biomass (6.8 Mg ha^{-1}) was obtained by three species diversity level i.e., LSC. In case of two species diversity level the highest (5.9 Mg ha^{-1}) biomass was obtained by LS followed by SC (4.7 Mg ha^{-1}) and LC (3.1 Mg ha^{-1}). The one species level of stevia produced high (3.2 Mg ha^{-1}) biomass with effect of PGPMs as compare to other one species level. The biomass production in all possible combinations were significantly affected by application of microbial consortium.

The highest changes in biomass production due to microbial consortium was observed in one species diversity level i.e., stevia (31.25%) followed by two species diversity level of LS (27.11%) and SC (24%) and three species diversity level of LSC (16.17%) (Figure 4.25 and 4.34).

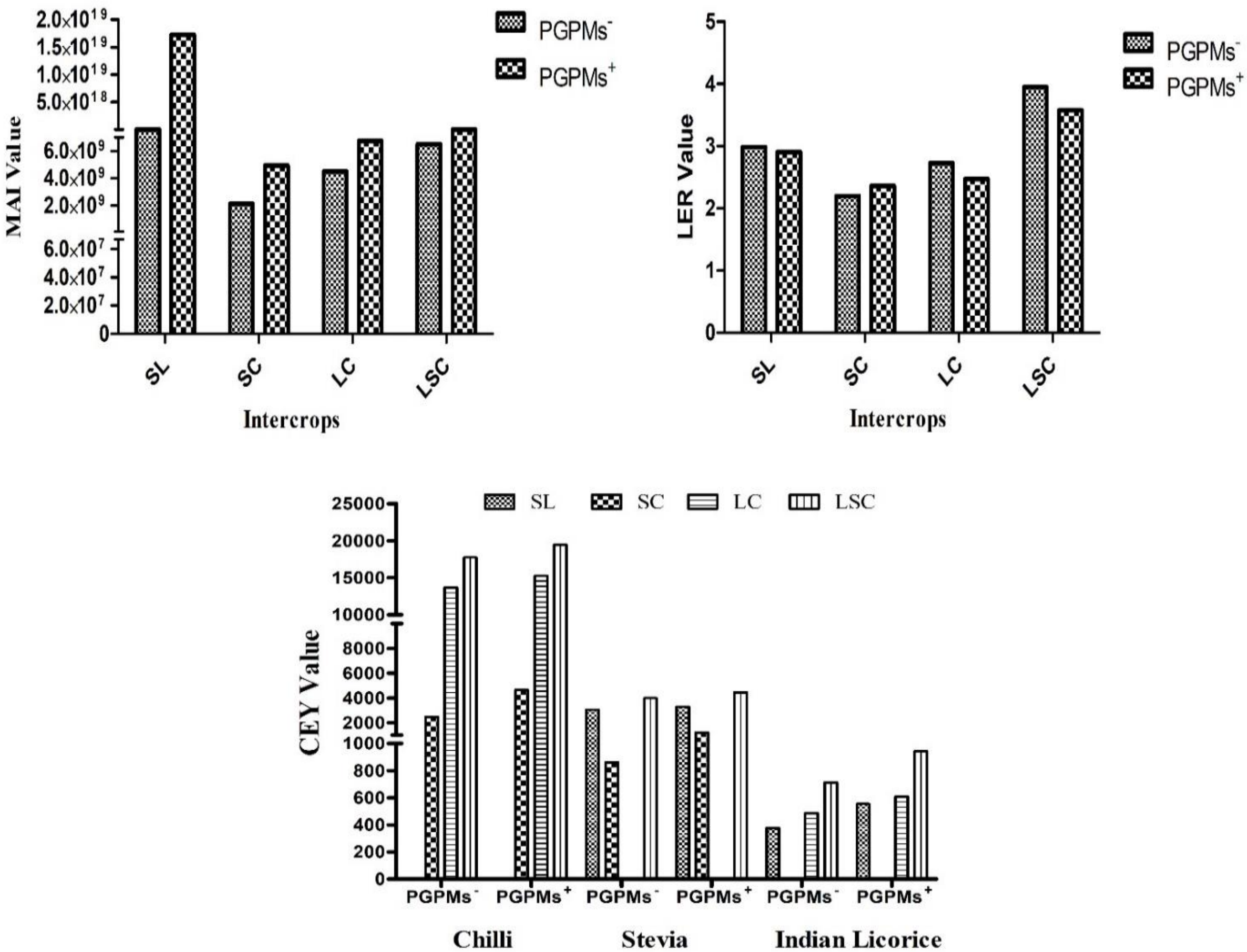


Figure – 4.23) Crop Equivalent yield (CEY) value (kg ha^{-1}), Land Equivalent Ratio (LER) and Monetary Advantage Index (MAI) of stevia-chilli and Indian licorice intercropping in a year. *S*stevia, C*chilli, L*Indian licorice, CL* chilli-Indian licorice, CS* chilli- stevia, CSL* chilli-stevia-Indian licorice, PGPMs$^-$*without microbial treatment, PGPMs$^+$*with microbial treatment, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

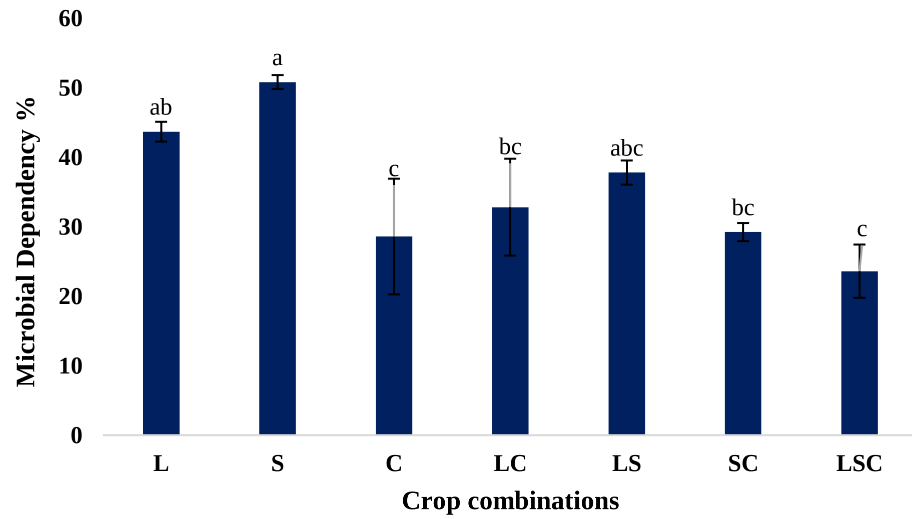


Figure- 4.24) Microbial dependency of crops at different crop combination levels. *Data are mean of three replicas ± standard error of means of two independent sets of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

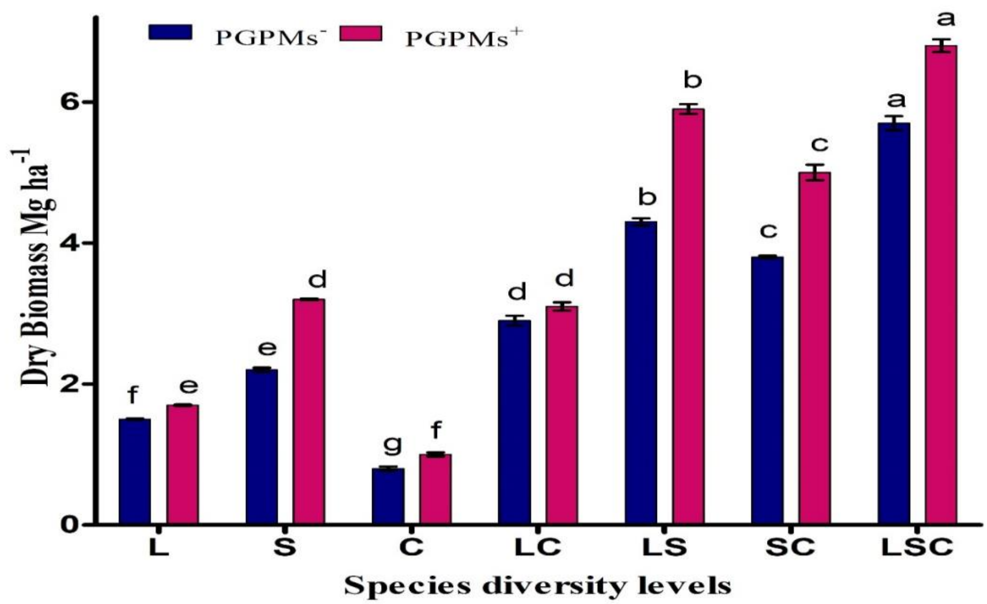


Figure – 4.25) Effect of species diversity levels and PGPMs application on total dry biomass in two years. *L* Indian licorice, S* Stevia, C*Chilli, PGPMs^{-}*without microbial treatment, PGPMs^{+}*with microbial treatment, Mg ha^{-1}* mega gram per hectare, Y^{-2}* per two-year, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

4.8 Changes in beneficial soil properties

Crop combinations at different diversity levels and microbial consortium both significantly affected the changes in soil physicochemical and microbial activities i.e., pH, EC (electrical conductivity), TOC (total soil organic carbon), N_{avl} (available nitrogen), P_{avl} (available phosphorous), K_{avl} (available potassium), alkaline phosphatase, DHA (dehydrogenase), CFU (Colony forming unit) and SMBC (Soil microbial biomass carbon). The changes in soil quality were very less during early months while steep at the end of the study.

The highest reduction in soil pH was recorded by the cultivation of sole Indian licorice, two crop combination LC and three crop combination LSC which was 14.84%, 14.44% and 11.02% higher after two years of study with PGPMs when compare to 0 month. Other crop combinations i.e., SC and LS also showed significant reduction in soil pH (9.69 and 10.55%) after two years over control (0 month). During initial 4 months changes in soil pH was recoded little bit faster like L, LC and LSC showed 5.42%, 4.69% and 4.21% reduced pH while SC and LS showed 1.14 and 2.38% reduction but later on 8th and 12th month of study it was reduced slowly than 4 months and showed 10.82%, 9.01%, 5.57%, 6.88% and 2.63% reduction by L, LC, LSC, SC and LS on 8th months while 12.77%, 11.97%, 9.29%, 8.54% and 5.65% higher reduction on 12th months of study over control (Table – 4.15 A). Similar to soil pH, EC was also highly decreased in two species diversity level LC followed by one species level of L and two species diversity level LS with microbial treatment at the end of the study. It was recorded highest 86.73% reduction in EC by LC while other crop combinations showed 84.04, 83.87, 81.52 and 77.65% reduction by LS, L, SC and LSC with PGPMs after 2 years over control.

*Crop combination LC, LS, SC is representing two species diversity levels while LSC shows Three species diversity level

At initial stage (4 months) the highest reduction was recorded for sole Indian licorice L (23.65%) followed by SC (20.65%), LS (20.21%), LSC (12.76) and LC (11.22%). At 8th months sampling the highest reduction moved towards by LC crop combination (41.83%) followed by LSC (41.48%), LS (39.36%), SC (39.13%) and L (37.63%) with PGPMs and After a year highest reduction were recorded by crop combination SC (70.65%), following LS (68.08%), LC (66.32%), L (65.59%) and LSC (62.76%) with PGPMs (Table – 4.15 B). These changes in pH were significantly higher ($p<0.05$) in soil of the same species diversity levels treated with microbial consortium as compare to non-treated plots.

The highest reduced bulk density was recorded by cultivation of two crop combination LC (45.85%) and three crop combination LSC (35.25%) followed by two crop combinations SC (30.43%) and LS (30.26%) with microbial amendments after two years of study (Table 15 C). These changes were very less during initial months and showed only 10.16 % by LC, 6.41% by LSC, 3.31% by L, 3.10% by S and SC, 2.70% by C and 2.63% decreased by LS on 4th month of study. Two crop combination LC and three crop combination LSC always showed highest decrease as compare to other intercrops and sole crops throughout the study. After 8th month study LC and LSC showed 15.28 and 9.61% reduction while others changed the trend of 4 months and showed 8.69% reduction by SC, 7.94% by L, 6.83% by S, 6.57% by LS and 4.72% by cultivation of sole chili crop with PGPMs over 0 months. The crop combination LC and LSC showed again highest 30.57 and 17.30% reduction in soil bulk density after a year while others were calculated for 12.5% reduction by LS, 11.25% by L, 10.55% by SC, 10.35 by S and 6.75% by C over control. The sole chilli cultivation always showed lowest reduction in BD throughout the study with and without PGPMs over control.

Cultivation of sole chilli crop with microbial amendments was recorded with highest available potassium (126.22%) than other sole crops and intercrops as compare to control at the end of the study. Other sole crops i.e., L and S showed 122.72 and 92.13% increase while intercrops i.e., SC, LS, LC and LSC showed 10.83%, 88.98%, 107.39% and 113.68% increase in AK after 2 years of study over control (Table 4.15 D). After 4 months of study the highest increase was found by cultivation of sole Indian licorice (77.01%) rather than sole chilli (69.41%) but chilli was higher than other crop patterns. Other intercrops i.e., SC, LS, LC and LSC showed 64.74%, 61.59%, 62.39% and 52.40% higher AK with PGPMs when compare to control soil. After 8 months sole chilli again continued with highest AK. The highest (100.24%) increase was recorded for cultivation of sole chilli followed by LC (89.77%), L (84.86%), LS (68.95%), LSC (68.33%), SC (66.04%) and S (62.34%) with PGPMs after 8 months over control. Changes in AK after a year followed the same trend as crop patterns follows at 8th month. It was highest by sole chilli (104.07%) then LC (92.96%), L (89.04%), LS (75.27%), LSC (73.35%), SC (70.63%) and S (66.21%) with effect of microbial inputs as compare to control soil.

The changes in alkaline phosphatase were recorded to be highest by the cultivation of sole Indian licorice (1097.13%) followed by three crop combination LSC (1048.21%), two crop combination LS (1270.39%), SC (1053.56%) and sole crop S (1099.43%) and C (1009.09%) while LC showed lowest increase (971.88%) in Alkn Phos with PGPMs after 2 years of experiment when compared with control soil (Table 4.15 E). During initial stage (4 months) these changes were recorded as 141.90% by LSC (highest), 120.12% by L, 104.76% by LC, 96.65% by C, 64.73% by SC, 45.37% by S and 21.66% by LS (lowest) over control soil. But after at 8 months interval the highest increament (770.60%) was calculated for SC crop combination followed by S (759.82%), LSC (746.87%), LS (746.18%), L (703.32%), C (668.19%) and LC (454.45%) over control. Changes in alkaline phosphatase enzyme were showing huge fluctuation throughout the study and after a year highest increase was recorded by two crop combination LS (1027.21%) followed by L (869.11%), S (841.72%), SC (821.82%), LSC (812.55%), LC (777.26%) and C (762.20%) with microbial amendments as compare to 0 months soil.

Total soil organic carbon enhanced significantly in all the possible groupings with and without microbial application. The maximum (457.16%) increases and highest significance ($p<0.001$) in soil organic carbon was found in two crop combination LC followed by SC (317.64%), LS (276.57%) and three crop combination LSC (279.48%) while sole crops showed 250.12% increased TOC by L, 208.64% by S and 206.32% by C with and without treatment after two years over control (Figure 4.26). Changes in TOC after 4 months of study showed highest by LC (64.78%) following L (65.78%), SC (61.76%), LSC (55.84%), LS (55.26%), C (46.83%) and S (41.97%) with PGPMs when compared with control soil. The changes on 8th months were observed highest by same combination as 4 months i.e., LC (161.97%) following LSC (142.85%), SC (122.05%), L (82.89%), C (78.57%), LS (77.63%) and S (70.37%) with microbial inputs over control. After a year change in total organic carbon by crop combination with effect of microbial application showed different pattern which was always recorded highest by two crop combination LC (411.26%) while other crop combinations benefited 240.25% higher carbon to the soil by cultivation of three crop combination LSC, 258.82% and 236.82% increase by two crop combination SC and LS and sole crops L, S and C profited 197.36%, 160.66% and 160.75% higher carbon to the soil over control soil.

The highest decrease in available nitrogen was recorded by cultivation of two crop combination LC (204.05%) followed by sole crop L (194.78%), two crop combination LS (186.17%), sole crop S (175.64%), C (170.08%), three crop combination LSC (164.58%) and two crop combination SC (242.24%) with PGPMs after 4 months of study when compared with control soil (Figure- 4.27). While after 8 months it was again highest by LC combination (365.57%) following LSC (311.90%), SC (242.24%), LS (228.40%) and sole L (225.04%), S (223.48%), C (217.99%) with microbial amendments over control soil. Changes was always recorded to be highest by LC combination throughout the study, after a year LC showed 438.33% higher AN in soil treated PGPMs followed by LSC (316.77%), LS (295.85%), L (289.24%), SC (270.65%), S (264.15%) and C (304.71%) compared to control. At the end of the study changes in AN followed the same trend as a year back and showed highest 496.02% increased by LC, 365.81% by LSC, 350.03% by LS, 341.13% by L, 311.46% by SC, 316.17% by S and 304.71% by sole chilli cultivation with PGPMs when compared with 2 years old field soil (control).

At initial stage (4 months), changes in available phosphorus recorded highest (81.04%) by sole stevia cultivation while others showed 73.59% increase by LSC, 53.99% by L, 52.42% by LC, 48.88% by SC, 37.73% by LS and 30.13% increase by sole chilli cultivation with effect of PGPMs (Figure 4.28). After 8 months, the highest increase was calculated by two crop combination LC (371.45%) following sole stevia (344.20%), three crop combination LSC (285.29%), two crop combination SC (274.28%), sole C (262.50%), L (240.69%) and LS (135.55%) with PGPMs over control. At the end of the year, the highest increase was recorded by cultivation of sole stevia i.e., 456.78% while others added 443.76% higher AP by cultivation of LC, 347.08% by C, 346.32% by SC, 345.03% by

LSC, 339.56% by L and 305.44% by two crop combination LS with PGPMs. The pattern of changes in AP was again varied with different crop combination when compare with control soil at the end of the study (2 years) which was 620.47% by LC, 617.46% by S, 552.39% by LS, 518.90% by L, 511.49% by LSC, 470.69% by SC and 458.15% by sole chilli cultivation with microbial inputs.

Soil microbial activities were also showed significant changes with all the possible plant groupings after two years of plantation. The highest positive changes in dehydrogenase enzyme in soil was calculated by cultivation of two crop combination LC at the end of the study, it showed 522.11% increase followed by SC (392.91%), sole stevia S (382.75%), sole chilli C (375.28%), three crop combination LSC (289.50%), LS (279.24%) and sole L (274.58%) with PGPMs over control (Figure 4.29). But at 4 months, the highest increase was recorded by sole chilli cultivation with microbial inputs (161.79%) while others showed 93.10% increased DHA by sole S, 83.46% by SC, 69.61% by L, 69.23% by LC, 62.43% by LSC and 51.57% by LS with amended microbes as compare to control. Cultivation of sole stevia found highest positive increase after 8 months of study, i.e., 168.96% following 158.65% by LC, 155.11% by SC, 132.04% by sole L, 122.65% by LSC, 108.80% by LS and 101.12% increase by sole chilli over control soil while after a year it was recorded to be highest increase by LC (327.88%) which was constantly highest till two years of study. Others were recorded with 228.34% increase as SC cultivation, 217.24% by sole S, 183.97% by LSC, 162.43% by sole L, 157.30% by sole C and 153.45% increase by cultivation of two crop combination LS as compare to 0 months soil.

The two-crop combination LC always recorded for best positive changes in soil properties with and without PGPMs. The soil with maximum increased microbial biomass carbon (306.42%) was calculated by cultivation of Indian licorice and chilli at the same unit area (LC) over control soil with microbial inputs for two years (Figure 4.30). Meanwhile other crop combinations were also recorded with increased microbial biomass carbon i.e., LS found with 224.66% increase, sole L with 224.49%, LSC with 209.80%, sole C with 203.76%, sole S with 191.23% and SC with 121.53% increased MBC with addition of microbial inputs over 0 months soil. At initial stage (4 months), the highest increased biomass carbon was found by cultivation of LC (69.87%) followed by LSC (56.57%), LS (52.63%), sole C (33.25%), L (32.56%), S (26.67%) and SC (11.14%) with PGPMs over control. After 8 months study, the highest increament was again found by cultivation of LC (86.32%) following LS (82.95%), LSC (65.23%), L (62.69%), S (55.66%), C (44.40%) and SC (28.11%) as compare to control soil. At the end of the years 261.28% increased SMBC was recorded by two crop combination LC with PGPMs which was highest and others showed 184.09% increased biomass carbon by LS, 183.96% by L, 169.07% by LSC, 165.59% by C, 152.43% by S and 87.47% increase by SC over before plantation soil (control).

The cfu increased with time spam and highest increase was recorded by the same crop combination as soil microbial biomass carbon at the end of the study i.e., 344.60% by LC which followed sole C (276.37%), LS (274.71%), L (268.54%), LSC (238%), S (235.28%) and SC (198.37%) with microbial application significantly over control soil (Figure – 4.31). At 4 months, LC showed 91.90% increased cfu (highest) followed by LS (75.65%), C (69.45%), L (67.41%), LSC (43.16%), S (32.70%) and SC (29.01%) with PGPMs over 0 months soil while at 8 months the soil cultivated with LC found to have139.41% higher CFU followed by C (117.31%), LS (112.83%), L (109.41%), S

(71.77%), LSC (68.56%) and SC (60.61%) than initial soil. Two crop combination LC promoted 236.51% increased cfu to the soil at the end of the year with combine effect of microbial inputs over 0 months soil and other crop combinations recorded with 180.84% increased cfu by LS, 175.76% by C, 168.54% by L, 150.94% by S, 141.83% by LSC and 120.09% by two crop combination SC with microbial amendments over soil before plantation.

The two-way anova analysis showed significant changes on soil properties due to species diversity levels (Factor-A), PGPMs (Factor-B) and interactions (Table-4.16). The time varying significant changes were observed in most of the soil properties excluding K_{avl} with effect of species diversity levels and microbial consortium. The interaction between species diversity levels and microbial consortium was not significant ($p>0.050$). N_{avl} was also increased in same manner of TOC as compare to initial stage of field experiment (Table 4.16).

4.9 Effect of plant combinations and microbial consortium on carbon sequestration

The plant species diversity levels, microbial consortium and their interactions showed significant changes for above ground C- sequestration and below ground C- sequestration (Table 4.16). Similar species diversity levels i.e., LSC and LS were found to assimilate highest carbon in plant biomass with and without microbial consortium (Figure 4.32A). The soil C- sequestration potential was calculated by all possible crop combination level with and without PGPMs and we found that cultivation of two species diversity levels i.e., LC, SC and LS have the highest potential of creating sink for 35 Mg ha^{-1}, 31 Mg ha^{-1} and 29 Mg ha^{-1} C- sequestration among all possible groupings (Figure 4.32 B).

Table -15 A-E). Changes in soil properties with and without PGPMs at different species diversity levels and time intervals in two years (A) pH

pH								
Species Diversity Levels		**L**	**S**	**C**	**SC**	**LS**	**LC**	**LSC**
0 month	**Control**	8.22±0.05[ab]	8.24±0.02[a]	7.78±0.05[d]	7.84±0.03[cd]	7.96±0.07[bcd]	8.10±0.03[ab]	8.07±0.19[abc]
4 months	**PGPMs$^-$**	7.77±0.02[a]	7.88±0.15[a]	7.77±0.03[a]	7.74±0.03[a]	7.87±0.09[a]	7.91±0.06[a]	7.74±0.05[a]
	PGPMs$^+$	7.72±0.07[a]	7.73±0.06[a]	7.68±0.11[a]	7.75±0.07[a]	7.77±0.03[a]	7.72±0.03[a]	7.73±0.05[a]
8 months	**PGPMs$^-$**	7.55±0.03[abcd]	7.61±0.13[abc]	7.53±0.09[abcd]	7.53±0.09[abcd]	7.67±0.03[a]	7.62±0.14[ab]	7.64±0.08[ab]
	PGPMs$^+$	7.33±0.03[d]	7.40±0.06[bcd]	7.30±0.06[d]	7.30±0.06[d]	7.75±0.10[a]	7.37±0.03[cd]	7.62±0.03[ab]
12 months	**PGPMs$^-$**	7.30±0.01[a]	7.41±0.09[a]	7.47±0.07[a]	7.40±0.06[a]	7.29±0.01[a]	7.30±0.06[a]	7.47±0.08[a]
	PGPMs$^+$	7.17±0.02[bc]	7.29±0.04[bc]	7.27±0.03[bc]	7.17±0.03[bc]	7.51±0.06[a]	7.13±0.03[c]	7.32±0.10[b]
16 months	**PGPMs$^-$**	7.20±0.06[abcd]	7.32±0.08[ab]	7.37±0.07[a]	7.30±0.06[ab]	7.38±0.06[a]	7.23±0.07[abc]	7.39±0.06[a]
	PGPMs$^+$	7.07±0.02[cd]	7.22±0.02[abcd]	7.17±0.03[bcd]	7.13±0.04[bcd]	7.22±0.02[abcd]	7.04±0.03[d]	7.24±0.11[abc]
20 months	**PGPMs$^-$**	7.17±0.03[cd]	7.29±0.09[abc]	7.33±0.03[ab]	7.27±0.03[abc]	7.34±0.03[ab]	7.15±0.08[cde]	7.36±0.03[a]
	PGPMs$^+$	7.05±0.03[de]	7.18±0.02[bcd]	7.14±0.03[cde]	7.13±0.03[cd]	7.19±0.01[bcd]	7.00±0.06[e]	7.21±0.09[abcd]
24 months	**PGPMs$^-$**	7.13±0.03[abcde]	7.22±0.07[abc]	7.27±0.03[ab]	7.16±0.03[abcd]	7.28±0.03[ab]	7.08±0.06[abcd]	7.29±0.01[a]
	PGPMs$^+$	7.00±0.10[de]	7.15±0.03[abcd]	7.04±0.09[cde]	7.08±0.02[bcde]	7.12±0.04[abcde]	6.93±0.12[e]	7.18±0.08[abcd]

B) Electrical Conductivity (EC)

EC (dS m^{-1})								
Species Diversity Levels		**L**	**S**	**C**	**SC**	**LS**	**LC**	**LSC**
0 month	**Control**	0.93±0.01[bc]	0.94±0.00[b]	0.92±0.00[c]	0.92±0.00[c]	0.94±0.00[b]	0.98±0.01[a]	0.94±0.01[b]
4 months	**PGPMs$^-$**	0.82±0.00[c]	0.91±0.00[a]	0.82±0.00[c]	0.81±0.00[c]	0.81±0.00[c]	0.90±0.01[a]	0.89±0.00[b]
	PGPMs$^+$	0.71±0.00[c]	0.73±0.00[c]	0.68±0.00[d]	0.73±0.00[c]	0.75±0.01[c]	0.87±0.03[a]	0.82±0.00[b]
8 months	**PGPMs$^-$**	0.66±0.01[c]	0.6±0.01[c]	0.6±0.01[e]	0.6±0.01[cd]	0.6±0.01[de]	0.7±0.01[a]	0.7±0.00[b]
	PGPMs$^+$	0.58±0.01[a]	0.57±0.00[ab]	0.48±0.01[c]	0.56±0.01[ab]	0.57±0.01[ab]	0.57±0.01[ab]	0.55±0.00[b]
12 months	**PGPMs$^-$**	0.46±0.01[a]	0.49±0.03[a]	0.34±0.01[b]	0.47±0.02[a]	0.46±0.01[a]	0.46±0.01[a]	0.48±0.01[a]
	PGPMs$^+$	0.32±0.01[ab]	0.36±0.01[a]	0.32±0.00[ab]	0.27±0.02[c]	0.30±0.02[bc]	0.33±0.01[ab]	0.35±0.02[a]
16 months	**PGPMs$^-$**	0.40±0.02[ab]	0.44±0.04[a]	0.31±0.02[cd]	0.45±0.03[a]	0.39±0.03[abc]	0.39±0.03[abc]	0.41±0.03[a]
	PGPMs$^+$	0.27±0.02[d]	0.32±0.02[bcd]	0.29±0.03[d]	0.24±0.03[d]	0.26±0.03[d]	0.27±0.01[d]	0.32±0.03[bcd]
20 months	**PGPMs$^-$**	0.32±0.03[abcd]	0.39±0.08[a]	0.26±0.02[bcd]	0.37±0.03[ab]	0.33±0.02[abc]	0.32±0.03[abcd]	0.38±0.05[ab]
	PGPMs$^+$	0.21±0.01[cd]	0.29±0.04[abcd]	0.22±0.01[cd]	0.21±0.01[d]	0.21±0.02[cd]	0.22±0.03[cd]	0.28±0.05[abcd]
24 months	**PGPMs$^-$**	0.22±0.03[abcd]	0.29±0.08[ab]	0.23±0.02[abcd]	0.31±0.01[a]	0.23±0.02[abcd]	0.22±0.03[abcd]	0.28±0.05[abc]
	PGPMs$^+$	0.15±0.03[d]	0.22±0.02[abcd]	0.19±0.03[bcd]	0.17±0.03[cd]	0.15±0.02[d]	0.13±0.02[d]	0.21±0.03[abcd]

C) Bulk Density (BD)

BD (g cm^{-3})								
Species Diversity Levels		L	S	C	SC	LS	LC	LSC
0 month	Control	1.51±0.03^{ab}	1.61±0.01^{a}	1.48±0.06^{b}	1.61±0.05^{a}	1.52±0.02^{ab}	1.57±0.01^{ab}	1.56±0.04^{ab}
4 months	PGPMs^{-}	1.48±0.02^{abcd}	1.59±0.01^{a}	1.46±0.06^{bcd}	1.60±0.01^{a}	1.50±0.02^{abcd}	1.44±0.04^{bcd}	1.48±0.05^{abcd}
	PGPMs^{+}	1.46±0.02^{bcd}	1.56±0.01^{abc}	1.44±0.06^{cd}	1.56±0.01^{ab}	1.48±0.02^{abcd}	1.41±0.04^{d}	1.46±0.05^{bcd}
8 months	PGPMs^{-}	1.41±0.03^{bcde}	1.53±0.01^{a}	1.44±0.06^{abcd}	1.50±0.01^{ab}	1.45±0.02^{abcd}	1.37±0.05^{de}	1.45±0.02^{abcd}
	PGPMs^{+}	1.39±0.03^{cde}	1.50±0.01^{ab}	1.41±0.05^{bcde}	1.47±0.01^{abc}	1.42±0.02^{bcde}	1.33±0.03^{e}	1.41±0.02^{bcde}
12 months	PGPMs^{-}	1.38±0.03^{bcd}	1.47±0.01^{a}	1.41±0.05^{abc}	1.46±0.02^{a}	1.38±0.01^{bcd}	1.16±0.03^{f}	1.34±0.02^{cde}
	PGPMs^{+}	1.34±0.03^{cde}	1.44±0.02^{ab}	1.38±0.03^{bcd}	1.44±0.01^{ab}	1.33±0.01^{de}	1.09±0.00^{g}	1.29±0.01^{e}
16 months	PGPMs^{-}	1.31±0.01^{abcd}	1.34±0.03^{abc}	1.37±0.03^{a}	1.35±0.02^{ab}	1.28±0.02^{bcd}	1.09±0.02^{e}	1.24±0.02^{d}
	PGPMs^{+}	1.28±0.02^{bcd}	1.33±0.05^{abc}	1.34±0.03^{abc}	1.34±0.02^{abc}	1.26±0.03^{cd}	1.04±0.02^{e}	1.24±0.04^{d}
20 months	PGPMs^{-}	1.21±0.05^{a}	1.25±0.05^{a}	1.27±0.03^{a}	1.24±0.03^{a}	1.18±0.02^{a}	1.03±0.01^{bc}	1.16±0.06^{a}
	PGPMs^{+}	1.18±0.07^{a}	1.23±0.01^{a}	1.26±0.03^{a}	1.22±0.05^{a}	1.16±0.03^{a}	0.98±0.04^{c}	1.14±0.04^{ab}
24 months	PGPMs^{-}	1.14±0.08^{ab}	1.18±0.05^{a}	1.20±0.01^{a}	1.17±0.04^{ab}	1.11±0.02^{ab}	0.96±0.05^{bc}	1.10±0.09^{ab}
	PGPMs^{+}	1.12±0.06^{ab}	1.16±0.08^{ab}	1.19±0.05^{a}	1.12±0.05^{ab}	1.06±0.03^{ab}	0.85±0.13^{c}	1.01±0.06^{abc}

D) Available potassium (K_{avl})

K_{avl} (kg ha^{-1})								
Species Diversity Levels		L	S	C	SC	LS	LC	LSC
0 month	Control	440.24±4.57^{a}	497.41±31.78^{a}	439.98±29.77^{a}	487.60±17.29^{a}	466.67±11.35^{a}	470.78±10.03^{a}	468.85±1.30^{a}
4 months	PGPMs^{-}	779.27±108.09^{a}	787.52±102.64^{a}	745.39±60.30^{a}	803.29±37.20^{a}	754.13±79.10^{a}	764.50±71.42^{a}	714.57±36.54^{a}
	PGPMs^{+}	881.93±13.96^{a}	858.31±67.40^{a}	762.05±62.66^{a}	904.31±32.37^{a}	735.13±34.61^{a}	869.43±71.62^{a}	906.27±29.96^{a}
8 months	PGPMs^{-}	813.93±93.97^{a}	807.52±90.15^{a}	881.04±35.38^{a}	809.62±35.70^{a}	788.46±18.70^{a}	893.43±58.69^{a}	789.23±98.08^{a}
	PGPMs^{+}	905.27±6.01^{a}	884.98±64.59^{a}	901.04±48.14^{a}	925.63±36.58^{a}	801.46±86.54^{a}	902.17±58.20^{a}	933.92±27.27^{a}
12 months	PGPMs^{-}	832.23±86.82^{a}	826.75±87.25^{a}	897.88±34.76^{a}	832.04±34.46^{a}	817.96±82.84^{a}	908.42±62.79^{a}	812.77±93.35^{a}
	PGPMs^{+}	920.65±14.87^{a}	902.64±59.35^{a}	942.02±31.21^{a}	937.24±31.73^{a}	828.59±31.94^{a}	923.06±46.90^{a}	948.55±25.87^{a}
16 months	PGPMs^{-}	858.90±85.29^{a}	840.09±79.25^{a}	911.21±42.79^{a}	845.37±32.78^{a}	831.30±85.88^{a}	921.75±67.05^{a}	826.10±87.90^{a}
	PGPMs^{+}	933.98±21.18^{a}	915.97±61.89^{a}	955.36±34.18^{a}	950.57±29.41^{a}	841.92±28.07^{a}	936.39±34.95^{a}	961.88±19.10^{a}
20 months	PGPMs^{-}	882.23±86.97^{a}	863.42±67.48^{a}	934.55±48.54^{a}	868.70±26.33^{a}	854.63±63.29^{a}	945.08±48.53^{a}	849.44±75.14^{a}
	PGPMs^{+}	963.98±35.49^{a}	939.30±72.82^{a}	978.69±48.62^{a}	973.91±41.19^{a}	865.26±29.94^{a}	959.73±36.68^{a}	985.2±11.47^{a}
24 months	PGPMs^{-}	898.90±75.91^{a}	880.09±60.56^{a}	951.21±32.10^{a}	885.37±9.90^{a}	871.30±76.33^{a}	961.75±31.95^{a}	866.10±67.25^{a}
	PGPMs^{+}	980.65±47.01^{a}	955.97±56.40^{a}	995.36±33.46^{a}	993.91±47.79^{a}	881.92±13.32^{a}	976.39±20.02^{a}	1001.88±5.39^{a}

E) Alkaline phosphatase (Alkn Phos)

Alkn Phos (µg g^{-1})								
Species Diversity Levels		L	S	C	SC	LS	LC	LSC
0 month	Control	12.92±1.63^{a}	12.32±0.70^{a}	12.86±0.41^{a}	13.61±0.08^{a}	12.97±0.33^{a}	14.69±1.43^{a}	13.46±2.23^{a}
4 months	PGPMs^{-}	14.18±4.16^{d}	12.68±1.41^{d}	16.94±0.43^{cd}	18.23±4.08^{bcd}	14.20±1.85^{d}	30.08±3.93^{bcd}	16.54±4.07^{cd}
	PGPMs^{+}	28.42±7.71^{abc}	17.91±2.38^{bcd}	25.29±5.32^{abcd}	22.42±4.70^{abcd}	15.78±1.28^{cd}	18.97±3.07^{ab}	32.56±0.68^{a}
8 months	PGPMs^{-}	89.80±3.40^{ef}	91.01±7.14^{ef}	100.72±4.65^{cde}	99.26±5.72^{cde}	137.91±8.06^{f}	96.30±2.96^{cde}	100.47±2.96^{cde}
	PGPMs^{+}	103.79±1.07^{bcde}	105.93±1.17^{bcde}	98.79±3.10^{cde}	118.49±7.54^{b}	109.75±3.28^{bcd}	81.45±5.57^{def}	113.99±8.72^{bc}
12 months	PGPMs^{-}	110.76±6.16^{cde}	105.20±2.83^{e}	105.58±2.65^{de}	104.28±3.35^{e}	120.11±6.80^{bcde}	114.56±5.49^{bcde}	109.73±4.07^{cd}
	PGPMs^{+}	125.21±5.86^{bc}	116.02±4.08^{bcde}	110.88±3.92^{cde}	125.46±8.82^{bc}	146.20±6.66^{a}	128.84±3.26^{b}	122.83±5.10^{bcd}
16 months	PGPMs^{-}	116.40±5.86^{cde}	111.88±2.67^{e}	112.26±2.37^{de}	110.76±3.26^{e}	127.01±6.49^{bcde}	120.82±5.58^{bcde}	116.21±4.00^{cde}
	PGPMs^{+}	131.47±5.60^{bc}	122.70±3.95^{bcde}	117.56±4.06^{bcde}	131.93±8.74^{bc}	152.67±6.86^{a}	134.48±2.90^{b}	129.30±5.01^{bcd}
20 months	PGPMs^{-}	128.72±6.07^{cd}	123.16±1.48^{d}	125.00±2.30^{d}	123.50±3.18^{d}	137.24±4.50^{bcd}	133.15±5.54^{bcd}	128.11±3.40^{cd}
	PGPMs^{+}	141.71±3.46^{bc}	134.19±2.95^{bcd}	128.22±3.73^{cd}	144.47±8.40^{b}	165.00±7.01^{a}	145.34±1.60^{b}	142.04±5.22^{bc}
24 months	PGPMs^{-}	141.46±5.86^{bcd}	136.95±2.67^{d}	137.32±2.37^{d}	135.82±3.26^{d}	149.98±4.41^{bcd}	145.89±5.58^{bcd}	141.27±4.00^{cd}
	PGPMs^{+}	154.45±3.65^{bc}	147.77±3.95^{bcd}	142.63±4.06^{bcd}	157.00±8.74^{bc}	177.74±6.86^{a}	157.46±1.40^{b}	154.55±4.91^{bc}

0 month; before plantation, PGPMs^{-}; without microbial consortium, PGPMs^{+}; with microbial consortium, dS m^{-1}; decisimen per meter, kg h^{-1}; kilogram per hectare, g cm^{-1}; gram per cubic centimeter, µgg-1; Microgram per gram, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)

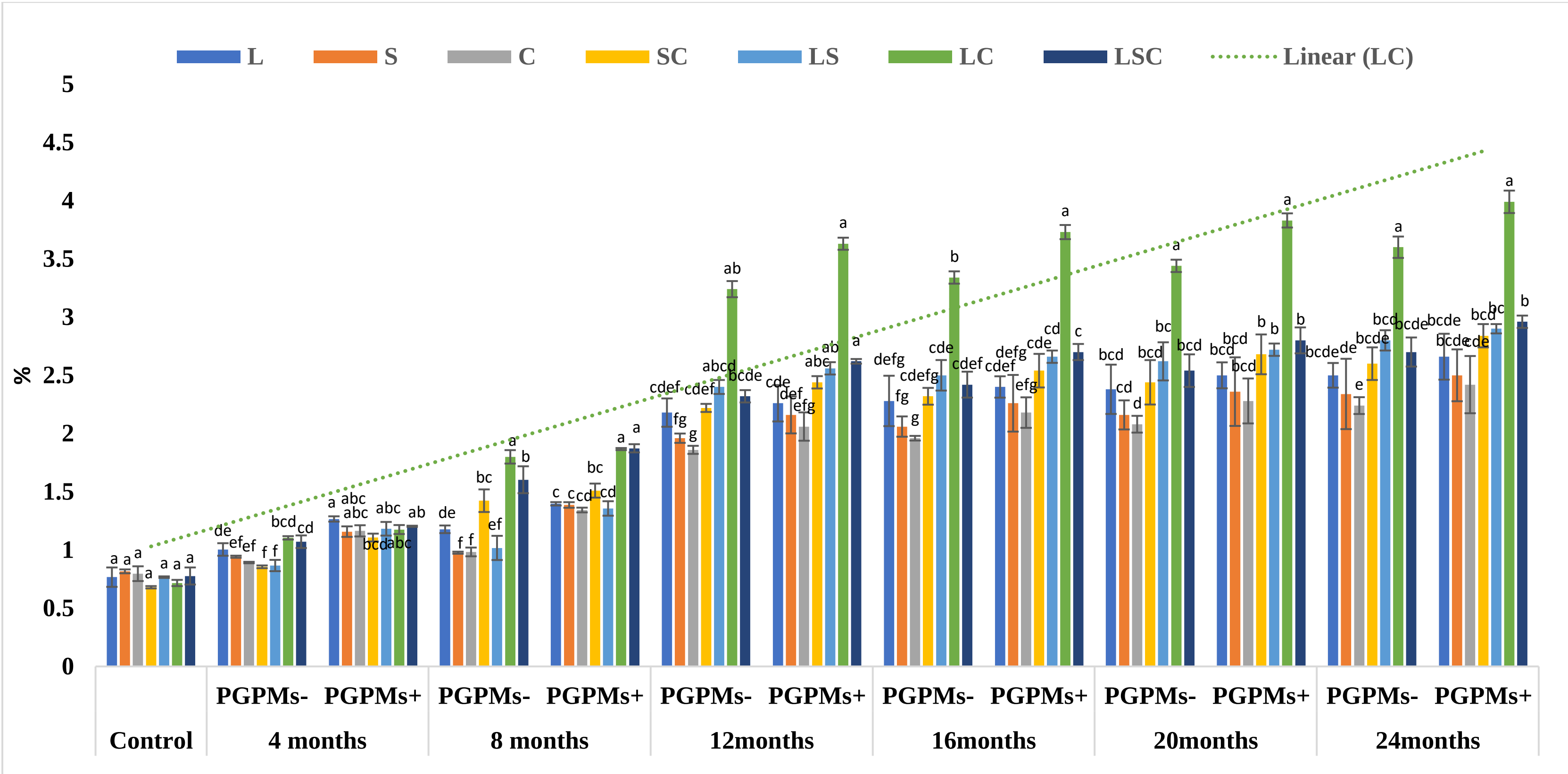


Figure – 4.26) Changes in soil total organic carbon (TOC) with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

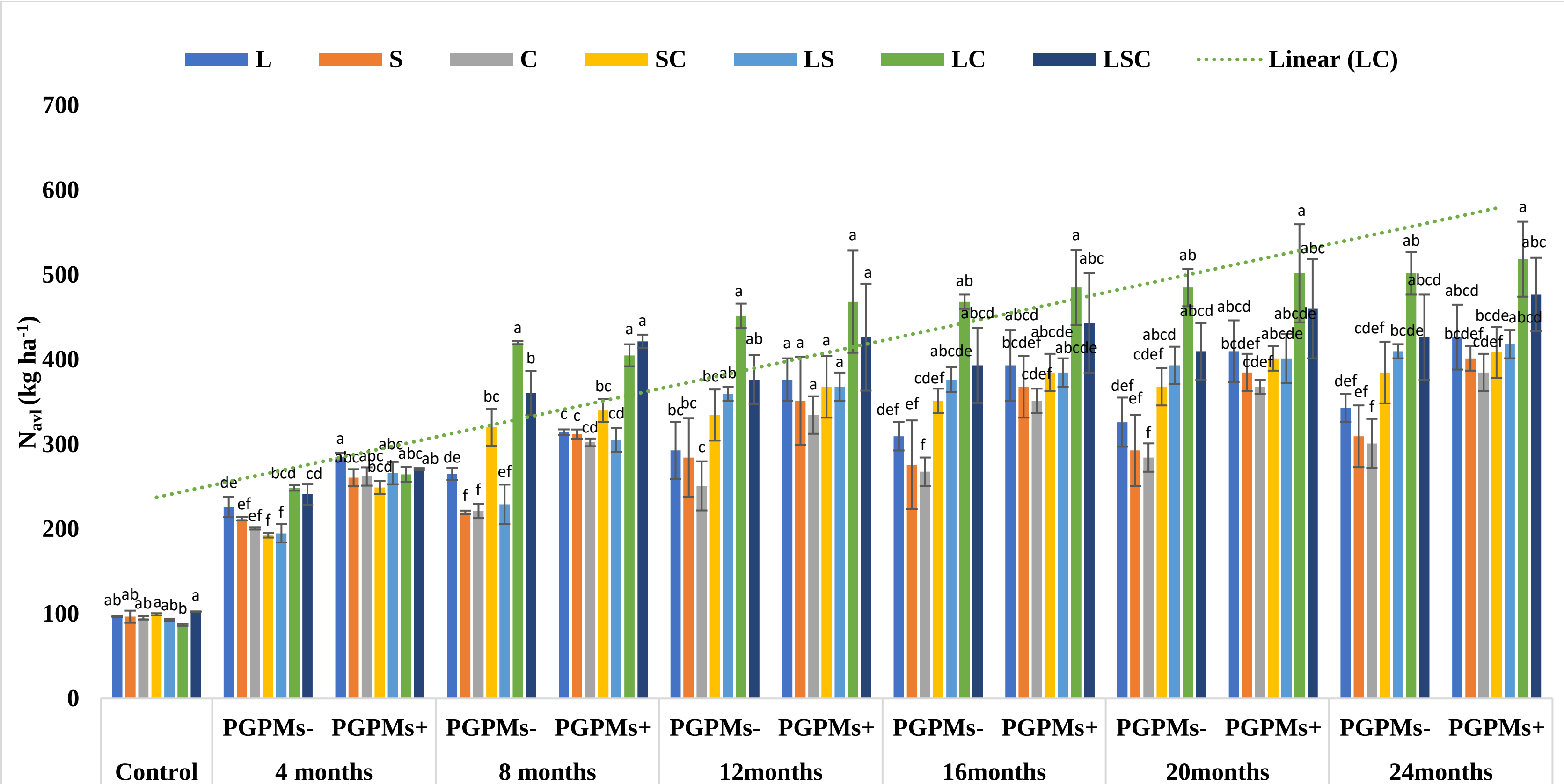


Figure – 4.27) Changes in Available Nitrogen (N_{avl}) with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium, kg h^{-1}; kilogram per hectare, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

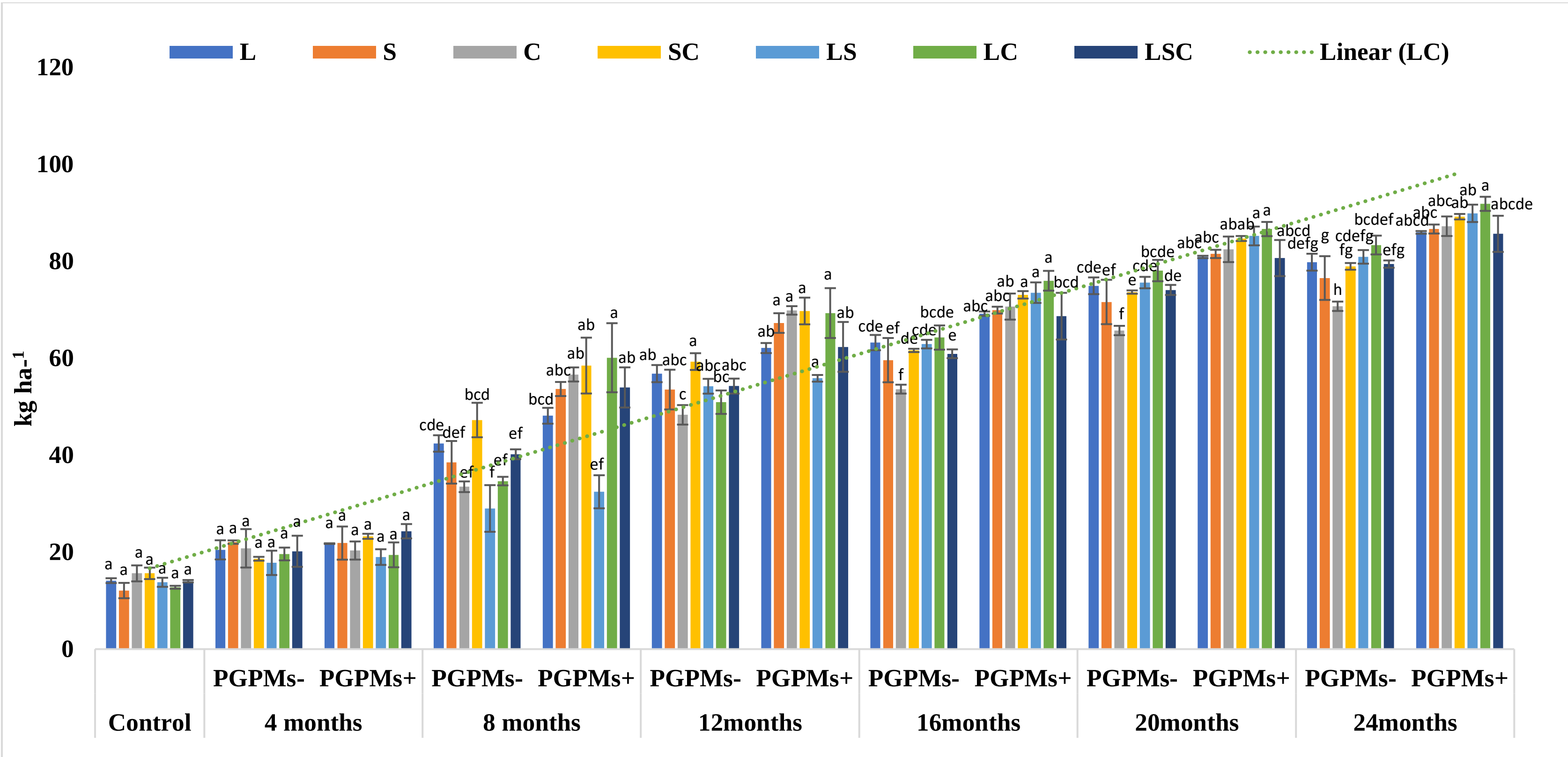


Figure – 4.28) Changes in Available Phosphorus (P_{avl}) soil properties with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium, kg h^{-1}; kilogram per hectare, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

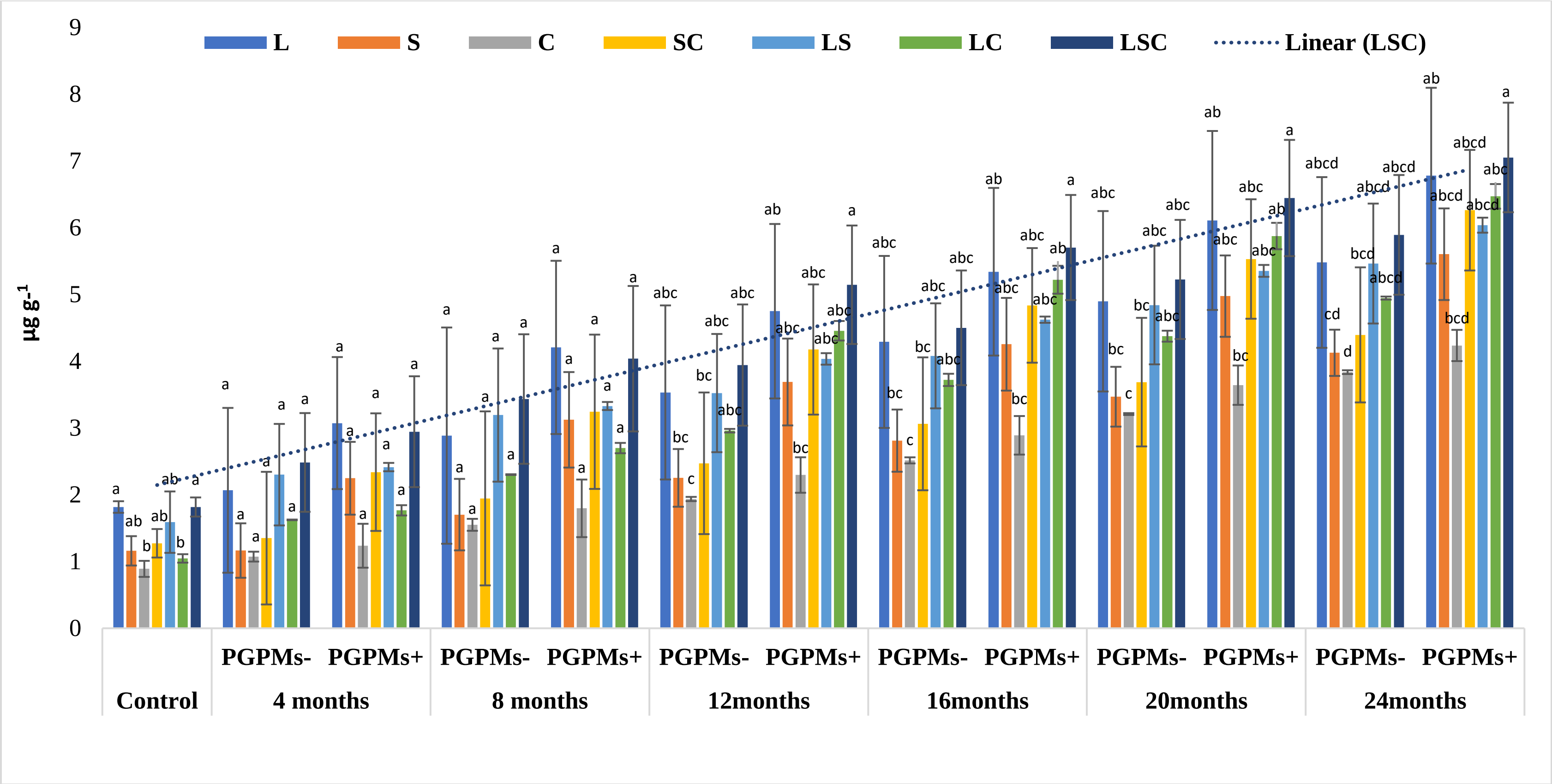


Figure – 4.29) Changes in Dehydrogenase (DHA)soil properties with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium,µgg^{-1}; Microgram per gram, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

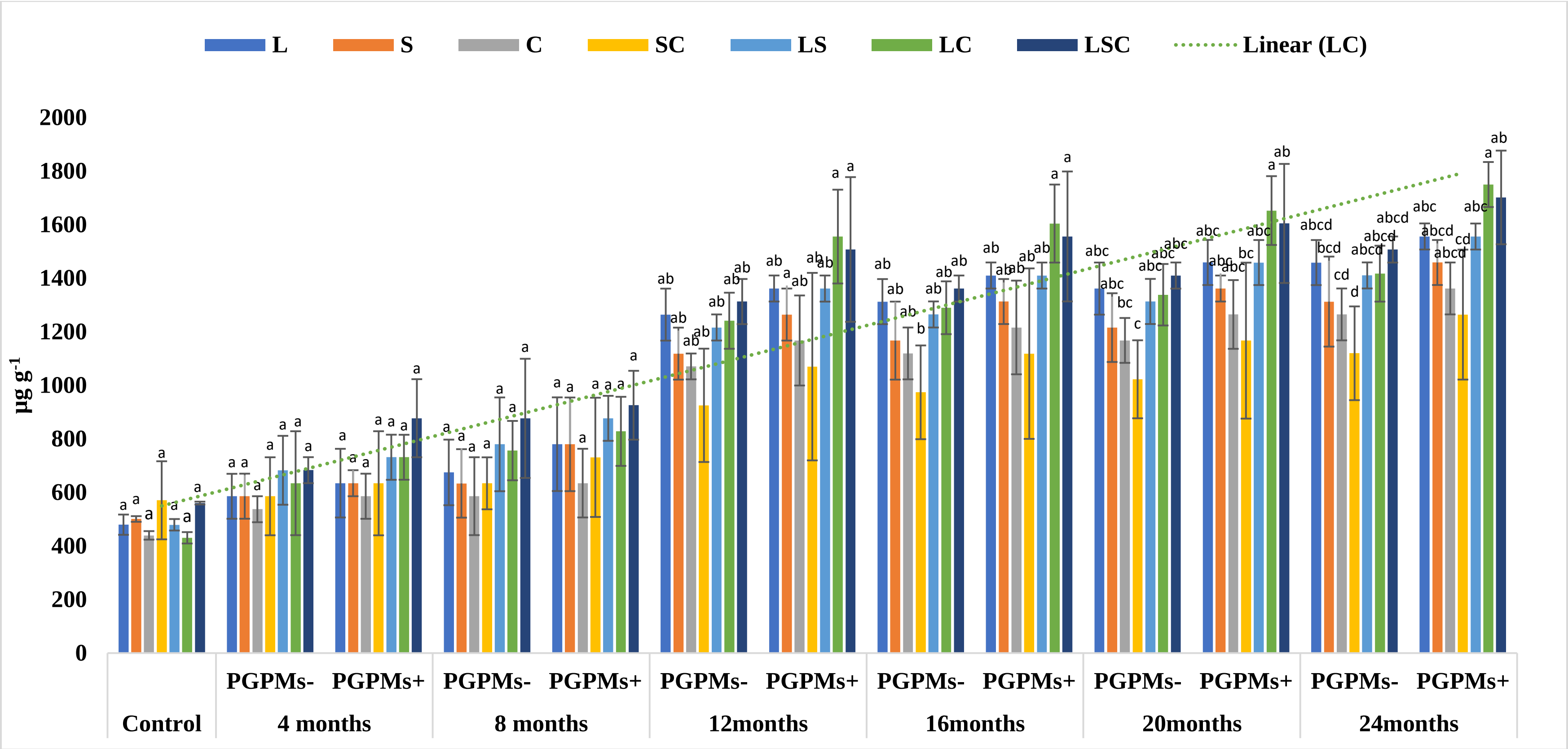


Figure – 4.30) Changes in Soil microbial Biomass Carbon (SMBC) with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium, µgg^{-1}; Microgram per gram, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

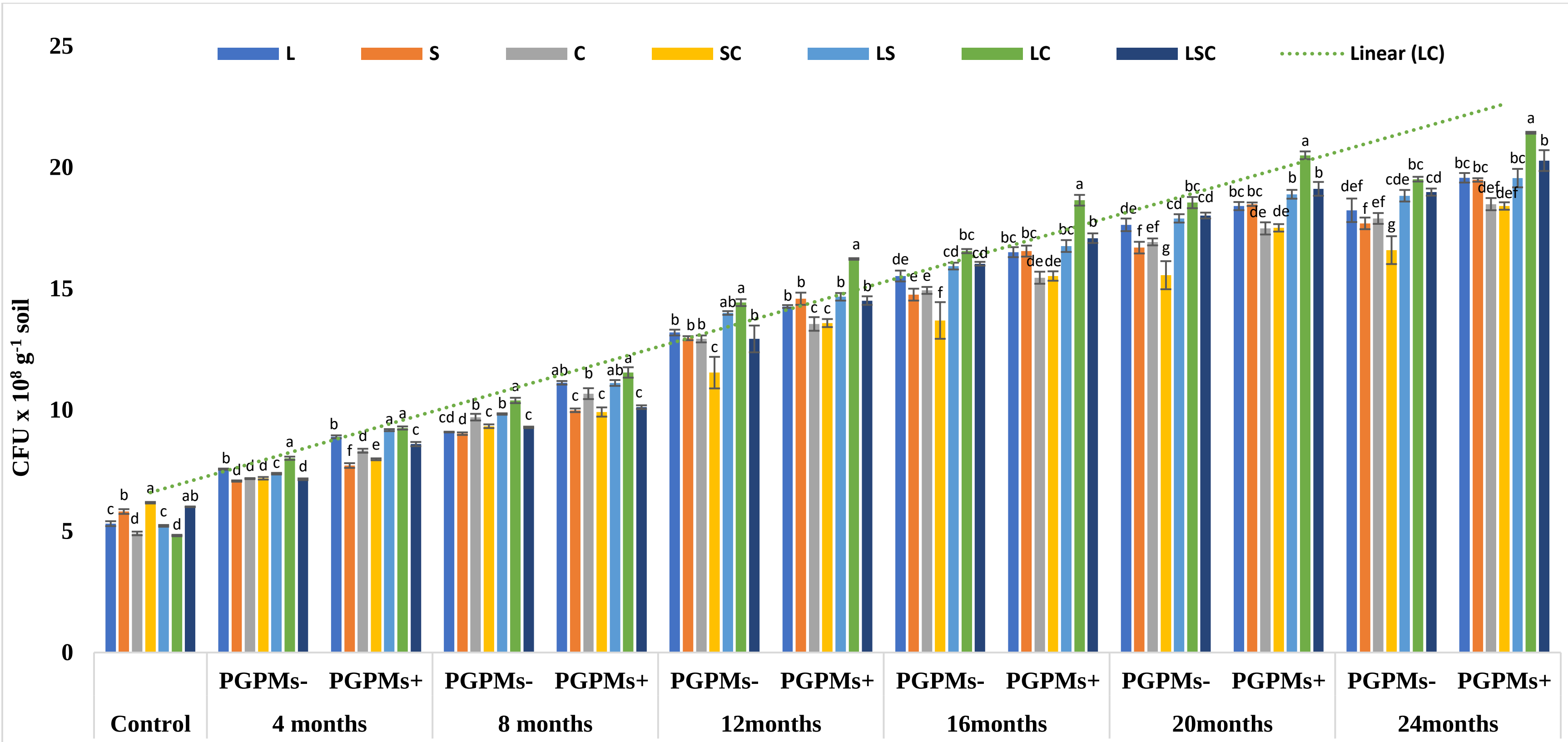


Figure – 4.31) Changes in soil bacterial Colony forming unit (CFU) with and without PGPMs at different species diversity levels and time intervals in two years *control; before plantation, PGPMs$^-$; without microbial consortium, PGPMs$^+$; with microbial consortium, L; Indian licorice, S; Stevia, C; Chilli, SC; stevia- chilli, LS, Indian licorice- stevia, LC; Indian licorice- chilli, LSC; Indian licorice- stevia-chilli, Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

Table- 4.17) Two-way anova for effect of species diversity levels and microbial consortium on soil properties, biomass production and carbon sequestration at different time intervals

Time interval	Dependent variables	Species diversity levels (Factor A)			PGPMs (Factor B)			Interaction (Factor A*Factor B)		
1st year (2019)		**DF**	**F-value**	**P- value**	**DF**	**F- value**	**P- value**	**DF**	**F-value**	**P-value**
	pH	6	0.710	**	1	4.879	***	6	0.495	**
	EC (dS m^{-1})	6	18.508	***	1	215.214	***	6	1.556	***
	OC (%)	6	6.106	***	1	116.991	***	6	2.584	NS
	BD	6	48.532	***	1	11.486	**	6	0.254	NS
	N_{avl} (kg ha^{-1})	6	6.148	**	1	117.87	*	6	2.645	NS
	P_{avl} (kg ha^{-1})	6	0.787	*	1	1.666	***	6	0.474	**
	K_{avl} (kg ha^{-1})	6	0.804	NS	1	5.688	*	6	0.573	NS
	Alkn Phosh (µg g^{-1})	6	2.135	***	1	18.571	***	6	1.017	NS
	DHA (µg g^{-1})	6	1.315	NS	1	2.349	**	6	0.209	NS
	MBC (µg g^{-1})	6	0.817	NS	1	1.476	NS	6	0.109	NS
	CFU x 10^8 g^{-1} soil	6	116.237	***	1	1529.111	***	6	23.467	NS
2nd year (2020)										
	pH	6	3.352	**	1	16.872	***	6	0.358	NS
	EC (dS m^{-1})	6	1.789	NS	1	19.462	***	6	0.325	NS
	OC (%)	6	19.899	***	1	6.701	**	6	0.189	NS
	BD	6	4.863	**	1	2.405	NS	6	0.196	NS

N_{avl} (kg ha^{-1})	6	6.617	***	1	9.08	**	6	0.622	NS
P_{avl} (kg ha^{-1})	6	3.845	*	1	80.067	***	6	1.536	NS
K_{avl} (kg ha^{-1})	6	0.948	NS	1	7.092	**	6	0.491	NS
Alkn Phosh (µg g^{-1})	6	5.281	***	1	33.166	***	6	1.208	NS
DHA (µg g^{-1})	6	2.291	NS	1	8.421	**	6	0.238	NS
MBC (µg g^{-1})	6	3.007	*	1	6.42	**	6	0.22	NS
CFU x 10^8 g^{-1} soil	6	21.619	***	1	73.621	***	6	1.625	NS
Biomass production and C-sequestration									
TDB Y^{-2}	6	1952.76	***	1	392.93	***	6	35.91	***
C-seq in Biomass	6	562.165	***	1	482.813	***	6	18.152	***
Soil C-seq	6	2.054	NS	1	0.226	NS	6	0.089	NS

*PGPMs; microbial consortium, EC; Electrical conductivity, TOC; Total organic carbon, N_{avl}; Available nitrogen, P_{avl}; Available phosphorus, K_{avl}; available potassium, Alkn Phos; alkaline phosphatase, DHA; Dehydrogenase, SMBC; Soil microbial biomass carbon, CFU; colony forming units, µgg^{-1}; Microgram per gram, TDB Y^{-2}; total dry biomass in two years, NS; not statistically significant at $p < 0{\cdot}05$. ***$p < 0{\cdot}001$. **$p < 0{\cdot}01$. *$p < 0{\cdot}05$. Data are mean of three replicas ± standard error of means of two independent set of experiments (n=6) Means, followed by the same letter in a column are not significantly different by Duncan's multivariate test (DMRT)*

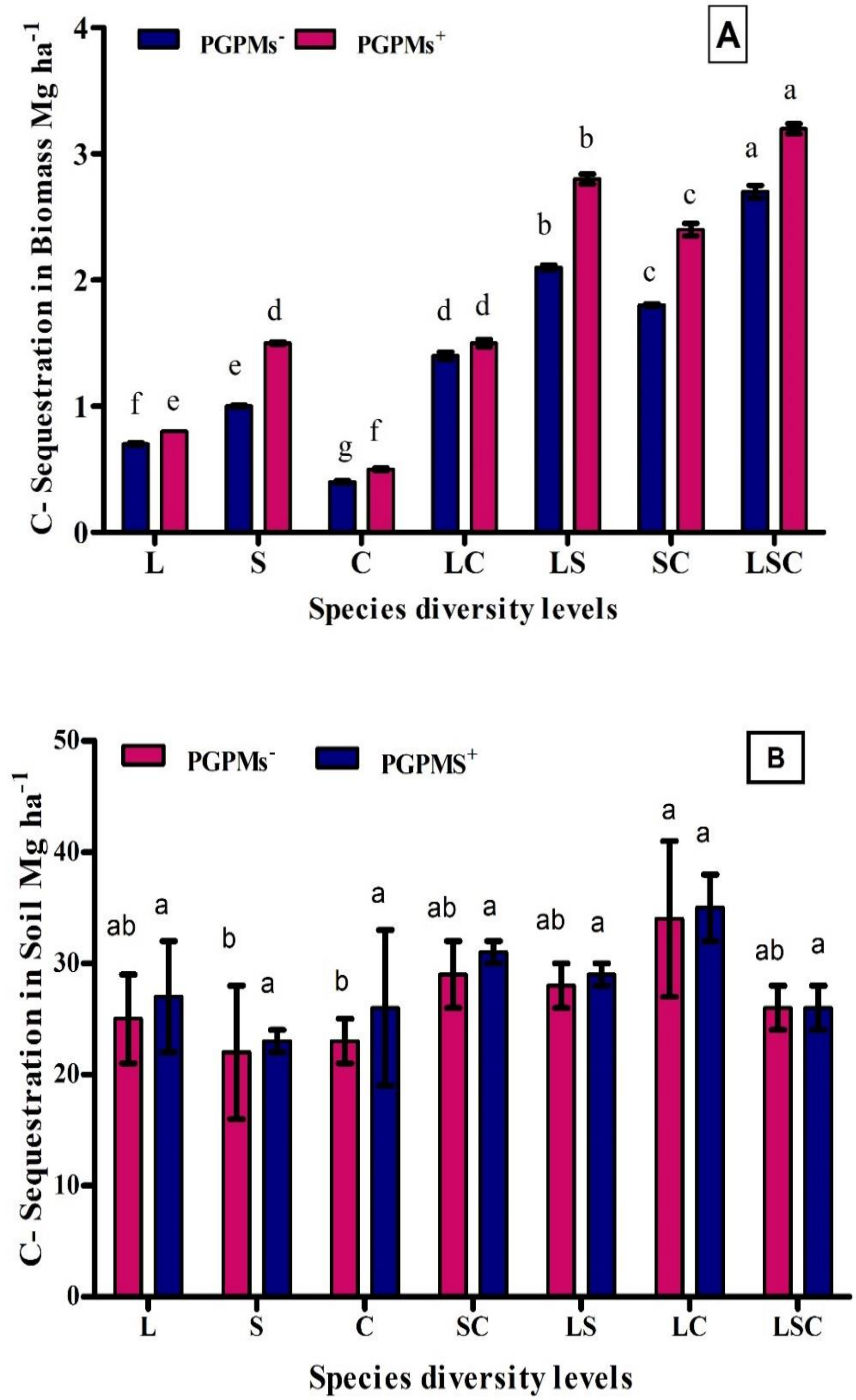


Figure- 4.32 A & B) Effect of species diversity levels and PGPMs application on Above ground and Below ground Carbon sequestration in two-year.

4.10 Economics of designed plant community

The highest gross return was observed in two species diversity level of LS (9.9 lacs) and LC (8.5 lacs) ha^{-1} y^{-2} with PGPMs (Figure 4.33). The income of LS was 25.13%, 25% and 36% higher than one species diversity level of Indian licorice, stevia and chilli while 14.14 and 34.34 % higher than the other two species diversity levels i.e., LC and SC. In case of one species diversity level highest (7.4 lacs ha^{-1} y^{-2}) cost was generated by Indian licorice with and without PGPMs as compare to other crops. The microbial consortium was highly significant for agriculture income with all possible. Three species diversity level LSC was not significantly effective for higher income generation.

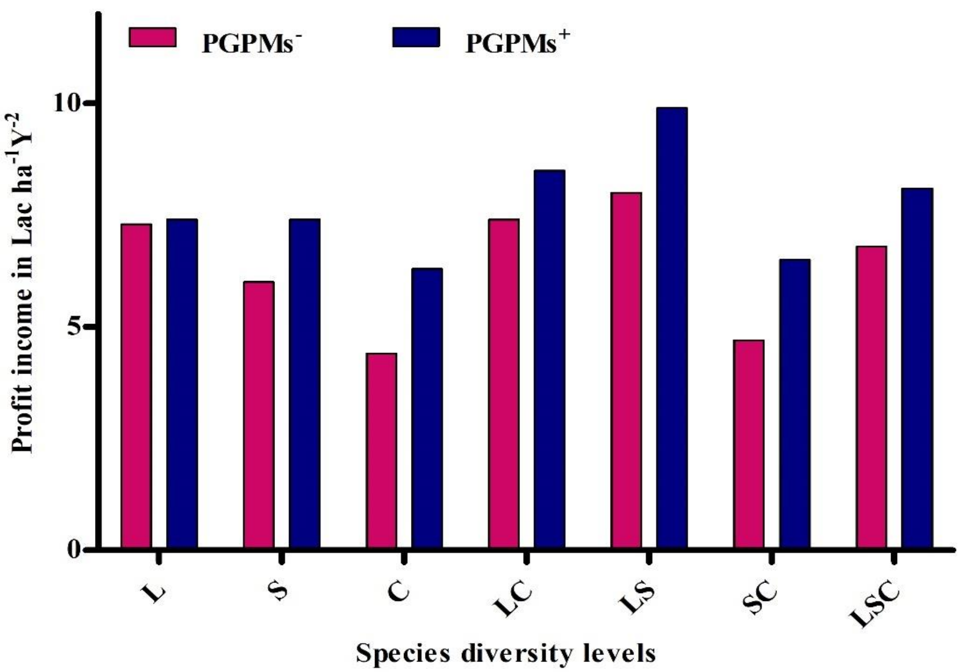


Figure – 4.33) Effect of species diversity levels and PGPMs application on total monetary benefits of the system (INR) in two-year. *cost was calculated by current market price of per kg of value products, Stevia dry leaves- 390INR, Chilli fruit (green)- 40INR (summer) and 80INR (winter), Indian licorice dry root- 1000INR*

Figure – 4.34) Experimental field with designed plant community; *A-Indian Licorice, B-Stevia and C-Chilli*

Discussion

Rural population density on marginal land is essentially a barometer of overall economic progress. They absorb rural migrants, growing population, and displaced unskilled labor from elsewhere in the economy as long as there is ample marginal land for cultivation. Smallholders that practice traditional agriculture get no rents because there is an endless supply of marginal land with low production (Barbier et al., 2014). The demand for biomass to drive a developing global bio-based economy is skyrocketing. As a result, various perennial biomass crops are already grown on marginal terrain (Francesco et al., 2020; Amaducci et al., 2017) but its cultivation with combination of other crops (Seasonal or annual) have never been cultivated yet which could be a novel approach to utilize such kind of marginal lands. Chemical fertilizers are crucial for global food production because they act as a fast food for plants, helping them to develop more quickly and efficiently. However, continued and imbalanced use of conventional chemical fertilizers disrupts soil ecology, degrades soil fertility, and, as a result, has negative health consequences and contaminates ground water. For these reasons, biofertilizers have been identified as a non-toxic input that helps to protect soil health and crop quality (Suhag, M. 2016).

5.1 Microbial diversity and their unique properties as biofertilizer

The inconsistent efficacy of microbes applied as biostimulants in soil, is one of the major bottlenecks for its commercialization and adoption as biofertilizers by the growers. The inconsistency may be due to their agility and consistency with endemic microbial populations, as well as their sensitivity to changes in soil physicochemical properties (such as pH, moisture, availability of minerals, C/N ratio and toxic materials)/ Microbes living in harsh climates may have developed genetic potentials for tolerance to abiotic stresses such as drought, salinity, temperatures, and toxic chemicals. The evolution of their nutrient acquiring capacities might have supported their co-existence in the rhizospheric niche of the plants growing in that climate. Certain reports are available on the microbial diversity and distribution of the microbial communities on ancient stone monuments and stones from mining sites., However, the studies on the microbial diversity of such rocky plateaus lying in between the human settlements and agricultural lands are rare (Kraková et al., 2012; Coutinho et al., 2013). Further, the microbial communities isolated from the rocky sites have rarely been explored for their PGP activities. The microflora on external surfaces of these stones usually represents a complex ecosystem with various types of bacteria, fungi, algae, lichens and protozoa etc. It is also reported that the composition and abundance of microflora were dependent on environmental conditions and detection methods.

The presence of fungi accelerates weathering of rocks and stones by its physical and chemical actions, such as a spread of fungal filaments and mycelia which penetrate underlying fissure and cracks of stones with secreting organic acids and enzymes (Li et al., 2016). The comparatively low biodiversity of fungi was observed (than bacteria) in the samples collected at genera level. Most isolated bacterial and fungal strains showed multiple plant growth promoting activities and tolerance to abiotic stresses (Table-4.4 and 4.7). various genera of fungus and bacteria are present in our sample which tend to produce siderophore for the iron chelating possibility (because the stone and soil samples consist high amount of iron).

We have isolated and cultured 5 bacterial and 4 fungal genera from these samples which were dominated by *Bacillus* (approximately 50% of all isolated bacterial communities). Similar results have been reported in the microbial isolated from rhizosphere soil of Dampa Tiger Reserve Forest and Reiek Mountain hill forest from

Mizoram, India (Passari et al., 2017) which indicate that such ecological niche may be suitable for plant growth on the rocky soils. The *Bacillus firmus*, *B. pseudomycoides*, *B. subtilis* and *B. filamentous* have been reported earlier as PGPR, isolated from the other sources (Han et al., 2014; Ahmad et al., 2019). In the present study, both strains of *staphylococcus* have shown the ability for ammonia production, IAA production and nitrogen fixation, while *S. hominis* exhibited phosphate solubilization and *S. haemolticus* showed siderophore production activities. Orhan et al. (2016) have also reported PGP activity in some species of *Staphylococcu*s, while Przemieniecki et al. 2019 have reported ammonia production and phosphate solubilization properties in *Staphylococcus hominis*. Our isolate *Alcaligens faecalis* has shown PGP activity similar to earlier reports, that impact the soil rhizoremediation and may benefit the adaptive processes of plants in extreme environments (Egamberdieva, 2008; Naseema and Bano, 2014). The bacterial strain *Pseudomonas stutzeri* and *Paenochrobactrum gallinarii* have shown nitrogen fixation, siderophore production and IAA production and these bacterial strains have never been reported as PGPM. Only a few studies are available on PGP activities of *A. aculeatus* (Narsian and Patel, 2000; Xie et al., 2017), *A. terreus* (Prajapati et al., 2013; Abdel-Ghany and Alawlaqi, 2018) and *A. luchuensis* (Yamada et al., 2016).

Our study for the first time reveals that bacterial strains i.e., *Paenochrobactrum gallinarii, Pseudomonas stutzeri* and *Staphylococus haemolyticus* and fungal strains i.e., *Penicillium polonicum, Fusarium chlamydosporum*, *Neurospora voucher*, *Epicoccum sorghinum* and *Aspergillus luchuensis* possess plant growth promoting activities and tolerance for salt, drought and temperature. Further, most of the microbes isolated in this study were able to tolerate stress (salt, drought, temperature and pesticide) but only fungal isolates had mineral solubilization potential along with tolerance against all the stresses. Among all the isolated strains, *B. firmus* showed highest salinity tolerance upto 25% NaCl, while most of the cultures were showing tolerance upto 15%. Similar study was assessed by El-Esawi et al., 2018 for the effect of highly salt tolerant species (*B. firmus* SW5*)* on growth and productivity of NaCl stressed soyabean plant. In our study, *B. filamentosus*, *B. pseudomycoides, B. firmus, staphylococcus faecalis* and *Paenochrobactrum gallinarii* showed water stress tolerance upto 30 % PEG level while other bacterial strains e.g., *pseudomonas putida* and *B. megaterium* produced higher IAA at highly stressed drought condition (60% PEG concentration) (Marulanda et a., 2009) which shows that these bacterial strains have developed some mechanism to cope with drought stress. The stone samples from which microbes were isolated consist of various minerals salt, low water availability, high temperature variability and poor vegetation on it, thus all these factors influence the microbes to evolve such properties for their survival.

The selected PGPMs e.g., *B. pseudomycoides* RS6B, *B. firmus* RS7B, *A. luchuensis* RS6F and *A. tamarrii* RS8F were able to enhance the survival and germination of chilli seeds over control. In similar study, an approximately 84.7% and 92.3% germination rate of moong and wheat was recorded by the application PGPMs (Mishra and Sundari, 2013). The PGPMs regulate various aspects of plant life, including seed germination, nutrition, growth, and response to biotic and abiotic challenges, through their multifunctional activities (Subramaniam et al., 2020a, b). The bioassay studies indicate that plant development and protection may be aided by PGPMs through direct and indirect mechanisms. Direct mechanisms help the plant grow either by providing nutrients or producing growth regulators by microbes, whereas indirect mechanisms help the plant grow healthy under abiotic stresses or protect it from infections, parasites, or certain predators as reported previously (Pandey et al., 2018; Maddhesiya et al., 2020; Arora et al., 2020; Mishra et al., 2021). Further, the enhanced seed germination rate and survivability were recorded by collective application of microbial strains as a consortium of two or more strains rather than single strains application which shows biofertilizer consisting microbial consortium would be more promising growth promoter than individual application (Jayshree and Jagadeesh, 2017; Bradacova et al., 2019).

The production of ammonium and IAA by the selected microbes were recorded upto 50% and 120% higher than the freshly isolated culture (1 year old) which indicates that these bioinoculants have gained certain PGP activities during the storage and reculture. The retention and enhancement of PGP activities for such a long duration give additional significance to these microbes to be used as a commercial bioinoculant for sustainable crop production. However, the microbial consortium of these selected microbes was found to produce 7-9 times higher IAA and ammonium as compare to the individual strains which indicate that these PGPMs are more effective if applied collectively. Many recent studies have demonstrated that the PGPMs consortia perform better than the individual PGPMs and it appears that commercialization may more adaptable for crop growers if such effective and long lasting bioinoculants will be formulated (Roshani et al., 2020).

5.2 Organic carrier, an effective tool for commercialization of biofertilizer

Carrier material supports the growth and delivery of microbes to the rhizosphere and have potential to preserve the microbes till their application (Brahmaprakash and Sahu, 2012). The two most essential criteria in carrier material selection are availability and price. Easy availability, cost-effectiveness, physical and chemical stability, non-toxicity, biodegradability, must be pollutant-free, easy processing, good buffering, and high moisture-holding capacity are all important factors that has to be consider when choosing carrier materials (Pacheco-Aguirre *et al*., 2017). Carrier suitability is primarily investigated to see if carriers can provide a desirable micro-environment for microbial strains injected into the rhizosphere to increase their survival and effectiveness. The carrier material may be organic (e.g., compost, peat, biogas slurry, crushed corn cob) or inorganic (e.g., zeolite, talc, perlite) in nature (Gunjal *et al*., 2012). Study of Abd-El-Fattah *et al*., 2013 revealed that due to their ease of use and long-term stability, carrier-based microbial compositions are extremely effective. Plant by-products, coal, talc, agricultural waste, organic waste, and bentonite were formerly employed as carrier materials for bioformulation but did not receive adequate market recognition (Stephens and Rask, 2000). Similarly, rhizospheric administration of liquid microbial inoculum does not improve crop productivity or growth (Zafar-ul-Hye 2019). Hence, In present study, mixing of microbes individually or in consortium with dry powder of cow dung and jaggery in the ratio of 9:1 increased cell/spore density up to 260 % in 15 days (Table 4.11). It has been demonstrated that cow dung powder provides a more suitable micro-environment and avoids rapid decrease of cells during competition with the better adopted native soil microflora (Sahu and Brahmaprakash, 2016). It appears that the organic source cowdung and jaggery increase the multiplication rate of the microbes if used as a carrier and may help the microbial inoculants to get established better in rhizospheric soil if applied in field conditions. As evidenced by the quality control study results obtained by Bello et al., 2019, cow dung is an excellent feedstock for the creation of biofertilizers. Use of cowdung as carrier is not new it has been previously used an organic carrier for preparation of bioformuation

(Gopalakrishnan et al., 2016).

5.3 Application of bioinoculant on plant growth and secondary metabolite production

Because of their role in nutrient cycling, environmentally friendly microbes have proven to be beneficial in plant growth promotion. Several studies have discovered that rhizobacteria affect the aroma of fruits such as strawberries and grapes (Verginer et al.010). Selected PGPR strains have been demonstrated to minimise illness in plant parts by inducing defensive chemicals, and members of the endomicrobiome, in particular, have been implicated in bioactive component production (Gutierrez et al., 2012). Bacteria and fungi have been identified as manufacturers of active compounds in various medicinal plants. Endophytes were shown to manufacture paclitaxel and maytansine, two key anticancer lead compounds. Because of its microbial endophytes, indigenous medicinal plants utilised in traditional remedies have been demonstrated to be active (Zhao et al., 2012).

In our study, the application of microbial inoculants mixed with the organic carrier in the earthen pots increased growth, biomass, and GA production of the roots, shoots, and leaves of *Abrus precatorius* by different magnitude (Table 4.12 anf figure 4.16). The bacterium, *B. firmus* RS7B as a PGPM was found best performing for the increase in growth and metabolite production in Indian licorice as an individual inoculant over others and control. With favourable microbial inputs, the diversity of rhizospheric and nodule bacteria has been found to be very high (Gyorgy et al., 2010). Study took also support from Swami et al., 2016 that application of rhizospheric microbes stimulates secondary metabolite accumulation in plants. Enhanced plant growth and metabolite production might be facilitated by better nutrient mobilization with microbial bioinoculants as reported in other plants (Awasthi et al., 2019; Maddhesiya et al., 2021). These solubilized nutrients are translocated from root to shoot and leaves following mechanisms of nutrients-transpiration xylem pathways. Several studies have shown that PGPRs and nitrogenfixing bacteria have a beneficial synergistic effect on plant growth and yield (Ipek et al., 2014). Swami et al., 2016 also suggested that application of plant growth promoting microbes can help medicinal aromatic plants to produce more physiologically active secondary metabolites. More research has been focused in recent years on the use of biologically active microbial inputs e.g., PGPMs in the cultivation of medicinal and aromatic plants to boost plant production (Karthikeyen et al., 2013). PGPMs boosted plant growth and development by regulating atmospheric N_2, expanding root surface area, and forming additional beneficial symbiotic interactions with the host plant (Vessey, 2003).

Many studies on medicinal and aromatic plant improvement employing rhizospheric microorganisms and the mechanisms involved are currently being conducted in order to choose the optimum microbial strain to be used as a commercial biofertilizer (Solaiman and Anwar, 2015). Meanwhile, in current study, the consortium of all four selected PGPMs (RSC4) showed the highest beneficial changes in plant growth as well as metabolite production (GA content) in different parts of the plant over single strains application. Thus, it may be recommended to develop bioformulation using a consortium of suitable bioinoculants in place of single microbial preparation. The consortia of PGPMs are well known to improve seed germination, plant growth, secondary metabolites, and plant productivity in many other plants (Rastegari et al. 2020a; Kour et al. 2020b; Rana et al. 2020b; Jayashree and Jagadeesh 2017; Rajasekhar et al., 2016 and Schoebitz et al., 2016). The treatment with consortium enhanced rosmarinic acid content in fresh basil by

110 percent, as per quantification of the profiles for the major phenolic acids (Ernesto et al., 2021). Many recent research has demonstrated the potential of microbial consortia, rhizobacteria, and rhizofungi as plant biostimulants (De Varies and Wallenstein., 2017; Wallenstein, 2017; Kong et al., 2018).

5.4 Effective bioinoculant for changes in soil fertility

The effect of prepared bioinoculants were further assessed for changes in soil fertility of pot inoculated soil after 4 months of study. The pH of the soil which was slightly alkaline before plantation decreased after cultivation of Indian licorice significantly which lowered further by the bioinoculation with various magnitude (Table- 4.13). The maximum decrease in pH, EC and organic carbon of soil with the microbial application may be due to the higher solubility of the salt in presence of these inoculated microbes. Babalola, 2010 has reported that the decomposed fine roots release organic exudates that reduce soil pH and increases soil organic carbon. The application of phosphate-solubilizing microorganisms can solubilize insoluble phosphate in soil by different mechanisms including enzyme production, secretion of organic acids, and excretion of siderophores making phosphates available for uptake in the plants (Rawat et al., 2020). It appears that during this study, the phosphate solubilizing fungal strain i.e., *A. tamarii* RS8F showed maximum increase in alkaline phosphatase individually as well as in consortium. Further, AN, AK and soil enzymes e.g., dehydrogenase (DHA), microbial biomass carbon (MBC), and CFU of the soil-applied with bioinoculants increased very significantly with a maximum enhancement in the pot applied with microbial consortium. The PGPM inoculants promoted plant seedling growth by enhancing soil enzyme activity such as soil urease, alkaline phosphatase, soil organic carbon, and accessible nutrient content such as available nitrogen, potassium, and phosphorus, among other things (Li et al., 2020). Increased SMBC (soil microbial biomass carbon) is directly related to the microbial activities that depend on the labile carbon pool (Trivedi et al., 2017). Hence, the highest microbial biomass carbon in soil treated with microbial consortium indicates that the application of microbes in combination promotes more microbial colonization and their positive interactions with the native microbes present in soil resulting in enhanced SMBC. Since a single strain and a single-function fertilizer can no longer fulfil the demands of modern agricultural development and provide the many nutrients required for crop growth and development, it is critical to develop a combination of PGPM (Li et al., 2020). When compared to a single strain in the environment, the combination of PGPM could potentially provide numerous roles and boost adaptability.

5.5 Effect of designed crop system on plant growth, Productivity and biomass generation

Our study indicates that ecological and economic benefits may require different kind of assembly of crop in the agroecosystem consisting of Indian licorice, stevia and chilli as cropping system. The maximum biomass production could be observed per unit area per annum when dry biomass of all three crops were added (Figure- 4.25 and 4.34). The hypothesis drawn for this study was to evaluate the beneficial impacts of crop diversity in the presence of PGPMs. There are only few perennial crops which can be grown on salt bearing land and usually trees are cultivated as agroforestry practices in such areas (Sera, 2017; Gyun, 2010). These tresses get matured in many years and therefore farmers do not get benefit of its cultivation consistently. Indian licorice can give economic benefits in a year or two after planting. On the other hands is a salt lover leguminous and perennial commercial crop which have buyers in pharmaceutical, cosmetics and other industries (Karawasar et al., 2010). The potential of commercialization of Indian licorice in marginal land along with other crops have not been explored out as per our database. We have attempted to cultivate another emerging crop stevia and chilli which have a great potential in growing market in all over the world including India.

In the present study, highly improved plant growth and yield was recorded for the crops grown with Indian licorice as two crop combination i.e., CL and SL over other combinations and sole crops (Figure- 4.22) which took support from Begum et al., 2015 and Ramesh et al., 2007 that mixing of chilli and stevia with other crops is preferable to growing chilli and stevia as a pure crop in terms of economic advantages and due to less competition among the component crops, legume-based intercropping has higher yield stability than the sole crops (Raseduzzaman and Jensen, 2017). The increased biomass of chilli and stevia in CL and SL combination was mainly due to overlapping of the emergence of the main crop with rapid growth and development of the combinations. Indian licorice was our main crop to utilize such kind of lands, which is slow growing at the initial stage so that other crops get the opportunity to grow faster due to lower competitive effect. Indian licorice is a leguminous crop that produces root exudates and makes surrounding soil more fertile to support other plants to grow well. This might be a region for the highest enhanced yield of chilli and stevia in CL and SL as compared to other combinations. Few other studies are available that indicate the growth and productivity of chilli in the intercropping/ combination systems are more beneficial than sole crop in many situations (Aravazhi et al.,1997; NataraJan, 1992; Sadashiv,2004).

The microbial consortium was significantly more effective for plant growth as compared to control crops which was supported by Vafadar et al., 2013 who reported similar findings for stevia growth and yield promotion. Thilagar et al., 2016 also supported our study that microbial consortia increase plant growth, dry weight of shoot, fruit yield and nutrient concentration of chilli. The microbial consortium may aid in the solubilization of plant nutrients from the native soil pool, the creation of helpful compounds (siderophores, hormones, and so on) for improved crop root growth, and the reduction of plant growth inhibiting hormones (Bahera et al., 2021). Due to the coverage of a varied collection of plant growth promotion and biological regulatory mechanisms, microbial consortia have been shown to boost positive features in plants when compared to individual strains. (Paredes et al., 2020; Ju et al., 2019). When compared to single inoculant treatments, a combination of two or more plant growth promoting bacteria (PGPB) can boost plant development in terms of leaf, root, and stem length, number of leaves, plant height, stress resistance, and pathogen control. To back up this assertion, research have demonstrated that inoculating plants with PGPB enhances their growth (Olanrewaju et al., 2019; Kumar et al., 2016; Shanmugam et al., 2013; Wang et al., 2012; Berendsen et al. 2018; Sharma et al., 2018).

The current study reveals that the highest biomass was generated by highly diverse species diversity level i.e., LSC which took favor from diverse mixture of legumes and other crops trends to generate higher biomass compare to monocrops (Yang et al., 2020 and Nabel et al., 2018). Cultivation of perennial leguminous plants on marginal lands could serve as a good alternative approach to generate higher biomass rather than annual crop on fertile lands (Nabel et al., 2018). As a result of diverse cropping, the three switchgrass mixtures produced the highest biomass and overall yield in the agronomic trial than the best performing monocultures (Morris et al., 2015). Due to the sampling effect, diversified combinations generally have higher productivity (Fargione et al., 2007) and may also promote niche complementarity (Cook-Patton et al., 2011), as is commonly found with wild and farmed interspecific mixtures (e.g., binary legume–grass mixtures) (Fargione et al., 2007; Nyfeler et al., 2009). In some other studies the plant mixtures are found to produce similar biomass that a highly productive monoculture can produce (Jungers et al., 2015). Tilman et al., 2006a; Picasso et al., 2011; Khalsa et al., 2012 have reviewed that enhancement of plant diversity is the basic approach for higher biomass yield from perennial crops. Tilman et al. (2006b) also found that biomass yields were more stable over time in polycultures than monocultures, and this stability improved with stand maturity. According to some study, adding plant species variety may help to overcome poor production restrictions by producing a more robust crop system (Bonin et al., 2018). Chapagagain et al., 2020; Couedel et al., 2018 a, b, c also suggested that legume and cereal treatment are the most effective strategies of producing increased biomass over a wide range of growth environments. Many studies reported that diverse cropping of perennial grasses is more productive than annuals for biomass generation (Maddheshiya et al., 2021; Chimyono et al., 2019; Manevski et al., 2017) but these are not fully agreed by Chen et al., 2022 as their findings on biomass yield and yield stability varied significantly over the 10 different cropping systems which might be differ in how they adopt to existing climatic conditions (Wendling et al., 2019), their resource use efficiency and strategies across various cropping system (Nouri et al., 2019). Hence, its need to aquire crop specific selection for higher biomass yield and stability according to existing agroclimatic conditions. According to research, diversifying agroecosystems and production forests has numerous advantages in terms of agricultural, forage, and wood production, yield stability, and a variety of regulating and supporting agroecosystem functions (Letourneau et al., 2011; Cardinale et al., 2012; Kremen and Miles 2012, Scherer-Lorenzen, 2014). Isbell et al., 2017 recommend using various mixtures even if yields are equivalent or slightly lower than the best monoculture because mixtures bring several additional benefits. Diversification could become an important tool for sustaining productivity and ecosystem services in croplands, production forests, and rangelands as demand for ecosystem services continues to rise (owing to population expansion and per capita consumption).

5.6 Intercropping Indices

A higher LER value than unity revealed more advantages of intercropping over sole crops. In our study LER values for all intercrops were higher than the standard which shows existing intercropping patterns are more profitable than other intercrops. Farhad et al., 2014 and Islam et al., 2004 also indicated that maximum land advantage has been reported by intercrops of chilli-garlic (1.35) and maize-bush bean (1.74) as compared to sole crop. Most intercropping indices covers only yield and agronomic advantages and do not pay attention for economic comparisons of intercroppings (Yayeh et al., 2014b). However, it is essential to accomplish some monetary valuations to satisfactorily comparison of the value of the yield advantages. The present study elucidates that intercropping of stevia with legume crop affected the MAI ($p < 0.05$) significantly. The positive and higher above one MAI value was recorded for all the intercropping (Figure 4.23). This proves that diverse intercropping systems were more economically profitable compared to sole cropping system. Indian licorice and stevia both are cash crops due to higher market price, they generated the highest monetary return in LS intercrop as compared to other intercrops and sole crops. This conforms to similar results by Dutta et al. (1994) on maize-rapeseed and maize- legumes combinations (Mekuanint, 2020).

5.7 Beneficial soil properties with effect of established crop system on marginal land

Generally increased soil carbon stock, microbial activities and high nutrients availability were observed during restoration of marginal lands (Mishra et al., 2003). These changes vary with applied restoration practices (physical, chemical and biological) (Singh and Abhilash., 2016). Likewise, in our study, improved soil nutrients, microbial enzymatic activities with enhanced carbon pool were observed after cultivation of established plant community over control (Table- 4.16). Several phytoremediation practices are already in use to combat marginal lands to restore its fertility (Singh et al., 2013c; Singh et al., 2012a, b; Haper et al., 2012; Vallejoa et al., 2012) among which the potential of leguminous trees (Family- Fabaceae) is also marked to improve biogeochemical characteristics of system through nutrient cycling (Kiran et al., 2009). In our study, significant changes in soil physicochemical and biological properties have been recorded after two years which may be possibly a consequence of consistent root formation by all the three plants in the variable combinations. Similar impacts were recorded with designed diversity of aromatic grasses in the similar agroecosystem by Madhhesiya et al., 2021. The highest increase was recorded by two species diversity level LC because legumes perform well in intercropping system by fixing atmospheric nitrogen, facilitation of water retention and availability (Gan et al.,2016 and Angus et al., 2015), improving soil organic matter and texture (Stagnari et al., 2017), phosphorous fixation and mobilization through secretion of organic acids like citrate, malate etc. (Shen et al., 2011) and reduced disease and pest pressure (Seymour et al., 2012). The by- products of legumes also favors development of plant growth promoting bacteria (Angus et al., 2015). Root nodules of *Abrus* were almost uniform throughout treatments, Hence, its specific impacts are not expected. Singh and Pandey 2012 also supported our study that rehabilitation of degraded lands through cropping practices improves soil fertility and promote restoration. In comparison to annual crops, perennial crops were found to maintain or increase soil C and N content (Culman et al., 2010; Taghizadeh-Toosi et al., 2014; Albers et al., 2020). The deeper and thicker roots, approximately 3-8 times more extensive to annual (Dietzel et al., 2017) with longer growing season may contribute to the greater root biomass which response of soil C pool to perennial crops over time (Ferchaud et al., 2016; Agostini et al., 2015). Greater root biomass also means higher rates of root exudation, which has been shown to promote soil aggregation and preserve soil C against microbial assault. Aggregation protects soil C by ensuring longer C residence durations, but it also benefits soil water holding capacity and water infiltration (Hernandez-Santana et al., 2013; Huang et al., 2014; McGowan et al., 2019). Furthermore, the reduced soil disturbance associated with perennial grasses as opposed to annual crops may contribute to the higher soil fertility associated with perennial crops (Carlsson et al., 2017; McGowan et al., 2019). Cultivation of perennial legumes-based intercrop (Maize-desmodium) promoted enhanced soil organic carbon and increased plant accessible phosphorus (Drinkwater et al., 2021). Perennial crops, whether harvested, grazed, or used for conservation plantings, have a high potential for conserving nitrogen for a variety of reasons (Moiser et al.,2021). Low post-senescence mortality Prior to senescence, perennial plants' ability to translocate nitrogen from aboveground leaves and stems to belowground roots, rhizomes, and root crowns is measured by N contents (Vergutz et al., 2012). The N will subsequently be re-translocated aboveground for usage during the next growing season, decreasing the demand for additional N (Yang and Udvardi, 2018). Perennial deep roots have a greater ability to collect inorganic N before it leaches from the soil profile, whether it is spontaneously mineralized from soil organic matter or supplied in fertilizer (Moiser et al., 2021). Biological nitrogen fixation is well known phenomenon in perennial legume crops. Legumes may meet up to 90% of their nitrogen needs using atmospheric N_2 in degraded soil through nodule bacteria.

Plant growth is limited by P, which is the second most limiting nutrient after N. P is required for the structure of DNA and RNA, enzyme synthesis, and the creation of ATP. Thus, in severely worn soils with inadequate P supply, P might limit plant productivity with N, or even directly limit output (Elser et al., 2007). Perennials have ability to reduce P losses and access P availability to plant (Crew and Brookes, 2014). Though perennial crops produce enhanced root biomass, soil organic matter as well as enhanced microbial biomass and activity, they can facilitate more available P to the plant through decomposition or dissolution of absorbed inorganic P (Gaxiola et al., 2011). These P can be released from minerals and organic material by microbial action and root exudation or inorganic acids. Plant residues and canopy cover will be retained for extended periods of time if degraded fields are perennialized. As a result, erosion, leaching, and runoff will be less frequent on these sites.

Organic fertilization increased bacterial diversity and boosted the growth of microbial groups (Firmicutes, Proteobacteria, and Zygomycota) that prefer nutrient-rich environments and are involved in the breakdown of complex organic molecules (Francioli et al., 2016). PGPMs (plant growth promoting microorganisms) are commonly used as biofertilizers. They help plants flourish by providing vital nutrients like nitrogen and phosphorus. Studies available on PGPMs as consortium application that plant growth is added by producing positive alteration in bulk soil and microbial communities around rhizosphere (Rilling et al., 2019). Over the fertilized uninoculated control, the application of microbial consortium resulted in considerably increased levels of accessible nitrogen (N), phosphorus (P), and potassium (K), as well as higher populations of Actinomycetes and Arthrobacter (Yaduwanshi et al., 2021). Enhanced nutrient content by the microbial application might be attributed to the highly multiplied soil microbes which converted organic to inorganic form as reported by Khandagle et al. (2020). Certain PGPMs, act as a nutrient enhancer, are able to solubilize P-minerals into soluble form via a variety of mechanisms including enzymatic oxidation-reduction, formation of chelates and complexes with amino acids, proteins, organic acids and so on. Besides producing siderophores and solubilizing P, they are known to create cocktail of enzymes which include amylase, cellulase, invertase, chitinase, lipase, peroxidase, keratinase, phytase, pectinase, protease, and xylanase which convert complicated nutrients into simple mineral forms. This nutrient cycling capacity makes them as an ideal microflora as natural fertilizers (Yaduwanshi et al., 2021).

Diverse microbial inputs (Consortium) and biomass carbon can improve soil porosity and aggregate stability (Kravchenko et al., 2019), which is aided by fungal hyphae and microbial extracellular chemicals (Helgason et al., 2010; Tiemann et al., 2015). PGPM-produced phosphatase enzymes selectively break P esters from organic matter, allowing P to be immobilized in microbial biomass (Richardson et al., 2009) which promote more P availability to soil after decay. Enhanced microbial biomass and activity by perennial legumes support higher release of plant available phosphorous. Microbes play an important role for converting total phosphorus into plant-usable forms by releasing adsorbed, inaccessible P from minerals and organic materials via metabolites and organic acids (Ingle and Padole, 2017). Because of higher root length, root surface area, and mycorrhizae substantially correlate with P uptake, having more roots and mycorrhizae in perennial systems enhances the likelihood of P uptake (Pang et al, 2010). As a result, having a healthy, diversified microbial population, microbial consortium will help in P availability and mobilization. The effect of microbial consortium integrated with 50% of recommended NPK found to be more effective in improved soil microbial biomass carbon, nitrogen, enhanced soil organic carbon, microbial population and respiration (Shukla et al., 2020).

5.8 Carbon Sequestration Potential of designed system

The terrestrial ecosystem has tremendous capacity to store atmospheric carbon for long periods of time. At the moment, terrestrial ecosystems are a net carbon sink of 3 GT year^{-1}, effectively buffering about a third of the annual rise in atmospheric CO_2 concentration due to greenhouse gas (GHG) emissions (Le Quere et al., 2018). On the other hand, agricultural ecosystems aone have the ability to store a significant amount of soil carbon (Sanderman and Baldock, 2010; Abdullahi et al., 2018). Hence, promoting sustainable crop cultivation has multiple benefits including increased crop and soil productivity, adaptation to climate change resilience, and high volume of above and below-ground biomass into the soil system for atmosoheric carbon sink. Improving plants to boost soil carbon sequestration represents an untapped and economically viable net carbon sink (Paustian et al., 2019a). Studies revealed that, the above ground plant leaves, stem, branches, fruits, forage, litter fall, wood and below ground plants dead roots, rhizospheric deposition, root exudates and microbial biomass carbon, all contributed directly to soil carbon sequestration. Carbon capture and storage has been particularly interested in abandoned agricultural sites because of their significant potential to carbon sequestration (Novara et al., 2017; Minasny et al., 2017). Soils store 3.5 times more carbon (C) than all living terrestrial plants in the form of soil organic matter, which is climatically relevant (Lal, R. 2004).

The legume-based crop pattern is well known to decrease soil disturbances and increase nitrogen availability and soil organic carbon sequestration. (Meena et al., 2019; Maillard et al., 2018; Srinivasarao et al., 2012). Likewise in our study, the highest below ground carbon sink was observed by cultivation of legume-based crop combination i.e., LC and monoculture of Indian licorice (L) (Figure 4.25) which is supported by Macedo et al., 2008 who explained these techniques are highly effective in atmospheric carbon sequestration along with recovering degraded lands. These cropping systems are also a mitigation strategy to combat climate change via storing atmospheric carbon in below ground soil. Perennial leguminous plants secret organic acids like citrate, malate etc. through its root to the soil promote higher atmospheric carbon storage (Shen et al., 2011). Macedo et al., 2008 also reported that leguminous tree uses for recovering degraded land are highly effective technique for sequestration of atmospheric carbon dioxide at high rates, respectively.

The highest above ground carbon sequestration with different Species diversity levels is directly related to higher above ground biomass generation. Yang et al., 2019 suggested that restoration of degraded land through highly diverse system (Diverse grass land) leads to accelerating 70% greater annual carbon storage than in monocultures which is related to higher above ground biomass production. According to the study conducted by He et al., 2021, diversified crops that incorporate canola and legume crops have the potential to capture more soil organic carbon than wheat-based cropping. Yang et al., 2018 also found that highly diverse grasslands have higher biomass yields resultant greater soil carbon capture, and root mass. In the near term, perennial diversification in the crop system considerably improved the annual soil C balance compared to diversified annual species (Martinez-Mena et al., 2021). Higher above ground species richness and belowground root biomass has a consistent positive influence on soil carbon capture in grasslands, forests and shrublands (Chen et al., 2018). Similar to our study, Jannson et al., 2021 has built a crop community with the dual functionality of being extremely productive for food, fuel, and feed while also improving the below-ground ecology and facilitating higher contributions to soil carbon.

5.9 Microbial Dependency

Plant microbe interactions are well known phenomenon to influence soil physicochemical and biological properties (Dubey et al., 2016). Therefore, the application of beneficial microbial bioagents with suitable formulation may be an appropriate option for sustainable agriculture (Kanchiswami et al., 2015 and Bhardwaj et al., 2014). As Smith- Hall et al. (2012) have also explained not to use chemicals for cultivation of medicinally important crops. So, in present study beneficials PGPMs (Consortium of four microbes) with suitable carrier were applied to the established plant community to replace chemical fertilizers.

In the present study, the highest microbial dependency was recorded for stevia as a sole crop which is supported by the studies that agriculturally beneficial PGPMs have higher efficacy on ecological cultivation of medicinal crops by promoting the availability of micronutrients to the host plant (Figure 4.24) (Mishra et al., 2021; Tahami et al., 2017).

Intercropping of Indian licorice were found less dependent on external microbial inputs which might be due to its belongingness to the Leguminosae family that can produce diverse root exudates in the form of compounds like organic acids, sugars, amino acids, structural carbohydrates and secondary metabolites (Walker et al., 2003) to enhance soil carbon pool and attract other beneficial soil microbes for growth promotion. Exudates may also boost the availability of some nutrients, like phosphorus (P) by releasing phosphatases and allowing P to be absorbed by plants (Dakora ana Phillips, 2002). Microbes surrounding the roots get their carbon energy from legumes, while legumes get their nutrition from nitrogen and phosphorus (Biate et al., 2015). This symbiotic association between plant and microbes makes them less dependent on biofertlizer. The root nodules of *Abrus precatorius* L. contain higher amount of ascorbic acid, ascorbate peroxidase and glucose than non-nodulated roots, which protects legume root nodules from the activated form of O_2 used for N_2 fixation for proper nodule functioning (Ghosh et al., 2014).

Application of microbial consortium showed significant effect on other plant species and their combinations for growth, productivity and biomass production over uninoculated controls. Highly improved soil quality was recorded by the effect of designed crop system with addition on microbial inoculants as a consortium. Several studies have been done on the beneficial effects of microbial consortium on plant growth promotion and soil fertility of polluted soil. Agriculturally beneficial PGPMs are highly effective for ecological cultivation of medicinal crops (Tahami et al., 2017 and Akhazari et al., 2015). These PGPMs promote the availability of micro nutrients to the host plant by assembling growth promoting chemicals, improved growth pattern of roots which releases divers root exudates (organic compounds) to enhance soil carbon pool and attract other soil microbes

(Drogue et al., 2013). A recent study published by Mishra et al., 2021 supported current findings that microbial consortium of *Bacillus pseudomycoides* RS6B, *Bacillus firmus* RS 7B, *Aspergillus luchuensis* RS6F and *Aspergillus tamarrii* RS8F has a great potential to improve soil fertility of marginal lands and production of secondary metabolites along with plant growth. Jansson et al., 2021 also supported our experimental findings and suggested that the significant improvement in soil carbon can be achieved by designing crop system with deeper root strength along with microbial consortium for better rhizospheric sink.

5.10 Economics of the system

Cultivation of bio-based crops on marginal lands are one amongst the best options to utilize such lands which are not suitable for conventional agriculture, have huge potential to provide agronomic and economic benefits (Zhu et al., 2016; Schmidt et al., 2015; Carlsson et al., 2017; Mehmood et al., 2017). At the economic levels, the crop selection and practices should sustain larger yield of high - quality products under favorable conditions, minimum input requirements and promote ecological restoration of marginal lands (Allwright and Taylor, 2016). Hence, industrial crops can supply ample renewable biomass feedstocks for the production of high-value bio-based commodities (such as bio-lubricants, bio-plastics, bio-chemicals, medicines, and so on) as well as bioenergy. The majority of these crops are versatile that may be utilised in a cascade bio refinery to produce a variety of value-added bio products and bioenergy, boosting the bio-based economy. Several studies have identified perennial crop mixes cultivated under low-input agriculture as viable cropping systems for higher agronomic and economic benefits (Carlsson, et al., 2017; Mehmood et al., 2017; Robertson et al., 2017). Its production can help farmers diversify their income and provide jobs, improving the living and economic standards of local populations (Valentine et al., 2012).

In present study, a crop system is designed with mixtures of two highly important medicinal, commercial perennials and one commercial seasonal crop to get benefited with higher economic advantages throughout the year which were cultivated on marginal land in all possible combination with microbial consortium as fertilizer source. However, the income generation per annum from the same unit area was maximum if only two crops i.e., Indian licorice and stevia when grown together, possibly due to higher market value (Figure 4.26). The cost was calculated as per year per hectare yield generation basis. Chilli produced highest annual yield but due to less market price, it could not generate high income. Chilli is also a commercial spice and with combination of other cash crops supported enhanced income to the growers.

Cultivation of such kind of designed crop system have multiple benefits which includes -

1- It offers nations to increase domestic production of feedstock for the growing bio-based economy by reducing its dependency on imports.

2- Improves the livelihood in marginal areas by placing abandoned marginal land in use again, thus improving farm income.

3- Mitigate the competition between the production of bio based raw materials and food production.

4- Creates new business models for value chains, leading to economic growth and job creation.

5- Mitigate further environmental and ecological risks by soil abandonment such as soil erosion and natural hazards.

6- Decreases pest pressure, including diseases, insects and weeds

7- Enhances beneficial pollinator populations.

Summary and Conclusion

Increasing the income of crop growers per unit area and restoring the fertility of marginal soils are major emerging challenges of agricultural sustainability in Indian agrarian economy. Agriculture, as a whole has been suffering with the increasing difficulty of low income and ecosystem degradation. As a result, farmers are leaving the farming as occupation. One of the major cause of land degradation is to excessive use of chemical fertilizer and pesticides which are toxicating the land resources, resulting in large economic and associated environmental consequences. Nearly 80 percent of the farmers in India belong to the marginal or small land holding category with 1-2 ha land or even less. Approximately 6.73 million ha land categorized as marginal land due to high alkalinity/ salinity which has lost its fertility for conventional agriculture. Rebuilding of such kind of degraded and marginal lands require sustained economic support to the farmers with better management of cropping patterns and product marketing. There are multiple biological and chemical practices are in use to improve the quality of marginal and degraded land. Several salt or alkali tolerant varieties of crops like rice, wheat, mustard, Acacia sps., date, palm, sorghum, biofuel crops and useful perennial grass species have been developed which can be grown on the marginal soil. The ecological and economic cultivation of Indian licorice, stevia and chilli as a highly paying cropping system in slightly alkaline land has been attempted in this study. Selection of species with its comparative commercial value, compatibility and the ability to withstand a variety of agroclimatic conditions including salt and drought stresses has recently made significant progress in reimagining, recreating and redesigning the degraded agroecosystems.

Our question was to assess the role of climate resilient plant growth promoters in supporting growth and productivity of high valued perennial and short - term crops like

Indian licorice, stevia and chilli in a suitable crop combination on a slightly alkaline soil (pH- 8.3, EC- 1.0 ds m^{-1}, ESP – 19 percent) with economic and ecological benefits to the growers and soil fertility.

We hypothesized that assembling novel food and non-food crops in a cropping system and rhizo-engineering of soil inoculants can enhance the productivity of multiple crops at the same time and can improve soil fertility for sustainable agricultural production without use of agrochemicals. The increase in soil microbial biomass can increase carbon sequestration in soil and the same field can be utilized for different annual and perennial organic agriculture products.

Microbial communities are essential for healthy soil growth and biological interactions, as well as the construction of sustainable plant communities and the proper functioning of the ecosystem. These bacteria have the potential to replace agrochemicals, which have a variety of detrimental effects on agricultural output, human health, biodiversity, and ecosystem function. Beneficial soil bacteria can assist in fulfilling the United Nations' 2nd Sustainable Development Goal, which aims to boost agricultural output and incomes by 2030 in order to end poverty and malnutrition while also ensuring sustainable food production systems. Beneficial soil microorganisms derived from various ecological niches have been treated exogenously to boost plant output in agricultural fields. Due to rhizospheric pressures and variability in specific agro-climatic variables such as salinity, pH, and other abiotic stresses, the efficiency of these exogenously applied microbial inoculants is typically inconsistent. Though native soil microbes are often thought to be better soil inoculants because of their habitat suitability and stability. Hence, A good number of climate resilient agriculturally beneficial bacterial and fungal strains have been isolated from dry plateau to Ralegarh Siddhi, Maharashtra and demonstrated for their plant growth promotion activities and high tolerance to salinity, drought, temperature variability and pesticide. The bioassay of the selected biostimulants as a consortium indicates that these microbes have good potential to develop climate resilient biofertilizers for enhanced plant growth, productivity and secondary metabolites (glycyrrhizic acid) production in Indian licorice. A successful climate resilient PGPM formulation for Indian licorice can help reduce a load of agrochemicals in the agroecosystems during its cultivation. The microbial bioinoculants developed by us have also improved soil fertility very significantly in pot conditions. Further its efficacy was assessed for changes in soil fertility of marginal land along with newly established crop community.

The objectives of the present study were aimed to find out the benefits of crops diversity levels among the selected crop species of high economic values in an ecological cultivation system in which beneficial soil microbes can be used as sole external amendments. In present study total seven crop combinations of Indian licorice (*Abrus precatorius* L.), Stevia (*Stevia rebaudiana* B.) and Chilli (*Capsicum annum* L.) were cultivated in all possible diversity levels with and without microbial application for two years and the benefits of these designed plant communities and microbial consortium of four plant growth promoting bacteria and fungi (*Bacillus pseudomycoides* RS6B, *Bacillus firmus* RS7B, *Aspergillus luchuensis* RS6F and *Aspergillus tamarii* RS8F) were assessed on production of Plant growth, productivity, biomass generation, above ground and below ground carbon sequestration, income of growers and improvements in soil fertility in two years. The maximum increases in biomass (6.8 Mg ha^{-1}), CEY (17765), LER (3.94) value and above ground carbon sequestration (3.2 Mg ha^{-1}) were found in three species diversity levels LSC (Indian licorice-stevia-chilli) with plant growth promoting microbes (PGPMs) whereas the highest below ground carbon sequestration (35 Mg ha^{-1}) and maximum soil fertility benefits e.g. pH (1.16 times), Electrical conductivity (7.53 times), Bulk density (1.84 times), soil total organic carbon (3.99 times), available nitrogen (5.95 times), dehydrogenase (6.16 times), microbial biomass carbon (4.06 times) and colony forming unit (4.44 times) were recorded at two species diversity level LC (Indian licorice-chilli) with and without PGPMs over the soil properties before cultivation of these plants. The highest net income (9.9 Lac ha^{-1} Y^{-2}) was estimated for the two species diversity level possessing Indian licorice and stevia (LS) with effect of PGPMs. The highest productivity was recorded for chilli in two Species diversity LC (Indian licorice-chilli) and the highest microbial dependency was found for sole stevia cultivation i.e., 50.81%.

Indian licorice was first time cultivated with combination of other crops on marginal land which improved soil fertility along with multiple ecological and economic benefits. It is suggested that Indian licorice can be cultivated with other compatible crops like stevia and chilli with suitable microbial amendments of beneficial soil microbes. It will help in economic and ecological utilization of marginal and degraded land and give better productivity. The crop cultivation with increased crop diversity can be considered as an attempt to improve the delivery and stability of ecosystem services with enhanced crop productivity and income. Considering the experimental findings, it is concluded that cultivation of Indian licorice and stevia as combine cropping system may be the best combination for maximum economic return and highly diverse cropping pattern i.e., LSC would become a highly profitable cropping pattern for maximum biomass production, above ground carbon sequestration as well as land equivalent ratio and crop equivalent yield over other less diverse crop pattern and sole crops. Designed diverse cropping practices are more efficient in the utilization of resources under stress conditions than the conventional monoculture crop practices.

Key findings of the study

- Newly established plant community of *Abrus precatorius* L., *Stevia rebaudiana* Bertoni and *Capsicum annum* L. are highly effective for biomass generation, income of growers and soil fertility of marginal lands.
- The amended microbial consortium of bacteria and fungi to the plant community produces more income with enhanced soil fertility.
- Highest below ground and above ground carbon sequestration was recorded by two and three crop combination levels of LC and LSC, respectively.
- Three crop combination levels of LSC produced highest biomass, crop equivalent yield (CEY) and land equivalent ratio (LER).
- Two crop combination levels of LS benefited with maximum monetary advantages.
- The highest productivity was recorded for chilli crop in two crop combination of LC.
- The highest improvement in soil fertility was achieved by cultivation of two crop combination LC with and without microbial inputs.
- The highest microbial dependency was found for sole stevia cultivation.

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Publications

Research articles

Roli Mishra, Priya Dubey, Rana Pratap Singh (2021). Assessing the efficacy of climate resilient microbial inoculants for enhanced phytochemical production from Indian licorice (*Abrus precatorius* L.). ***Medicinal Plants***, 13(2), 330-338.

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Research Article

Assessing the efficacy of climate resilient microbial inoculants for enhanced phytochemical production from Indian licorice (*Abrus precatorius* L.)

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ABSTRACT

Chemical fertilizers adversely affect beneficial micro-organism of the soil with high residual contamination in the plants which subsequently raises questions on the quality of useful metabolites of medicinal plants. The study investigates the efficacy of plant growth-promoting microbes, i.e., *Bacillus pseudomycoides* RS6B*, Bacillus firmus* RS7B*, Aspergillus luchuensis* RS6F*,* and *Aspergillus tamarii* RS8F, individually as well as in consortium on growth and glycyrrhizic acid content of Indian licorice (*Abrus precatorius* L). The maximum increase in biomass of root, stem, and leaves was obtained as 2.48 folds, 2.40 folds, and 2.41 folds along with 4.95, 4.63 and 2.61 folds in glycyrrhizic acid content, respectively on the rhizo-engineering of the consortium of all the 4 microbes. The microbial inoculants also increased soil organic carbon (1.44 times), available phosphorus (3.06 times), and microbial biomass carbon (3.01 times) in the soil after 120 DAS over non inoculated control. It appears that the microbial consortium of these 4 PGPMs is a highly efficient growth promoter over no microbes and individual PGPM for Indian licorice. It can be developed as a potential bioinoculant for the ecological cultivation of this important and commercial medicinal plant.

Keywords: Glycyrrhizic acid, plant growth-promoting microbes, soil microbial biomass carbon, sustainable agriculture

Abbreviations: PGPMs = Plant growth promoting microorganisms; **GA** = Glycyrrhizic acid; **PGP** = Plant growth promotion; **HPLC** = High performance liquid chromatography

INTRODUCTION

Agrochemicals and pesticides have been indiscriminately used not only in food crops but also in aromatic and medicinal crops to increase crop growth and productivity since their adoption during the green revolution (Pereira *et al.*, 2019). However, they adversely affect native beneficial micro-organisms of the soil with high residual contaminations in the plant which subsequently raise questions on the quality of its useful metabolites and has been suggested to avoid chemical fertilizers and pesticides for the cultivation of medicinal and aromatic plants (Arora *et al.*, 2018). Applications of agriculturally beneficial microbes popularly known as plant growth-promoting microbes (PGPMs) or bioinoculants have been reported to be highly effective in the cultivation of medicinal plants (Tahami *et al.*, 2017). Some microbial bioinoculants have recently been reported to increase the stomatal density and

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Abstract

Increasing the income of crop growers per unit area and restoring the fertility of marginal soils are major emerging challenges of agricultural sustainability in Indian agrarian economy. This study investigates the benefits of plant communities consisting of Indian licorice (*Abrus precatorius* L.), Stevia (*Stevia rebaudiana* B.), Chilli (*Capsicum annum* L.) and microbial consortium of four plant growth promoting bacteria and fungi (*Bacillus pseudomycoides*, *Bacillus firmus*, *Aspergillus luchuensis* and *Aspergillus tamarii*) on production of plant biomass, income of growers and improvements in soil fertility in two years. The three crop species were planted in all possible groupings at one, two and three species diversity levels with three replication and two treatments i.e., with PGPMs and without PGPMs. The maximum increases in biomass (6.8 Mg ha^{-1}) and above ground carbon sequestration (3.2 Mg ha^{-1}) were found in three species diversity levels of LSC (Indian licorice-stevia-chilli) with plant growth promoting microbes (PGPMs) whereas the highest below ground carbon sequestration (35 Mg ha^{-1}) and maximum soil fertility benefits e.g. pH (1.16 times), Electrical conductivity (7.53 times), Bulk density (1.84 times), soil total organic carbon (3.99 times), available nitrogen (5.95 times), dehydrogenase (6.16 times), microbial biomass carbon (4.06 times) and colony forming unit (4.44 times) were recorded at two species diversity level of LC (Indian licorice-chilli) with and without PGPMs over the soil properties before cultivation of these plants. The highest net income (9.9 Lac ha^{-1} Y^{-2}) was estimated for the two species diversity level possessing Indian licorice and stevia (LS) with effect of PGPMs.

Supporting Information

Document Information

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Sources included in the report

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