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Response of microorganisms and soil enzyme activity in groundnut rhizosphere under different nutrient management system and its in *vitro evaluation* of antagonistic efficiency against foliar pathogen *Alterneria porri*

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ABSTRACT

The effect of different nutrient management practices on microorganisms and soil enzyme activities in groundnut rhizosphere was investigated in the present study. This research faced challenges such as variability in soil microbial populations under different farming systems and the difficulty of isolating effective biocontrol agents. Despite these challenges, the study provides significant contributions by identifying specific bacterial isolates with potential antagonistic effects against foliar pathogens and offering insights into the efficacy of nutrient management practices. The effect of different nutrient management practices on microorganisms and soil enzyme activities in groundnut rhizosphere was investigated in the present study. Depending on the present farming trend, the microbial distribution in soil practices with natural farming, organic farming, conventional farming and farmer's practice was analyzed. A total of twenty soil samples at different intervals were collected from different types of management practices under groundnut at Zonal Agricultural and Horticultural Research Station (ZAHRS), Hiriyur, Karnataka, India.Three years pooled data revealed that, Among the different nutrient management practices at harvest stage, the chemical farming practice (RPP) showed higher number of bacteria, (42.07 x 10-5cfu g-1 soil), fungi (13.22 x 10-4cfu g-1 soil), actinomycetes (8.07 x 10-3cfu g-1 soil) and beneficial organisms viz., Rhizobiumsp,Azotobactersp, PSB, Pseudomonassp(11.16,12.08, 8.33 and 13.43 x 10-5cfu g-1 soil respectively) and Trichodermasp (2.25 x 10-4cfu g-1 soil).Similarly, soil enzyme activities viz., dehydrogenase (90.35 µg TPF g-1 soil day-1), Urease (35.65 µg NH4+ g-1 soil 2 hr-1) acid and alkali phosphatase (20.54 and 16.18 µg PNP g-1 soil) compared to other practices. A total of thirty twobacterial isolates.e. eight isolates from each practice were selected based on their morphological and biochemical characteristics and tested their antagonistic effect on groundnut foliar pathogen Alterneriaporri under in vitro dual culture bioassay technique.Out of that, only seven isolates from different practices were inhibited foliar pathogen Alterneriaporri. The maximum inhibition ofpathogen by NA-2 isolate from natural farming practices (47.02 %), followed by Azo-2 isolate from organic farming practice (46.30 %), NA-1 isolate from chemical farming practices (41.11 %) and PSB -1 isolate from organic farming practice (40.74 %). These seven isolates were identified at the species level based on the 16S rRNA gene. Molecular identification based on 16S rRNA gene revealed seven species belonging to Pseudomonas, Serratia, Azotobacter, and Bacillus genus.

Keywords: *Groundnut, Soil enzyme activity, Alternariaporri, Azotobacter, Natural farming, Organic farming, Pseudomonas, Sustainable agriculture*

INTRODUCTION

Soil microorganisms play an important role in soil fertility not only because of their ability to carry out biochemical transformation but also due to their importance as a source and sink of mineral nutrients [14]. Soil microbes, the living part of soil organic matter, function as a transient nutrient sink and are responsible for releasing nutrients from organic matter.

The broad spectrum of agricultural practices further limits comparability among different studies [9],[13]. Whereas, natural farming promotes a natural catalyst of biological activity in the soil by application of beejamrutha, jeevamrutha and mulching and natural protection from pests and diseases by spraying Neemastra, Agniastra *etc* [23]. Organic systems are lacking the application of synthetic fertilizers and pesticides, the definition of conventional farming and farmer's practiceare more variable. Fertilization and plant protection practices as well as crop rotation and soil tillage strategies often vary across both the systems. Commonly, conventional management practices includes both chemical farming and farmers' practice relies on the use of synthetic fertilizers and pesticides and often avoids the use of organic fertilizers in farmer's practices. However, a combination of organic amendments and the use of balanced nutrition (synthetic fertilizers) have been shown to exert positive effects on various soil properties [25].

Groundnut (*Arachishypogaea* L.) is a leguminous plant used for human consumption all over the world. Groundnut kernels contain 40-50% fat, 20-50 % protein and 10-20 % carbohydrate [31]. In the rhizosphere, the populations of microorganisms may fluctuate from few thousands to millions,where roots release large quantities of metabolites from living root hairs or fibrous root systems.

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These metabolites act as chemical signals for motile bacteria to move to the root surface but also represent the main nutrient sources available to support growth and persistence in the rhizosphere [22]. This plant-microbe interaction has shown many beneficial effects on plant growth and development [26], [29], Plant growth promoters increased uptakes and availability of nutrients and disease suppression [10], [20] and [21]. However, increased resistance to abiotic and biotic stresses leads to increases in crop productivity and soil sustainability as well as nutrient availability [2], [39].

A field experiment was conducted to study the response of rhizosphere microorganisms to different farming practices in groundnut will assist with providing a theoretical basis for scientific, rational and effective fertilization strategies, which in turn will enhance the microorganisms and soil enzyme activities in the rhizosphere and also reduce the disease of groundnut.

MATERIALS AND METHODS

The field experiment was conducted during the summer seasons of 2018-19, 2019-20 and 2020-21 at Zonal Agricultural and Horticultural Research Station, Babbur farm, Hiriyur, Karnataka, India under irrigated conditions. The soil of the experimental plot was block in texture and slightly alkaline in reaction. The soil has an organic carbon content of 0.43 percent and was low in available nitrogen 207 kg/ha, high in phosphorus 23.0 kg/ha and potash 320 kg/ha. The experiment was laid out in a randomized block design with five replications. The experiment consisted of four treatments *viz*., T_1: Natural farming practice, T_2: Organic farming practice T_3: Recommended package practice (chemical farming), and T_4: Farmer's practice. A recommended dose of fertilizers and plant protection measures were taken up and the crops were grown to maturity and harvested (Table-1).

Table:1 List of Fertilizers and Plant protection chemicals applied to the different management practices.

Treatments	Seed treatment	Soil application		Plant protection measures		Soil conservation
		Basal	Split	Insect	Diseases	
T_1: NFP	Beejamrutha @20 L/acre	Ghanajeevamrutha 1000 kg/ha	Jeevamrutha 500 L/ha @ 15 days intervals	Neemastra 5 ml/lit, Bhramastra-5 ml/lit	Neem leaf and seed extract 5 ml/lit	Add Ragi straw residues in-between the rows
T_2:OFP	Microbial consortia 20g/kg	FYM 10 t/ha	-	Azadirachtin 0.03 EC	Azadirachtin 0.03 EC	Nil
T_3:RPP	Thiram- 2g/kg and Microbial consortia 20g/kg	FYM 10 t/ha NPK: 12.5:50:37.5 kg/ha	N: 12.5 kg/ha	imidacloprid 17.80 SL	Hexaconazole 5 % EC	Nil
T_4:FP	Thiram- 2g/kg	5bags DAP(18:46:0) kg/ha	-	imidacloprid 17.80 SL	Hexaconazole 5 % EC	Nil

** **NFP**: Natural Farming practices, **OFP**: Organic farming practices, **RPP**: Recommended package of practices (Chemical farming) and **FP**: Farmer's practice.
* Gypsum:500 kg/ha were applied to OPF,RPP and FP

Soil sampling and analysis

Total twenty soil samples in each year from different farming practiceswere collected to know the total microbial counts. Soil samples from rhizosphere of groundnut were collected by grubbing up plants. The samples were taken around the roots at initial and harvest stage to analyze microorganisms and enzyme activities.

The population of rhizosphere microorganisms in soil was determined by serial dilution plate count method [5]. Rhizosphere soil samples were collected treatment-wise at initial and harvest stages. Ten grams of soil were weighed and mixed in 90 ml sterilized water blank to give 10^{-1} dilutions. Subsequent dilutions up to 10^{-5} were made by transferring serially 1 ml of each dilution to 9 ml sterilized water blanks. The population of bacteria, fungi, actinomycetes, *Rhizobium* sp., *Azotobacter* sp., *Pseudomonas* sp., phosphate solubilizing bacteria and *Trichoderma*sp were determined by using respective mediums. Plates were incubated at 30 ± 1^0 C for a week and the colonies which emerged were counted.

Soil enzyme activities

Dehydrogenase activity was measured using triphenyl tetrazolium chloride (TTC) as a substrate [6], where the 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution of 3 % was mixed with 2 g of moist soil and incubated for 24 h at 30 °C. After incubation, 10 ml of methanol was added, and absorbance was determined at 485 nm using a spectrophotometer. The activity of dehydrogenase was expressed as µg TTC g^{-1} h^{-1}.The urease activity was determined by using urea as a substrate [7],[32].

Two grams of moist soil was incubated with 0.2 ml of toluene, 9 ml of THAM buffer and 1 ml of 0.2 M urea for 2 hr at 37 °C. After 2 hr, the stoppers were removed and approximately 35 ml of KCl-Ag_2SO_4 solution was added. Then, the flasks were swirled again and allowed to stand until the contents had cooled to room temperature. The volume was then made up to 50 ml by the addition of KCl-Ag_2SO_4 and absorbance was determined at 578 nm using a spectrophotometer. The activity of urease was expressed as µg NH_4^+ g^{-1} soil 2 hr^{-1}. Acid and alkali phosphatase activity was analyzed using p-nitrophenyl phosphate (p-NPP) as substrate [7]. One gram of moist soil was mixed with 4 ml of modified universal buffer (pH 6.5 and 11) and 1 ml p-NPP and incubated at 37 °C for 60 min. After incubation, 1 ml of $CaCl_2$ and 4 ml of 0.2 M NaOH were added to terminate the reaction. The absorbance was determined using the spectrophotometer at 420 nm. The activity of phosphatase was expressed as µg p-NPP g^{-1} h^{-1}.

Morphological and Biochemical Characterization of Bacterial Isolates

Colony morphology, size, shape, color and growth pattern were recorded after 24 h of growth on respective selective media plates at 28 ± 2°C [27]. Cell size was observed by light microscopy. The Gram reaction was performed [33]. A series of biochemical tests were conducted to characterize the isolated bacteria using the criteria of Bergey's Manual of Systematic Bacteriology [3]. Catalase and oxidase tests were performed [12],[24].

In Vitro Screening for Antagonism

Source of pathogens

The culture of foliar leaf spot of fungal pathogens *Alterneriaporri* was isolated from the same experiment of groundnut at Zonal Agricultural and Horticultural Research Station, Babbur farm, Hiriyur, Karnataka, India.

Dual culture technique

Seven days old culture *i.e.,* mycelial disc (5 mm) from *Alterneriaporri* was placed on a PDA plate at one end. Then isolated bacterial isolates were inoculated at the centre by streaking the culture aseptically on the PDA plate opposite to the pathogen. They were maintained equidistant from the periphery of the plate and were incubated at 30 °C. After 7 days of the incubation period, radial growth of *Alterneriaporri* was recorded and percentage inhibition was calculated in relation to control.

$I = (C-T)/C \times 100$

Where, I = Inhibition of radius mycelial growth

C = radius growth measurement of fungus in control

T = Radius growth measurement of fungus in the presence of bacterial and yeast isolates

Microbial Identification using 16S rRNA gene based molecular method

The selected bacterial isolates were also characterized by molecular level at Barcode Bioscience Company, Bangalore which are responsible for impartingbiocontrol abilities against foliar plant pathogenic fungi.

DNA was isolated from the culture and its quality was evaluated on 1.0 % agarose gel, a single band of high-molecular weight DNA has been observed. The fragment of 16S rRNA gene was amplified by 16SrRNA-F and 16SrRNA-R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 16SrRNA-F and 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The consensus sequence of 16S rRNA gene was generated from forward and reverse sequence data using aligner software. The 16S rRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on the maximum identity score first ten sequences were selected and aligned using the multiple alignment software program Clustal W. Distance matrix and phylogenetic tree constructed using MEGA 10.

Statistical analysis and interpretation of data

The experimental data were analyzed via "Analysis of variance" (ANOVA) [8]. Wherever, F-test was significant critical difference (CD) values were worked out for comparison amongst the treatments. All the data were analyzed and the results are presented and discussed at a probability level of 5 per cent.

EXPERIMENTAL RESULTS

In the present investigation, with the aim to isolation and identify potential rhizobacterial inoculants that can enhance the growth and yield of groundnut along with suppression of foliar fungal pathogen of groundnut using *in vitro* bioassay study.

Rhizosphere microbial load of groundnut

The rhizospheremicroflora of groundnut was significantly influenced by the different nutrient management system at different intervals and pooled over three year's results and percent of increase over the year are presented in graphs (fig 1 to 3). Among the different management practices, the recommended dose of fertilizers has constantly maintain the showed highest number of bacteria (42.07 x 10^{-5}cfu g^{-1} soil), fungi (13.86 x 10^{-4}cfu g^{-1} soil), actinomycetes (8.07 x 10^{-3}cfu g^{-1} soil) and beneficial microorganisms like *Rhizobium* sp (11.51 x 10^{-5}cfu g^{-1} soil), *Azotobacter*sp (12.15 x 10^{-5}cfu g^{-1} soil), Phosphate solubilizing bacteria (8.33 x 10^{-5}cfu g^{-1} soil) *Pseudomonas*sp (13.43 x 10^{-5}cfu g^{-1} soil) and *Trichoderma*sp (2.25 x 10^{-4}cfu g^{-1} soil) compared to farmer's practice.

Further,percent increase over the years (fig 1, 2 and 3), the organicfarming practices has shown the highest microbial populations *viz*., bacteria (111 %), fungi (105 %), actinomycetes (116 %) and beneficial microorganisms like *Rhizobium* sp (115 %), *Azotobacter*sp (112 %), Phosphate solubilizing bacteria (109 %) *Pseudomonas*sp (113 %) and *Trichoderma*sp (114 %) as compared to other nutrient management practices.In contrast lowest percent of population over the years *viz*., bacteria (78 %), fungi (28 %), actinomycetes (51 %), and beneficial microorganisms like *Rhizobium* sp (39 %), *Azotobacter*sp (57 %), Phosphate solubilizing bacteria (41 %), *Pseudomonas*sp (42 %) and *Trichoderma*sp (55) were recorded in the farmer's practice.

The beneficial microorganisms like *Rhizobium* sp (97 %), *Azotobacter*sp (95 %) and *Pseudomonas*sp (96 %) populations were decreased over the consecutive years in the recommended package of practices (fig 2 and 3).

Soil enzyme activity

The pooled data at harvest of selected enzymes activity is shown in Fig. 4. The dehydrogenase (90.35 µg TPF g^{-1} soil day^{-1}), urase (35.65 µg NH_4^+ g^{-1} soil 2 hr^{-1}) and alkaline phosphatase (16.18 µg PNP g^{-1}soil) activities were significantly higher in recommended package of practice compared to other practices. Where as highest acid phosphatase (20.77 µg PNP g^{-1} soil)activity was observed in the organic farming practices.

In vitro evaluation of antagonistic effect on foliar fungal pathogen

A total of 28 rhizobacterial isolates were screened for antifungal activities against the foliar fungal pathogen (Fig 1) of groundnut *i.e*. *Alterneriaporri*. Out of 28 isolates, only 07 isolates from the different nutrient management systems showed antifungal activity against *Alterneriaporri*pathogen. Out of seven isolates from the different practices only four isolates showed >40 % percent of inhibition and the rest showed 30-40 percent inhibition. The maximum per cent of inhibition was recorded in *Serratiamarcescens* (47.04) which was isolated in the treatment of natural farming practice, followed by *Azotobacterchroococcum* (46.30) isolated from the organic farming practice, *Pseudomonas aeruginosa* (41.11) isolated from the recommended package of practice and *Pseudomonas putida* (40.74) which was isolated in the organic farming practice (Table-2 and Plate-1).

Molecular characterization of isolated strains

We obtained a total of 27 rhizobacterial strains from the rhizosphere soil of groundnut under different nutrient management practices. Seven isolates – NA-1, NA-2, AZO-1, AZO-2, PSB-1, PSB-2 and PF-2 were selected based on their ability to show in vitro antagonism against foliar fungal pathogen *Alterneriaporri* in a preliminary screening (Table 2).

In all the three years Azo-1 and Azo-2 isolates showed similar morphological characteristics *viz.,* circular, glistening colony, coccus, gram negative, catalase, oxidase and indole utilization positive. In contrast NA-1 and NA-2 isolates showed similar morphological and biochemical characteristics except for colony morphology, elevation and oxidase test. Whereas, PF-2 showed morphological characteristics *viz.,* circular, smooth colony, cream colour and rod in shape. Gram reaction negative, catalase and oxidase utilization positive, indole, methyl red and vogesproskauer test negative. The PSB-1 and PSB-2 showed some typical characters. They were circular, smooth colony, waxy white in colour, convex and flat when they are grown on media and rod in shape.

Based on the 16S rRNA sequences showed that the selected isolates were mainly members of the genus *Pseudomonas, Serratia, Azotobacter, Bacillus*. The sequences of the isolates NA-1 had 100 % homology with *Pseudomonas aeruginosa* (T_3), PSB-1 had 98 % sequence homology and identified as *Pseudomonas putida* (T_2), and was submitted to GenBank under accession numbers MW467369 and MW467371 respectively. Isolate NA-2, had 98% homology with *Serratiamarcescens*(T_1) and was submitted to GenBank under accession number MW467370. Isolate PF-2, AZO-1, AZO-2 and PSB-2 identified as *Pseudomonas* sp (T_1), *Azotobacterchroococcum* (T_3), *Azotobacterchroococcum* (T_2) and *Bacillus*sp (T_3) respectively (Table-2).

DISCUSSION

However, the beneficial microbial populations were slightly decreased over the years except for bacteria, fungi and actinomycetes. This may cause rising soil acidity by use of acidifying nitrogen fertilizers or incomplete cycling of nitrogen species in the soil and increasing years of consecutive monoculturing resulted in a significant increase in abundance of pathogens and a decrease in beneficial microorganisms in the rhizosphere of plants [33].

We found that the long-term use of organic fertilizer significantly increased the microbial populations in terms of species richness. Our data are in agreement with the findings [28], who reported that the NPK chemical fertilizers caused a significant decrease in bacterial diversity. Significant differences in soil bacterial composition were also observed in tea orchards under long-term treatment with chemical or organic fertilizers. We speculate that long-term application of chemical fertilizers decreased soil pH, promoted the proliferation of some specific microbes and activated the heavy metal ions in soil.

The positive plant-soil feedback depends on beneficial interactions between plant roots and microorganisms for growth promotion, nutrient acquisition and disease suppression [1]. Several studies reported that increasing years of consecutive monoculturing resulted in a significant increase in abundance of pathogens and a decrease in beneficial microorganisms in the rhizosphere of plants [33], [34]. A significant decrease in beneficial plant bacteria was also observed in the rhizosphere soil of continuously monocultured crops.

Soil enzyme activity is influenced by the soil characteristics related to nutrient availability, soil microbial activity and land use management processes which modify the potential soil enzyme-mediated substrate catalysis [15]. Application of balanced or recommended amounts of nutrients and manures improved the organic matter and microbial biomass carbon status of soils, which corresponded with higher enzyme activity [17].

These microbes play a major role in controlling diseases, which is a form of biological control and is an environment-friendly approach. Many rhizosphere bacteria have been reported to produce antifungal compounds like HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, and pyoluteorin [4]. The biocontrol ability of the PGPR depends on a wide variety of traits, such as the production of various antibiotic compounds, iron chelators and exoenzymes [18], [19] and [37].

In this study, seven microbial isolates were classified as *Pseudomonas aeruginosa* (NA-1), *Serratiamarcescens* (NA-2), *Pseudomonas putida* (PSB-1) *Azotobacterchroococcum* (AZO-1, AZO-2), *Pseudomonas* sp (PF-2) and *Bacillus*sp (PSB-2) were isolated from the rhizosphere soil of groundnut. All the isolated strains were gram-negative except *Bacillus*sp and rods shape except *Azotobacterchroococcum* and tested positive for catalase activity except Bacillus sp and Oxidase activity except *Serratiamarcescens* [31], [35]. *Bacillus*, *Pseudomonas* and *Azotobacter* are the most frequently reported genera of PGPR [16], [11] and [36]. Similarly, most isolates in this study belong to genus *Pseudomonas*, *Bacillus*, *Serratia* and *Azotobacter* [30].

In conclusion, among thedifferent nutrient management practices, the recommended package of practices will retain the microbial population and organic farming and natural farming practices will slightly improve in the microbial load over the three years of groundnut. Our findings suggest that recommended package of practices can shape microbial composition and recruit beneficial bacteria into the rhizosphere of groundnut. These results provide a promising strategy to groundnutcrops by treatment with recommended dose of fertilizers.

Future Scope of the Study

The findings of this study provide valuable insights into the impact of different nutrient management practices on soil microbial populations, enzyme activities, and their antagonistic efficiency against foliar pathogens in groundnut cultivation. However, there are several areas that warrant further investigation:

Long-term Effects: Future studies should focus on the long-term effects of different nutrient management practices on soil health, microbial diversity, and crop productivity. This would help in understanding the sustainability of these practices over extended periods.

Field Trials: While in vitro studies provide useful preliminary data, field trials are essential to validate the efficacy of the identified microbial isolates in real-world conditions. This would help in assessing their potential as biofertilizers and biocontrol agents in large-scale agricultural settings.

Mechanistic Insights: Further research is needed to elucidate the mechanisms by which these microbial isolates inhibit foliar pathogens. Understanding the biochemical pathways and genetic factors involved could lead to the development of more effective biocontrol strategies.

Integration with Other Practices: The integration of these microbial isolates with other sustainable agricultural practices, such as crop rotation, intercropping, and conservation tillage, should be explored. This could enhance the overall resilience and productivity of agricultural systems.

Economic Analysis: An economic analysis of the cost-effectiveness of these nutrient management practices, including the use of microbial inoculants, would be beneficial. This would help farmers make informed decisions about adopting these practices.

Climate Change Resilience: Future studies should also investigate the potential of these microbial isolates to enhance crop resilience to climate change-induced stresses, such as drought, salinity, and extreme temperatures.

Conflict of Interest

The authors declare that they have no conflict of interest. This research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

Kumar Naik A. H conducted the experiment and analyzed the data; Sumana D conceptualized the research and guided throughout the experiment; Amith G helped in main manuscript writing and forming tables and data curation.

Table: 2. Morphological and biochemical characteristics of the isolated microorganisms from different management practices *Tr.-Treatments, CM- colony morphology, CS- cell shape, GR-Gram reaction, Endo-Endospore, Cat- Catalase, Oxi-Oxidase, Ind- Indole, MR- Methyl red, VP- Vogesproskauer, H_2S – H_2S production.*

Tr.	Isolates	Morphological characteristics				Biochemical characteristics							
		CM	Elevation	Opacity	CS	GR	Endo	Cat	Oxi	Ind	MR	VP	H_2S
T_1	NA-1	Cream, irregular, mucoid	undulate	opaque	Cocci	+	+	+	+	-	-	+	-
	NA-2	Round, glabrous creamy	Convex and slightly umbonate	Opaque	Rod	-	-	+	-	-	-	+	-
	Azo-1	Circular, Brown, mucoid	Convex	Translucent	Rod	-	-	+	+	+	+	+	+
	Azo-2	Circular, Dull white, mucoid	Convex	Opaque	Rod	-	-	+	+	+	+	+	+
	PSB-1	Cream, raised, spherical, dry	Undulate	Opaque	Rod	+	+	+	-	-	-	+	+
	PSB-2	Cream, circular, mucoid, entire	Raised	Opaque	Rod	+	+	+	-	-	-	+	-
	PF-1	Circular, smooth	Convex	Opaque	Coccus	-	-	+	-	-	-	-	-
	PF-2	Small circular Creamy whitish	Flat	Opaque	Rod	-	-	+	+	-	-	-	-
T_2	NA-1	Off white, wrinkled, dry,	Flat	Translucent	Rod	+	-	+	-	-	-	+	+
	NA-2	Cream, circular	Raised	opaque	Cocci	-	+	+	-	-	+	-	+
	Azo-1	Circular, Brown, mucoid	Convex	Translucent	Rod	-	-	+	+	+	+	+	+
	Azo-2	Circular, Glistening, Whitish mucoid	Convex	Translucent	Coccus	-	-	+	+	+	-	-	-
	PSB-1	Round, smooth creamy	Convex	Translucent	Rod	-	-	+	+	-	-	-	-
	PSB-2	Cream, circular, mucoid, entire	Raised	Opaque	Rod	+	+	+	-	-	-	+	-
	PF-1	Circular, smooth	Convex	Opaque	Coccus	-	-	+	-	-	-	-	-
	PF-2	Circular, smooth	Flat with irregular	Opaque	Rod	-	-	+	-	-	-	-	-
T_3	NA-1	Small circular Yellowish	Flat	Opaque	Rod	-	-	+	+	-	-	-	-
	NA-2	Cream, circular	raised	opaque	Cocci	-	+	+	-	-	+	-	+
	Azo-1	Circular, Glistening, Whitish mucoid	Convex	Translucent	Coccus	-	-	+	+	+	-	-	-
	Azo-2	Circular, Dull white, mucoid	Convex	Opaque	Rod	-	-	+	+	+	+	+	+
	PSB-1	Cream, raised, spherical, dry	undulate	Opaque	Rod	+	+	+	-	-	-	+	+
	PSB-2	Circular, Smooth, Whitish	Raised	Opaque	Rod	+	+	-	+	-	-	-	-

	PF-1	Circular, smooth	Convex	Opaque	Coccus	-	-	+	-	-	-	-	-
	PF-2	Circular, smooth	Flat with irregular	Opaque	Rod	-	-	+	-	-	-	-	-
T_4	NA-1	Cream, irregular, mucoid	undulate	opaque	Cocci	+	+	+	+	-	-	+	-
	NA-2	Cream, circular	raised mucoid	opaque	Cocci	-	+	+	-	-	+	-	+
	Azo-1	Circular, Brown, mucoid	Convex	Translucent	Rod	-	-	+	+	+	+	+	+
	Azo-2	Circular, Dull white, mucoid	Convex	Opaque	Rod	-	-	+	+	+	+	+	+
	PSB-1	Cream, raised, spherical, dry	undulate	Opaque	Rod	+	+	+	-	-	-	+	+
	PSB-2	Cream, circular, mucoid, entire	Raised	Opaque	Rod	+	+	+	-	-	-	+	-
	PF-1	Circular, smooth	Convex	Opaque	Coccus	-	-	+	-	-	-	-	-
	PF-2	Circular, smooth	Flat with irregular	Opaque	Rod	-	-	+	-	-	-	-	-

Table: 2. Identification and characteristics of the selected isolates against foliar fungal pathogen (A. porri). *Tr.-Treatments, CM- colony morphology, CS- cell shape, GR-Gram reaction, Endo-Endospore, Cat- Catalase, Oxi-Oxidase, Ind- Indole, MR- Methylred, VP- Vogesproskauer, H_2S – H_2S production.*

Isolates	Tr.	Gene bank accession number	Identification	Morphological characteristics				Biochemical characteristics								Per cent inhibition *A.porri*
				CM	Elevation	Opacity	CS	GR	Endo	Cat	Oxi	Ind	MR	VP	H_2S	
NA-1	T_3	MW467369	*Pseudomonas aeruginosa*	Small circular Yellowish	Flat	Opaque	Rod	-	-	+	+	-	-	-	-	41.11
NA-2	T_1	MW467370	*Serratiamarcescens*	Round, glabrous creamy	Convex and slightly umbonate	Opaque	Rod	-	-	+	-	-	-	+	-	47.04
Azo-1	T_3	-	*Azotobacterchroococcum*	Circular, Glistening, Whitish mucoid	Convex	Translucent	Coccus	-	-	+	+	+	-	-	-	32.96
Azo-2	T_2	-	*Azotobacterchroococcum*	Circular, Glistening, Whitish mucoid	Convex	Translucent	Coccus	-	-	+	+	+	-	-	-	46.30
PSB-1	T_2	MW467371	*Pseudomonas putida*	Round, smooth creamy	Convex	Translucent	Rod	-	-	+	+	-	-	-	-	40.74
PSB-2	T_3	-	*Bacillus*sp	Circular, Smooth, Whitish	Raised	Opaque	Rod	+	+	-	+	-	-	-	-	38.89
PF-2	T_1	-	*Pseudomonas sp*	Small circular Creamy whitish	Flat	Opaque	Rod	-	-	+	+	-	-	-	-	30.37

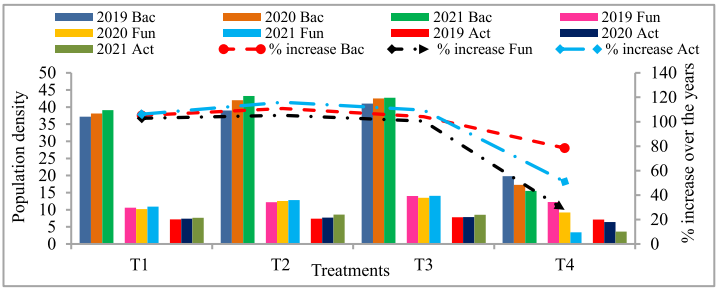

Fig :1 Population density and per cent increase over the years of (Bac-bacteria, Fun-fungi and Act-actinomycetes)

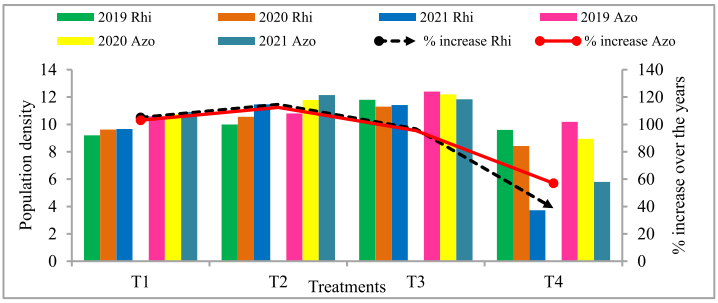

Fig : 2 Population density and per cent increase over the years of N2 –fixing microorganisms(Rhi-Rhizobium, Azo- Azotobacter)

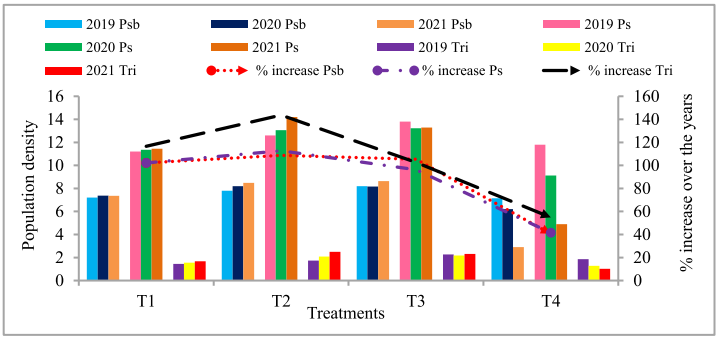

Fig : 3 Population density and per cent increase over the years (Ps-Pseudomonas, PSB –Phosphate solubilizing bacteria and Tri-Trichoderma)

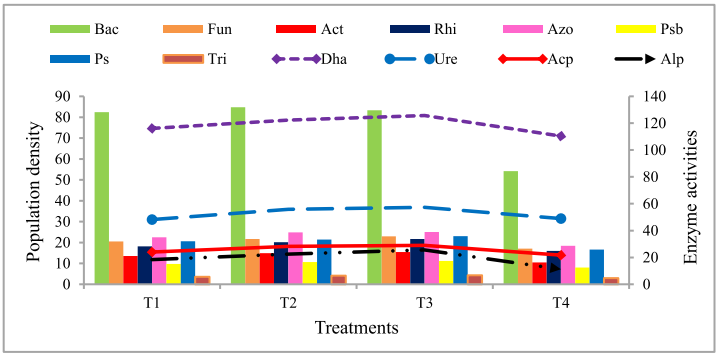

Fig: 4 Population density vsenzyme activity(Dha-Dehydrogenase, Ure-Urease, Acp-Acid phosphates, Alp-alkaline phosphatase)

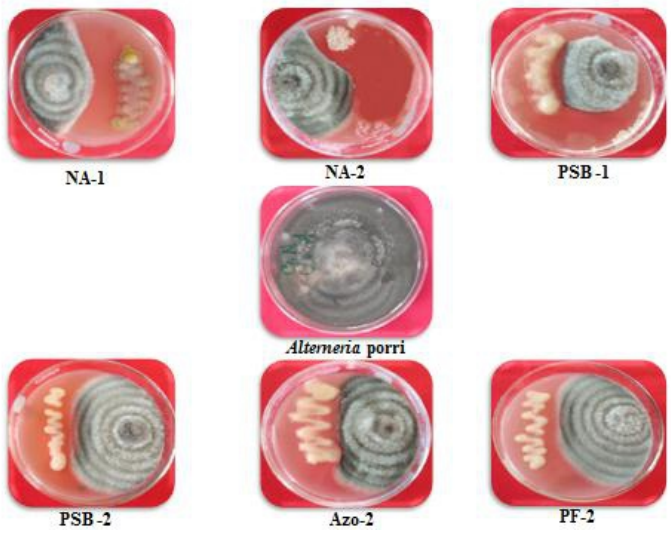

Plate 1: In vitro evaluation of different isolates against foliar plant pathogen of A. porri

REFERENCES

1. Arafat, Y., Wei, X., Jiang, Y., Chen, T., Saqib, H. A. S., and Lin, S. (2017). Spatial distribution patterns of root-associated bacterial communities mediated by root exudates in different aged ratooning tea monoculture systems. International Journal of Molecular Sciences, 18:1727.

2. Badri, D. V., Chaparro, J. M., Zhang, R., Shen, Q., and Vivanco, J. M. (2013). Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. Journal of Biological Chemistry, 288(7):4502–4512.

3. Bergey, D. H., Holt, J. G., and Noel, R. K. (1994). Bergey's Manual of Systematic Bacteriology, Vol. 1, 9th Edn. Baltimore, MD: Williams & Wilkins, 1935–2045.

4. Bhattachary, P. N., and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology, 28(4):1327–1350.

5. Bunt, J. S., and Rovira, A. D. (1955). Microbiological studies of subantarctic soil. Journal of Soil Science, 6:119–122.

6. Casida, L. E., Klein, D. A., and Santoro, T. (1964). Soil dehydrogenase activity. Soil Science, 98:371–376.

7. Eivazi, F., and Tabatabai, M. A. (1977). Phosphates in soils. Soil Biology and Biochemistry, 9:167–172.

8. Gomez, K. A., and Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. 2nd Ed. New York: John Wiley and Sons.

9. Gomiero, T., Pimentel, D., and Paoletti, M. G. (2011). Environmental impact of different agricultural management practices: conventional vs. organic agriculture. Critical Reviews in Plant Sciences, 30:95–104.

10. Haas, D., and Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology, 3(4):307–319.

11. Hallmann, J., and Berg, G. (2006). "Spectrum and population dynamics of bacterial root endophytes," in Microbial Root Endophytes, eds B. Schulz, C. Boyle, and T. Sieber. Heidelberg: Springer, 15–31.

12. Hayward, A. C. (1960). A method for characterizing Pseudomonas solanacearum. Nature, 186:405. doi:10.1038/186405a0.

13. Hole, D. G., Perkins, A. J., Wilson, J. D., Alexander, I. H., Grice, P. V., and Evans, A. D. (2005). Does organic farming benefit biodiversity? Biological Conservation, 122:113–130.

14. Jenkinson, D. S., and Ladd, J. N. (1981). Microbial biomass in soil measurement and turnover. In: Paul, E. A. and Ladd, J. N. (Eds.), Soil Biochemistry, Vol. 5. Marcel Dekker Inc., New York and Basel, 415–447.

15. Kandeler, E., Kampichler, C., and Horak, O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. Biology and Fertility of Soils, 23:299–306.

16. Laguerre, G., Attard, M. R., Revoy, F., and Amarger, N. (1994). Rapid identification of Rhizobia by restriction fragment length polymorphism analysis of PCR amplified 16S rRNA genes. Applied and Environmental Microbiology, 60:56–63.

17. Mandal, A., Patra, A. K., Singh, D., Swarup, A., and Masto, R. E. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. Bioresource Technology, 98:3585–3592.

18. Meena, V. S., Maurya, B. R., and Verma, J. P. (2014). Does a rhizospheric microorganism enhance K+ availability in agricultural soils? Microbiological Research, 169:337–347.

19. Meena, V. S., Maurya, B. R., and Bahadur, I. (2015). Potassium solubilization by bacterial strain in waste mica. Bangladesh Journal of Botany, 43:235–237.

20. Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van der Voort, M., Schneider, J. H., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A., and Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science, 332(6033):1097–1100.

21. Morrissey, J. P., Dow, J. M., Mark, G. L., and O'Gara, F. (2004). Are microbes at the root of a solution to world food production? EMBO Reports, 5(10):922–936.

22. Nihorimbere, V., Ongena, M., Smargiassi, M., and Thonart, P. (2011). Beneficial effect of the rhizosphere microbial community for plant growth and health. Biotechnology, Agronomy, Society and Environment, 15:327–337.

23. Palekar, S. (2014). Zero Budget Natural Farming: Spiritual Farming Techniques.

24. Rajat, R. M., Ninama, G. L., Mistry, K., Parmar, R., Patel, K., and Vegad, M. M. (2012). Antibiotic resistance pattern in Pseudomonas aeruginosa species isolated at a tertiary care hospital. Ahmedabad. National Journal of Medical Research, 2:156–159.

25. Rosen, C. J., and Allan, D. L. (2007). Exploring the benefits of organic nutrient sources for crop production and soil quality. Horticultural Technology, 17:422–430.

26. Singh, B. K., Millard, P., Whiteley, A. S., and Murrell, J. C. (2004). Unravelling rhizosphere–microbial interactions: opportunities and limitations. Trends in Microbiology, 12(8):386–393.

27. Somasegaran, P., and Hoben, H. J. (1994). Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. New York, NY: Springer-Verlag.

28. Sun, R., Zhang, X. X., Guo, X., Wang, D., and Chu, H. (2015). Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology and Biochemistry, 88:9–18.

29. Thayamini, H., and Seran, T. (2018). Effects of inorganic and organic nutrients combinedly used on yield and quality of groundnut (Arachis hypogaea L.). Bangladesh Journal of Scientific and Industrial Research, 53(4):289–296.

30. Tripura, C. B., Sudhakar, P. R., Reddy, M. K., Shashidhar, B., and Podile, A. R. (2007). Glucose dehydrogenase of a rhizobacterial strain of Enterobacter asburiae involved in mineral phosphate solubilization shares properties and sequence homology with members of Enterobacteriaceae. Indian Journal of Microbiology, 47:126–131.

31. Turnbull, P. C. B., Sirianni, N. M., LeBron, C. I., Samaan, M. N., Sutton, F. N., Reyes, A. E., and Peruski, L. F. (2001). MICs of selected antibiotics for Bacillus anthracis, Bacillus cereus, Bacillus subtilis, and Bacillus thuringiensis. Antimicrobial Agents and Chemotherapy, 45(3):759–762.

32. Vincent, J. M., and Humphrey, B. (1970). A manual of practical techniques for the study of root-nodule bacteria. *IBP Handbook No*. 15. *Blackwell Scientific Publications, Oxford*

33. Wu, H., Wu, L., Wang, J., Zhu, Q., Lin, S., and Xu, J. (2016). Mixed phenolic acids mediated proliferation of pathogens Talaromyces helicus and Kosakonia sacchari in continuously monocultured Radix pseudostellariae rhizosphere soil. Frontiers in Microbiology, 7:335. https://doi.org/10.3389/fmicb.2016.00335 PMID:27014250.

34. Wu, L., Wang, J., Huang, W., Wu, H., Chen, J., and Yang, Y. (2015). Plant-microbe rhizosphere interactions mediated by Rehmannia glutinosa root exudates under consecutive monoculture. Scientific Reports, 5:15871. https://doi.org/10.1038/srep15871 PMID:26515244.

35. Weisskopf, L., Abou-Mansour, E., Fromin, N., Tomasi, N., Santelia, D., Edelkott, I., and Ranelli, P. (2011). White lupin has developed a complex strategy to limit microbial degradation of secreted citrate required for phosphate acquisition. PLoS ONE, 6(5):e20693.

36. Zahid, M., Abbasi, M. K., Hameed, S., and Rahim, N. (2015). Isolation and identification of indigenous plant growth-promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (Zea mays L.). Frontiers in Microbiology, 6:207. doi:10.3389/fmicb.2015.00207.

37. Zolla, G., Badri, D. V., Bakker, M. G., Manter, D. K., and Vivanco, J. M. (2013). Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. Applied Soil Ecology, 68:1–9.