ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Inoculation Of Novel Consortium For Ameliorating Paddy Cultivation In Saline Soil

Manyata Sharma[1,2], Soni Chauhan[3], Saloni Rishi[2], Pankaj Kumar Srivastava[2,] Deepak Chand Sharma[1*]

[1] Dr. Shakuntala Misra National Rehabilitation University, Lucknow, India

[2] Environmental Technology Division, CSIR-National Botanical Research Institute, Lucknow, India

[3] Microbial Technology Division, CSIR-National Botanical Research Institute, Lucknow, India

***Corresponding Author:** Deepak Chand Sharma

Email: dcsharma@dsmnru.ac.in, dcsharma@gmail.com

ABSTRACT

A novel microbial consortium was investigated for promoting paddy growth in fields on saline soil without chemical fertilizers. Four popular rice varieties Sarjoo-52; NDR-359; Indrasan and Sambha Mahsuri (BPT 5204) studied for two consecutive years. Rice varieties were given novel fungal (T2) and bacterial (T3) consortium treatments. An un-inoculated treatment was also taken as control (T1). Changes in soil physico-chemical, biological properties and plant growth promotion were studied. Statistical analysis performed to assess significant differences in effects under different treatments. Sarjoo-52 and Indrasan rice varieties showed significantly higher growth and yield under the microbial treatments in two consecutive years. Statistical analysis showed significant ($p<0.05$) differences in various soil properties under different treatments, in different rice varieties, and their interactions. PCA analysis of the experimental results indicated that the total variance observed was 77%, 76%, 74%, 69% and 58% in the case of soil physical, chemical, enzymes, colony-forming unit (CFU) and plant growth promotion parameters, respectively. The T2 treatment was found more effective than the T3 treatment compared with the uninoculated control (T1). The study concluded that the novel fungal consortium has the potential to be applied as a biofertilizer in saline fields for improving paddy cultivation without chemical fertilizers.

Keywords: Field Efficacy, Fungal Consortium, Microbial Consortia, Rice Varieties, Soil Properties.

INTRODUCTION

Chemical fertilizer (CF) application in agricultural fields is the most adapted regime to enhance crop yields. However, extensive and prolonged usage of CF led to

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022**

buildup of soil toxicity. Thus, resulting in deteriorated quality and safety of agricultural products (Yang *et al*., 2020). Indiscriminate use of CF alters the physiological (*viz*., bulk density, moisture content and water holding capacity), chemical (*viz*., pH, electrical conductivity, total organic carbon, microbial biomass carbon available nitrogen, phosphorous, sodium, calcium and potassium) and biological properties (*viz*., microbial diversity and soil enzymes) (Ferrerase *et al*., 2006). Millions of tons of synthetic fertilizers added every year to the soil which does not fully get absorbed by the plants. Around 50% of N and 90% of P has been escaped into the environment (i.e in the atmosphere, water bodies and also with the runoff from the crop fields). This causes salinization of soil, eutrophication of water bodies and generation of greenhouse gases (Da Costa *et al*., 2013; Simpson *et al*., 2011). This has drawn the attention of the scientific community to explore the alternative of CF and transform our agricultural development into an eco-friendly and sustainable way. One such alternative has been organic farming. However, organic farming has been associated with lower crop yield and thus a higher cost (Durham and Mizik 2021). Therefore, to overcome this, plant growth-promoting microbes not only reduces the usage of chemical fertilizers but also enhances the crop yield without compromising the food quality and soil vigor (wang *et al*., 2016; Mahanty *et al*., 2017; Kour *et al*., 2010).

Many microbes *viz*, *Trichoderma* spp., arbuscular mycorrhizal fungi (AMF), plant growth-promoting rhizobacteria (PGPR), and endophytes have been commercially applied in agriculture as a promising biofertilizers (Malusa*et al*., 2016). Microbes mineralize micro and macronutrients present in soils by nitrogen fixation and also found effective in enhancing water holding capacity which in turn improves soil moisture content (Singh and Purohit, 2011). Diaz-Zorita and Fernandez-Canigia, (2009) reported an 8% enhancement in wheat yield after the application of *Azospirilum brasilense.* Likewise, microbial inoculants can also be applied as a biofertilizer such as *Frankia*, *Azospirillum*, *Pseudomonas*, and *Dyadobacter* etc using liquid or solid nutrient medium (Chaudhary *et al*., 2020).

Srivastava *et al*., (2012) tested four novel fungal strains as plant growth-promoting microbes and found them effective under glasshouse conditions. However, two newly explored bacterial strains NBRI-SD1K and NBRI-10S1H have not been explored so far. The aim of the study was to explore the plant growth promoting potential of newly explored bacterial strains. As it was isolated from the paddy rhizosphere of the saline affected region. Therefore, it can have the potential to enhance the yield of crops that have been grown in saline areas. The aims of the research were (1) To evaluate the effect of different microbial (fungal and bacterial) treatments on four locally grown rice varieties (Sarjoo-52; NDR-359; Indrasan and SambhaMahsuri (BPT 5204)

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

and (2) To evaluate the potential of microbial strains under saline stress when applied to rice varieties as well its effect on the physico-chemical and biological properties of the soil. This kind of field-based study of potential novel PGP microbial strains on rice under the saline condition without chemical fertilizers will evolve new avenues to cultivate different rice varieties.

2. Materials and Methods

2.1.Field experimental setup

Field experiment studies were conducted using four locally grown Indian rice varieties *viz*., Sarjoo-52; NDR-359; Indrasan and SambhaMahsuri (BPT 5204) for two consecutive years on a saline soil (pH 8.47, electrical conductivity 449.61 µS cm^{-1}) at Banthra Research Center of CSIR-National Botanical Research Institute (NBRI), Lucknow, India (26º68' N, 80º83' E). The experiment has been performed in a completely split-plot design. The bed size of 6 m^2. Row-to-row and plant-to-plant spacing were 25 cm and 20 cm respectively. All the four rice varieties have been given treatments of fungal (T2) and bacterial (T3) consortium. The untreated rice was taken as control (T1). Initially, rice seeds have primed with fungal and bacterial consortium by applying their cultures @10g per kg seeds. Roots of 3 weeks old seedlings have been treated with individual liquid consortium just before transplanting for 30 min, followed by sowing them in the respective treatment beds.

2.2.Microbial strains

Four fungal strains namely FNBR_3, FNBR_6, FNBR_13 and FNBR_19 have been used in this study, which have been isolated from paddy fields of West Bengal, India (Srivastava *et al*., 2012). The bacterial strains, NBRI-SD1K and NBRI-10S1H have been isolated as rice root endophytes from hybrid rice varieties. These bacterial strains have studied for exhibiting plant growth promotion traits *viz*., auxin production, siderophore production, phosphate solubilization, proline production (data not shown here).

Seeds of selected rice varieties were surface sterilized by using 0.1% $HgCl_2$ for 1 min. Bacterial and fungal cultures were grown in nutrient and mycological broths respectively for 48h at 28ºC under continuous shaking (100 rpm). The individual bacterial and fungal strain cultures have been mixed in equal proportions to make respective bacterial and fungal consortiums. These microbial strains in the respective consortium have been found compatible to grow with each other (data not shown here).

2.3.Physico-chemical analysis of the soil

The agriculture soil samples were collected at the time of rice harvesting for analysis of different physico-chemical parameters. The soil samples were air dried, sieved (sized-2 mm) and stored at cool temperature (4°C) (Black, 1965) for further analysis and each sample was analyzed in triplicate. The parameters such as bulk density (BD) was done using the pycnometer method and the available phosphorus, water holding capacity (WHC) (using perforated circular

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

brass boxes-keen's box method) was determined following the standard method (Kumar Srivastava *et al*., 2011). Total organic carbon (TOC) was done following Jackson's method (1962). Available nitrogen was done by Kjeldahl method (Kumar Srivastava *et al*., 2011), available potassium by flame photometer method using Systronix-128 and microbial biomass carbon (MBC) by chloroform-fumigation extraction technique (Vance *et al*., 1987). The pH and electrical conductivity (EC) were done using the Orion ion meter using soil samples and Milli-Q suspension a ratio of 1:25 (*w/v*).

2.4.Soil enzymatic assays

Soil enzyme assay were determined in moist and sieved (2mm) rhizospheric soil samples in triplicates (Dick, 2011). Dehydrogenase activity (DHA) was assessed by the measuring the reduction of 2, 3, 5-triphenyl tetrazolium chloride, and expressed as µg triphenyl formazan (g soil) $^{-1}$ h^{-1} (Pepper *et al*., 1995). Alkaline phosphatase (Eivazi and Tabatabai, 1977) and β-glucosidase (Eivazi and Tabatabai, 1988) were assessed by means of the substrate analogue ρ-nitrophenyl-β-D-glucopyranoside (ρ-NPG) based on quantifying the released ρ-nitrophenol by incubating soil sample (g) with the p-NPG solution for 1h at 37ºC. Protease activity (Kumar Srivastava *et al*., 2011) was measured using tyrosine as standard and folic-ciocalteau reagent. Fluorescein diacetate hydrolysis activity (FDA) was estimated through determining the amount of released fluorescein (µg g^{-1} h^{-1}) after incubating the soil sample with diacetyl-fluorescein (Dick *et al*., 1997). Cellulase activity has been assayed by measuring the reducing sugars released after incubating the soil samples with carboxy methyl cellulose (CMC) solution for 50°C for 24h (Dick, 2011).

2.5.Colony Forming Units

Colony-forming units (CFU) of soil samples have been determined by the serial dilution method (Elad*et al*., 1981) at different time intervals for 90 days. The dilutions have been taken as direct, 10^{-2}, 10^{-4} and10^{-6} for bacterial CFU count, and direct, 10^{-1} and 10^{-2} for fungal CFU count.

2.6.Assessment of rice growth parameters

Rice plants were harvested after six months of planting. Vegetative and yield parameters of rice varieties *viz*., flag leaf length (FLL), plant height (PHt), flag length width (FLW), numbers of panicles (PnN), panicle length (PnL), numbers of tillers, viability, the weight of seed (WOS), weight of seeds without husk (WOSWH), and viability of seeds have been determined to assess the effects of fungal and bacterial consortium treatments on paddy growth and yield.

2.7.Statistical analysis

Experimental data was subjected to univariate analysis for the least significant differences (LSD) observed at $p<0.05$ using the SPSS 10.0. Univariate analysis was used to define if there is a statistically significant correlation between dependent variables (soil physico-chemical parameter, soil enzymes, vegetative growth and yield of different rice varieties) and independent

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

variables (microbial consortium treatment and different rice varieties). The Principal Component Analysis (PCA) has been performed using the statistical XL-STAT to determine the principal components, which are responsible for changes in different soil parameters and plant growth upon microbial treatments given to rice varieties under study.

3. Results and Discussion

3.1.Soil physical parameters

Univariate statistical analysis of soil physical parameters *viz.*, BD, WHC and MC revealed significant differences ($p<0.05$) when treated with the fungal and bacterial consortium. A significant difference was observed in all four varieties in case of microbial treatments and rice varieties during both years (Table 1). The maximum reduction in the soil BD was observed in the Indrasan rice variety with the T2 treatment followed by the T3 and furthers in the T1 treatment. The application of fungal (T2) and bacterial (T3) treatments reduced soil BD by 77.03% and 12.93% respectively compared with the control (T1). Fungal consortium treatment (T2) showed better results than bacterial consortium treatment (T3), however, both treatments have showed reduced BD than the control. This may be because bacteria produces amino acids, polysaccharides, polyuronic acid that are negatively charged to the electrostatic charge on the surface of clay which brings together small aggregates of the soil (Naseem and Bano,2014). Similarly, extracellular polysaccharides, a crucial component of mucilaginous exudates, that have been released from the both the fungal and bacterial consortia would have helped in soil aggregate that enhances soil porosity which in turn reduces the bulk density of the soil. This reduction in BD also may be due to improved soil aeration because of aerobic microbial activities. Our result was in conformity with Reid and Goss, (2006) which showed that polysaccharides released from the growing roots had a determining part in the microaggregates stability in sandy loam and silt soil.

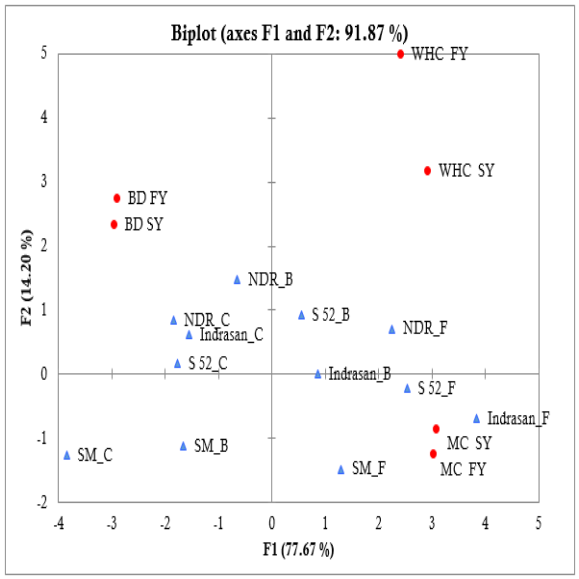
Similarly, maximum water holding capacity (WHC) and moisture content (MC) has been observed in T2 followed by T3 (data not shown). T2 and T3 value for WHC were 20.37% and 14.58% more that of the T1 (Control). In the case of MC, a similar observation has been recorded, T2 and T3 was 50.20%, 46.40% more respectively from the T1(control). Both fungal and bacterial consortium treatment enhanced the WHC and MC of the soil. This may be due to bacteria and fungi present in the soil adding steadiness to the soil aggregates (Daynes *et al.*, 2012). These aggregates determine various soil functions (Li *et al.*, 2017). Thus, governs soil moisture and water holding capacity. Statistical analysis also justified our results, PCA analysis revealed that the first principal component (PC1) was accounted for 77.67% variance representing WHC as the main soil parameter, and NDR as the main rice variety

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

influenced under the T2 (fungal consortium) treatment, and Sarjoo 52 with the T3 (bacterial consortium) treatment (Figure 1). Likewise, the soil moisture content was the most important contributor to the first principal component (F1) under the T2 treatment for all four rice varieties. Variance analysis depicts the effect of each variable and also evaluate the effect of other factors (Gouzou *et al.*, 1993), here variation in type III sum of square value (Table 1) inferred the significant changes due to treatments when compared with control.

Figure 1: PCA biplot of different soil physical parameters under different soil treatments on four rice varieties.



S52_C: Sarjoo-52 control treatment, S52_F: Sarjoo-52 fungal treatment, S52_B: Sarjoo-52 bacterial treatment, SM_C: Sambha Mahsuri control treatment, SM_F: SambhaMahsuri fungal treatment, SM_B: SambhaMahsuri bacterial treatment, Indarsan_C: Indarsan control treatment, Indarsan_F: Indarsan fungal treatment, Indarsan_B: Indarsan bacterial treatment, NDR_C: NDR control treatment, NDR_ F: NDR fungal treatment, NDR_B: NDR bacterial treatment, BD: Bulk Density; WHC : Water holding Capacity, MC: Moisture Content

Table 1: Univariate analysis of changes in soil physical parameters

		First Year			Second Year		
Soil Parameters		Treatment	Variety	Treatment * Variety	Treatment	Variety	Treatment * Variety
Bulk density	Type III Sum of Squares	1.331	0.116	0.096	1.991	0.126	0.129
	df	2	3	6	2	3	6
	Mean Square	0.666	0.039	0.016	0.996	0.042	0.022
	F	852.879*	49.689*	20.59*	274.457*	11.592*	5.929*
Water holding capacity	Type III Sum of Squares	23.733	164.874	164.001	218.607	183.682	19.392
	df	2	3	6	2	3	6
	Mean Square	11.866	54.958	27.333	109.304	61.227	3.232
	F	83.137*	385.039*	191.5*	24274.72*	13597.69*	717.775*
Moisture content	Type III Sum of Squares	568.398	408.776	398.263	789.99	420.748	345.95
	df	2	3	6	2	3	6
	Mean Square	284.199	136.259	66.377	394.995	140.249	57.658
	F	11075*	5309.9*	2586.7*	68364.49*	24273.93*	9979.335*

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

3.2.Soil chemical parameters

Univariate statistical analysis of soil chemical parameters *viz.*, pH, EC, microbial biomass carbon, total organic carbon, available nitrogen, available phosphorous, available potassium, available sodium and available calcium showed significant difference ($p<0.05$) after application of the T2 (fungal) and T3 (bacterial) treatments in case of four rice varieties in both the years (Table 2). Soil pH has a strong influence on the diversity and decomposition of bacteria and fungi. In our study, pH value has been decreased in the T2 and T3 treatments, as compared to the control soil. The pH value decreased from 8.7 to 8.01 for BPT, 8.57 to 8.04 for Indrasan, 8.49 to 8.12 for Sarjoo 52 and 8.45 to 8.06 for NDR attained in case of fungal treatment. The lowering of pH may be due to the ammonium ions acidification effect during its biotransformation in the soil. Nitrogen mineralization is the major source of acidification of soil and pH of soil alters due to enhanced microbial activities and associated beneficial impacts (MsimbiraLevini and Donald, 2020). The efficiency of the T2 (fungal) treatment compared with the T1 treatments in regulating soil EC was observed in a pattern of 103.13%> 66.64%> 61.15%> 57.02% in the case of the BPT> Sarjoo-52> NDR>Indrasanrice varieties, respectively. Fungal treatment showed enhanced EC than control. This might be because microorganisms (bacteria or fungi) and their metabolic process has a relationship with electrical conductivity (Krishna murti and Kate, 1951). Change in EC might be observed due to the increased microbial activity leading to organic matter decomposition, nutrient cycling and increased plant nutrients (Kim *et al.*, 2016). A similar trend has been observed in the case of TOC and available nitrogen. T2 (fungal) treatment displayed a significant ($p<0.05$) (Table 2) increase in the TOC and available nitrogen as compared to its control (T1).

The highest value for TOC has been recorded in Indrasan followed by NDR, BPT and Sarjoo-52 which was 75.96%, 51.25%, 49.65% and 39.81% respectively. The maximum significant ($p<0.05$) increase in the available nitrogen was observed in the T2 (800%), followed by the T3 (278.57%) when compared with the T1. Availability of Ca and K was increased by 78.76% (Sarjoo 52) and 106.6% (Indrasan) for T2 treatment in comparison with the T1 (control). Efficiencies of the treatments for increasing soil available Ca and K contents were observed to T2>T3>T1. The maximum MBC has been observed in the T1 (control) Indrasan rice variety under the T2 treatment with a 207% increase as compared to the T1. Likewise, soil available phosphorous was found 203% higher in the T2, and 94% in the T3 when compared with the T1. Available sodium content in soil was found to decrease upon the application of (fungal and bacterial) treatments. The T2 treatment was found comparatively more effective compared with the T3 treatment. The maximum decrease in available Na was

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022**

found as 60% in the T2 and 98% in the T3 compared with the T1. Application of the T2 (fungal) and the T3 (bacterial) treatments increased soil microbial activities and TOC contents. The resultant increase in soil TOC content might be due to microbial endo-cellulases, cellobiohydrolases and β-glucosidases, which might break down the complex organic carbon in soil (Medina *et al*., 2004). Bacterial and Fungal treatments can cause mineralization and mobilization of P, K, Fe reserves of soil, increase of organic matter and fixation of nitrogen from the atmosphere (Owen *et al*., 2015). MBC, TOC, N and P are the main soil chemical properties regulated upon the fungal (T2) and bacterial (T3) treatments under the current (data not shown) study. Availability of these nutrient contents revealed nutrient mineralization in the inoculated soil (Shukla *et al*., 2008). T2 (fungal) treatments in all studied rice varieties showed a significant increase in TOC, N, P and MBC (Table 2) than T3 (bacterial) treatment *viz*-a-*viz* both showed better results than control (T1). This might be due to the production of organo-polysaccharides and proteins (glomalin, mucilage's and hydrophobins) by microorganisms which help to promote stability of soil aggregate and enhance the micro and macronutrients uptake by the plants (Nadeem *et al*., 2009). However, in saline soil fungal treatment showed good results than bacterial treatment. This may be because, in elevated salt conditions, fungal dominance has been observed due to the accumulation of ergosterol in soil (Wichern *et al*., 2006). Our results have also been supported by statistical analysis (Figure 2). PCA analysis revealed that soil MBC, TOC, N and P have been the main soil properties that influenced the fungal (T2) treatment. PC1 accounted for 76·44% variance representing these soil properties (Figure 2). The first principal component showed the major influence of the fungal treatment (T2) on these properties compared with other treatments.

Figure 2: PCA biplot of different soil chemicals parameters under different soil treatments on four rice varieties.

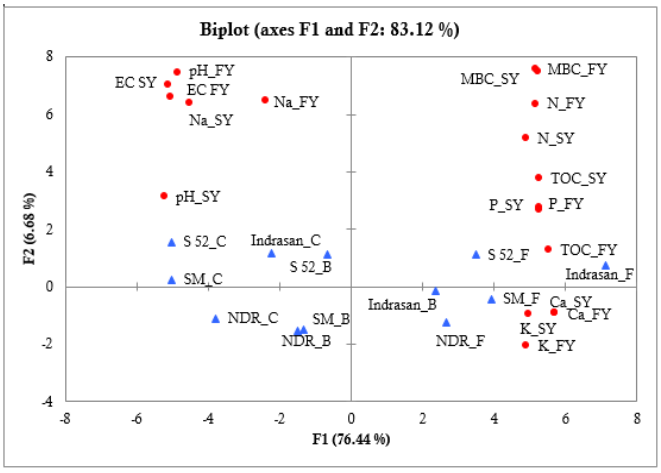


Table 2: Univariate analysis of changes in soil chemical parameters

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022**

Factors			First Year			Second Year	
		treatment	variety	treatment * variety	treatment	variety	tre
pH	Type III Sum of Squares	8.755	0.724	0.935	5.114	0.832	0.3
	df	2	3	6	2	3	6
	Mean Square	4.378	0.241	0.156	2.557	0.277	0.0
	F	280.419*	13.883*	8.963*	493.035*	53.49*	11.
Electrical conductivity	Type III Sum of Squares	58062.83	7508.334	3469.004	,	6174.99	25
	df	2	3	6	2	3	6
	Mean Square	29031.42	2502.778	578.167	26940.411	2058.33	43
	F	45638.31*	3934.447*	908.897*	34886.86*	2665.46*	55
Microbial biomass carbon	Type III Sum of Squares	218851.27	73388.541	5676.012	221406.767	73148.138	50
	df	2	3	6	2	3	6
	Mean Square	109425.63	24462.847	946.002	110703.384	24382.713	84
	F	170543.05*	38110.478*	1474.372*	53400*	11800*	40
Total organic carbon	Type III Sum of Squares	1.761	0.346	0.164	0.953	0.437	0.1
	df	2	3	6	2	3	6
	Mean Square	0.88	0.115	0.027	0.476	0.146	0.0
	F	351.394*	46.008*	10.909*	848.975*	259.662*	50.
Available nitrogen	Type III Sum of Squares	0.39	0.353	0.071	0.217	0.261	0.0
	df	2	3	6	2	3	6
	Mean Square	0.195	0.118	0.012	0.109	0.087	0.0
	F	92.898*	56.086*	5.607*	520.893*	417.867*	75.
Available phosphorous	Type III Sum of Squares	99569.3	21962.58	13345.76	100057.564	21978.196	13
	df	2	3	6	2	3	6
	Mean Square	49784.65	7320.859	2224.294	50028.782	7326.065	22
	F	75942.65*	11167.41*	3392.99*	58900*	8618.27*	26
Available potassium	Type III Sum of Squares	27736.829	13974.471	8503.11	27536.112	14543.13	73
	df	2	3	6	2	3	6
	Mean Square	13868.4	4658.16	1417.19	13768.056	4847.71	12
	F	1325.359*	445.165*	135.436*	32128.365*	11312.76*	28
							12
Available sodium	Type III Sum of Squares	23369.62	18929.16	32693.92	36987.955	16457.717	22
	df	2	3	6	2	3	6
	Mean Square	11684.81*	6309.719*	5448.987*	18493.978	5485.906	37
Available calcium	Type III Sum of Squares	2132965.8	269021.05	51533.158	49131.652*	14574.24*	98
	df	2	3	6	2111343.33	273296.92	50
	Mean Square	1066482.9	89673.685	8588.86	2	3	6
	F	63020.766*	5299.016*	507.534*	1055671.66	91098.976	84

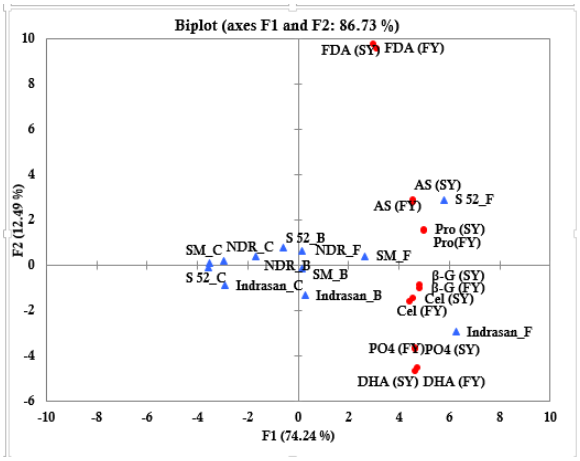
*p<0.05

EC: electrical conductivity; MBC: microbial biomass carbon; TOC: total organic carbon; P: available phosphorous; N: available nitrogen, Ca: available calcium, Na: available sodium, K: available potassium

S52_C: Sarjoo-52 control treatment, S52_F: Sarjoo-52 fungal treatment, S52_B: Sarjoo-52 bacterial treatment, SM_C: Sambha Mahsuri control treatment, SM_F: SambhaMahsuri fungal treatment, SM_B: SambhaMahsuri bacterial treatment, Indarsan_C: Indarsan control treatment, Indarsan_F:

Indarsan fungal treatment, Indarsan_B: Indarsan bacterial treatment, NDR_C: NDR control treatment, NDR_F: NDR fungal treatment, NDR_B: NDR bacterial treatment

Figure 3: PCA biplot of different soil enzymes under different soil treatments on four rice varieties.



DHA: Dehydrogenase; FDA: Fluorescein diacetate activity; PO_4: Phosphatase; β-G: β-glucosidase activity; AS: arylsulphatase; Pro: Protease; Cel: Cellulase S52_C: Sarjoo-52 control treatment S52_F: Sarjoo-52 fungal treatment

S52_B: Sarjoo-52 bacterial treatment SM_C: Sambha Mahsuri control treatment SM_F: SambhaMahsuri fungal treatment SM_B: SambhaMahsuri bacterial treatment. Indarsan_C: Indarsan control treatment, Indarsan_F: Indarsan fungal treatment, Indarsan_B: Indarsan bacterial treatment, NDR_C: NDR control treatment, NDR_F: NDR fungal treatment, NDR_B: NDR bacterial treatment

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Table 3: Univariate analysis of changes in soil enzyme activities

		First Year			Second Year		
Soil Enzymes		Treatment	Variety	Treatment * Variety	Treatment	Variety	Treatment * Variety
DHA	Type III Sum of Squares	88.819	50.971	20.932	152.313	62.251	39.501
	df	2	3	6	2	3	6
	Mean Square	44.91	16.69	3.489	76.156	20.75	6.583
	F	894.251*	339.141*	70.887*	1723.646*	469.638*	149.003*
FDA	Type III Sum of Squares	375.402	662.991	243.358	440.393	660.328	259.432
	df	2	3	6	2	3	6
	Mean Square	187.701	220.997	40.56	220.447	220.109	43.239
	F	60064.31*	70719.09 *	12979.11*	22850.8*	22815.84*	4481.981*
Protease	Type III Sum of Squares	1992.305	90.656	263.277	2185.332	82.786	284.848
	df	2	3	6	2	3	6
	Mean Square	996.153	30.219	43.879	1092.666	27.595	47.475
	F	21706.61*	658.475*	956.153*	45427.85*	1147.287*	1973.77*
Cellulase	Type III Sum of Squares	12.188	3.177	15.953	20.692	6.415	23.37
	df	2	3	6	2	3	6
	Mean Square	6.094	1.059	2.659	10.346	2.138	3.895
	F	3089.894*	536.892*	1348.159*	2228.979*	460.705*	839.139*
Phosphatase	Type III Sum of Squares	4483.935	3727.741	2015.367	4777.876	3865.825	2162.821
	df	2	3	6	2	3	6
	Mean Square	2241.967	1242.58	335.894	2388.938	1288.608	360.47
	F	21676.65*	12013.99 *	3247.624*	35295.898 *	19038.785 *	5325.834*
Aryl sulphatase	Type III Sum of Squares	5816.918	107.427	1373.829	6117.403	99.097	1460.181
	df	2	3	6	2	3	6
	Mean Square	2908.459	35.809	228.972	3058.701	33.032	241.697
	F	12625.19*	155.442*	993.932*	60167.886 *	649.781*	4754.433*
β-glycosidase	Type III Sum of Squares	1136.733	86.561	75.746	1250.496	80.854	83.174
	df	2	3	6	2	3	6
	Mean Square	568.367	28.854	12.624	625.248	26.951	13.862
	F	6323.381*	321.011*	140.453*	9532.835*	410.915*	211.351*

*p<0.05

3.3.CFU counts and soil enzymatic activities

Soil enzymes and microbial counts are directly associated with physico-chemical and biological characteristics of the soil. A significant ($p<0.05$) surge in the activity of DHA, phosphatase and β-glucosidase enzyme has been observed in the T2 (fungal) treatment as compared with T3 (bacterial) and T1 (control) treatment. Maximum activity of Dehydrogenase, Phosphatase and β-glucosidase activity was 154%, 51%, and 33% respectively (Table 3) as compared fungal (T2) treatment. Microbial treatment enhanced the microbial population, which term responsible for mineralization of N using protease, and also of P using phosphatase into their bioavailable forms (Parham *et al*., 2002). Dehydrogenase activity is considered a decent indicator for the microbial activity which affects the oxidative activity of microflora of the soil because this is only present in viable cells (Bolton *et al*., 1985). The current study showed not only enhanced dehydrogenase and β-glucosidase activity but also has shown elevated levels of cellulase activity in T2 and T3 treatment as compared to T1 (control).

All the studied soil enzymes enrich the soil quality. Thus, a decrease in BD (Table 1) has been observed in T2 (fungal) and T3 (bacterial) treatment as compared with T1 (control). T2 andT3 treatment showed higher enzyme activity than T1. This may be because enzyme activities occurred due to the catalysis of substrates present in the soil. This involves various processes like hydrolysis of ester-S, and mineral sulfur which may provide sulphur in available inorganic form, from the unavailable organic compounds present in the soil matrix (Makoi and Ndakidemi, 2008). Further, this soil sulphur content may improve plant growth as it is an active component of cysteine and methionine (sulphur-containing amino acids) and metabolites such as phytochelatins and glutathione (**Noji and Saito, 2003)**. Protease hydrolyses proteins and releases N for its availability to the plants. In this study T2 showed a significant increase protease activity than T1 (control) and it was 190% that of the control. Total N has also increased in T2 (Table 2) as compared to T1 (control). Similarly, phosphate enzyme activity showed a similar trend. A significant ($p<0·05$) increased in alkaline phosphatase has been recorded in

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

T2 treatment as compared to T1 (control) (Table 3), which was 51% that of the T1 (control). Phosphatase enzymes are important for releasing PO_4^{3-} from immobile organic phosphorous and improving the soil phosphorous availability to plants (Borase *et al*., 2020).

CFU count shows the microbial strength of a soil sample responsible for the decomposition of organic matter and facilitates microbial soil enzymes contributing to maintaining nutrient cycling. The application of both the T2 and T3 increased the population of fungi, bacteria, and actinomycetes and was observed maximum in the T2 as 47% (fungal) followed by 41% (bacterial), and 23% (actinomycetes) compared with the T1. Overall, the present study exhibited higher soil enzyme activity (DHA, FDA, arylsulphatase, cellulase, β-glucosidase, cellulase and phosphatase) in T2 (fungal) treatment than that of T3 (bacterial) treatment and T1 (control). The higher microbial population could be the reason for enhanced enzyme activity. Fungi contribute approximately 86% of soil cellulase activity (Ajwa and Tabatabai, 1994). So fungal treatment not only improves the soil physico-chemical properties (like BD, MC, N, TOC etc.) but also elevated the activity of soil enzymes. PCA analysis revealed that FDA, arylsulphatase and protease are the main factors contributing to the first principal component and accounted for 74.24% variance (figure 3). These results suggested that fungal treatment rendered better soil enzyme activities compared with the T1 and the T3 treatment. CFU counts have been increased upon both the microbial treatments, and PCA results confirmed it with a total variance of 89.27% (Figure 4). The Statistical analysis also confirmed that T2 treatment has a significant ($p<0.05$) difference (Table 3) with T1 (control) treatment. However, among the studied four rice varieties, soil enzyme assay from the rhizosphere of these four rice varieties showed insignificant differences.

3.4.Plant growth promotion

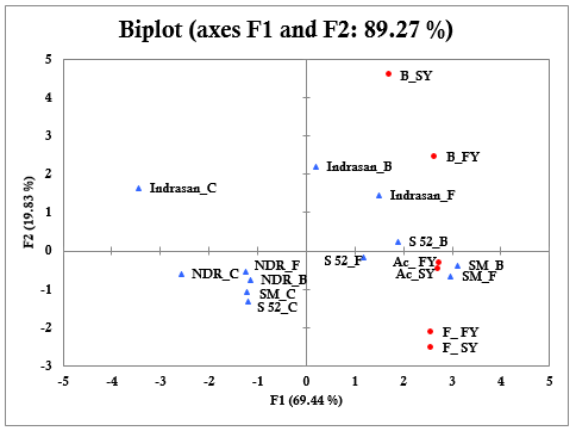
Treatments of different rice varieties with bacterial and fungal consortium significantly ($p<0.05$) increased paddy growth attributes (Table 4). A significant increase in vegetative growth has been observed in the plants of T2 treatments. The plant height (PHt), flag leaf width (FLW), flag leaf length (FLL), number of tillers, number of panicles (PnN), panicle length (PnL) and number of spikelets (SpL), has been increased around 150%, 9%, 83%, 72%, 61%, 27%, and 30% respectively (Table 4) compared with theT1 (control) plants. The maximum weight of seeds with husk per year was recorded in the T2 followed by the T3, and then in the T1 treatment. The weight of seeds without husk was elevated by 49% and 32%, respectively in the T2 and the T3 when compared with the T1 (control). The maximum increase in viability was 12% in the case of the T2, and 9% in the case of the T3 when compared with the T1. The data showed that the rice grain yield in the T2 and T3 was significantly ($p<0.05$) higher

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved, UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022**

compared with T1. Increase in the FLL, PHt, the viability of seeds (VBT), the weight of seeds (WOS), and numbers of tillers can be due to increased soil chemical and biochemical properties upon the application of bacterial and fungal consortium (Majeed *et al*., 2015). Masunaka*et al*., (2009) revealed that the applications of bacterial and fungal consortiums regulate the secondary metabolite production and release (phytohormones and biologically active substances), which enhances the plant growth similar. Our results were in conformity with Hashem *et al*. (2016) study, that reported bacteria (*Bacillus subtilies*) stimulated the root and shoot growth of *Acacia gerardia* under salt stress. Soil salinity decreases the phosphorous availability (Graltan and Grieve, 1998) and inhibits the uptake of Pi by roots and transport of Pi into the plants (Martinez *et al*., 1996). However, the presence of indigenous *Arbuscular mycorrhizal* improves the P uptake in maize and cotton (Liu *et al*., 2016). Similarly, in our results plants grown in T2 (fungal treatment) treatment demonstrated rich growth and yield than T3 (bacterial) treatment and T1 (control) control. Plants grown in both T2 and T3 showed enhanced growth in saline stressed than the T1 (control). Our results have been further validated by statistical analysis. PCA analysis revealed that FLL, PHt, FLW, VBT, WOS and numbers of tillers upon the fungal treatment (T2) contributing to the first principal component which was 58.95% of the total variance (Figure 5).

Figure 4: PCA biplot of CFU under different soil treatments on four rice varieties.

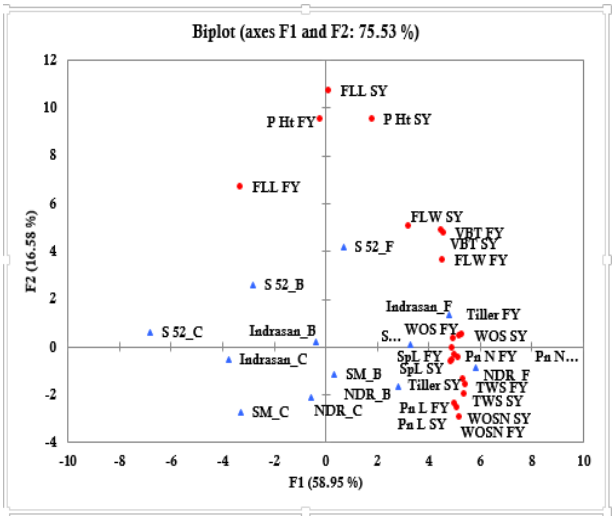


B_FY: Bacteria for first year; B_SY: Bacteria for second year; F_FY: Fungi for first year F_SY: Fungi for second year; Ac_FY: Actinomycetes for first year; Ac_SY: Actinomycetes for second year S52_C: Sarjoo-52 control treatment S52_F: Sarjoo-52 fungal treatment, S52_B: Sarjoo-52 bacterial treatment SM_C: Sambha Mahsuri control treatment, SM_F: SambhaMahsuri fungal treatment, SM_B: SambhaMahsuri bacterial treatment Indarsan_C: Indarsan control treatment Indarsan_F: Indarsan fungal treatment Indarsan_B: Indarsan bacterial treatment NDR_C: NDR control treatment NDR_F: NDR fungal treatment NDR_B: NDR bacterial treatment

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

Figure 5: PCA biplot of rice growth parameters under different soil treatments on four rice varieties



FLL: Flag leaf length; FLW: Flag leaf width; TWS: Total weight of seeds; WOS: Weight of seeds; WOSN: Weight of seeds without husk; VBT: Viability; PHt: Plant Height; PnN: No. of Panicles; Pn L: Panicles length; SpL: No. of Spikelets; Tiller)

Table 4: Univariate analysis of changes in growth parameters of rice

		First Year			Second Year		
Vegetative growth parameters		Treatment	Variety	Treatment * Variety	Treatment	Variety	Treatment * Variety
Flag leaf length	Type III Sum of Squares	10.407	12.792	1.914	207.946	468.556	49.772
	df	2	3	6	2	3	6
	Mean Square	5.203	4.264	0.319	103.973	156.185	8.295
	F	3065.774*	2512.384*	187.939*	688.89*	1034.83*	54.96*
Flag leaf width	Type III Sum of Squares	1.247	0.354	0.238	2.174	0.422	2.412
	df	2	3	6	2	3	6
	Mean Square	0.624	0.118	0.04	1.087	0.141	0.402
	F	385.672*	73.078*	24.494*	81.252*	10.514*	30.049*
Panicle length	Type III Sum of Squares	16.158	10.622	3.612	11.108	10.381	2.113
	df	2	3	6	2	3	6
	Mean Square	8.079	3.541	0.602	5.554	3.46	0.352
	F	3278.966*	1436.971*	244.351*	824.513*	513.704*	52.282*
No. of panicles	Type III Sum of Squares	53.137	48.437	19.719	51.219	45.435	21.385
	df	2	3	6	2	3	6
	Mean Square	26.569	16.146	3.286	25.61	15.145	3.564
	F	4953.249*	3010.069*	612.706*	4893.56*	2893.93*	681.03*
No. of tillers	Type III Sum of Squares	48.174	20.608	34.516	43.407	28.303	25.372
	df	2	3	6	2	3	6
	Mean Square	24.087	6.869	5.753	21.703	9.434	4.229
	F	4311.91*	1229.734*	1029.803*	5282.79*	2296.48*	1029.36*
Plant height	Type III Sum of Squares	2755.902	8455.052	5240.13	4597.509	2597.868	773.724
	df	2	3	6	2	3	6
	Mean Square	1377.951	2818.351	873.355	2298.755	865.956	128.954
	F	200267.4*	409610.9*	126930.9*	13073.49*	49248.68*	7333.87*
Viability	Type III Sum of Squares	436.094	137.151	40.961	413.191	135.269	42.515
	df	2	3	6	2	3	6
	Mean Square	218.047	45.717	6.827	206.595	45.09	7.086
	F	30531.66*	6401.442*	955.909*	32477.88*	7088.353*	1113.937*
Weight of seed	Type III Sum of Squares	253.3	131.908	37.619	248.163	137.83	53.428
	df	2	3	6	2	3	6
	Mean Square	126.65	43.969	6.27	124.082	45.943	8.905
	F	9719.469*	3374.321*	481.169*	70679.35*	26170.27*	5072.296*
Weight of seed without husk	Type III Sum of Squares	58.577	94.893	21.516	56.304	91.208	22.55
	df	2	3	6	2	3	6
	Mean Square	29.289	31.631	3.586	28.152	30.403	3.758
	F	11767.74*	12708.83*	1440.81*	41317.41*	44620.64*	5515.917*

*p<0.05

4. CONCLUSION

Salinity has a detrimental effect on the plants as well as on the soil microbial community resulting in decrease microbial biomass, its related decomposition and mineralization processes. However, after amelioration with the fungal and bacterial consortium treatment for two years in saline soil reflects the acclimatization of the microbial community that results in the robust growth of all the studied rice varieties (Sarjoo-52; NDR-359; Indrasan and SambhaMahsuri (BPT 5204) used in this experiment). Although Sarjoo-52 and Indrasan showed higher yields than NDR-

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

359 and SambhaMahsuri (BPT 5204). Fungal treatment countered the harmful effects of salt, as microorganism benefits from the substrate availability and can easily manage with high salinity. This fungal treatment also conditioned the soil, improved the soil's physico-chemical properties after 2 years of successive application. This study recommends the novel fungal consortium can be used as potential biofertilizers in fields for better soil fertility and improved paddy crop production.

Authors Contributions

MS, PKS, DCS: Conceptualization, Methodology, Software **MS, PKS, DCS, SR**: Data curation, Writing- Original draft preparation. **MS, SC**: Visualization, Investigation. **PKS, DCS***:* Supervision**. MS, SC, PKS**: Software, Validation. **MS, PKS, DCS:** Writing- Reviewing and Editing

Availability of data and materials

All the generated or analyzed data during the study are included in the manuscript.

Acknowledgements

We would like to thanks, Director CSIR-National Botanical Research Institute for providing the necessary facilities for this experiment. We also want to extend thanks to the farm in charge (Banthra, Lucknow) for providing all the facilities related to field trials.

REFERENCES

Ajwa H.A., Tabatabai M.A. (1994): Decomposition of different organic materials in soils. Biology and Fertility of Soils, 18:175–182.

Black C.A. (1965): Methods of soil chemical analysis and microbiological properties. Agronomy No. 9. American Society of Agronomy, Madison, Wisconsin USA

Bolton Jr, H., Elliott, L. F., Papendick, R. I., andBezdicek, D. F. (1985). Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil biology and Biochemistry*, *17*(3), 297-302.

Borase, D. N., Nath, C. P., Hazra, K. K., Senthilkumar, M., Singh, S. S., Praharaj, C. S., ... and Kumar, N. (2020). Long-term impact of diversified crop rotations and nutrient management practices on soil microbial functions and soil enzymes activity. *Ecological Indicators*, *114*, 106322.

Chaudhary T., Dixit M., Gera R., Shukla A.K., Prakash A., Gupta G., Shukla P. (2020): Techniques for improving formulations of bioinoculants. 3 *Biotech* 10, 199

Costa, P., Beneduzi, A., Souza, R., Schoenfeld, R., Vargas, L., and Passaglia, L., 2013. The effects of different fertilization conditions on bacterial plant

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

growth promoting traits: Guidelines for directed bacterial prospection and testing. Plant and Soil. 368. 10.1007/s11104-012-1513-z.

Daynes, C.N., Zhang, N., Saleeba, J.A., and McGee, P.A., 2012. Soil aggregates formed in vitro by saprotrophic Trichocomaceae have transient water-stability. Soil Biology and Biochemistry 48, 151–161.

Díaz-Zoríta M., FernandezCanígiaM.V. (2009): Field performance of a liquid formulation of *Azospirillumbrasilense* on dryland wheat productivity. European Journal of Soil Biology, 45: 3–11.

Dick R.P. (2011): Methods of soil enzymology. Issue 9, Soil science society of America Madison., USA.

Dick, R.P., Breakwell, D.P., Turco, R.F., 1997. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. Methods Assess. soil Qual. 49, 247–271.

Durham, T., and Mizik, T., 2021. Comparative economics of conventional, organic, and alternative agricultural production systems. *Economies*, *9*(2), 64.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606.

Elad Y., Chet I., Henis Y. (1981): A selective medium for improving quantitative isolation of *Trichoderma* Spp. from soil. Phytoparasitica, 9: 59-67.

Ferreras, L., Gomez, E., Toresani, S., Firpo, I., and Rotondon, R., 2006. Effect of organic

amendments on some physical, chemical and biological proper-ties in a horticultural soil.

Bioresour Technol., 97: 635–640.

Gouzou, L., Burtin, G., Philippy, R., Bartoli, F., andHeulin, T. (1993). Effect of inoculation with Bacillus polymyxa on soil aggregation in the wheat rhizosphere: preliminary examination. In *Soil Structure/Soil Biota Interrelationships* (pp. 479-491). Elsevier.

Grattan, S. R., and Grieve, C. M. (1998). Salinity–mineral nutrient relations in horticultural crops. *Scientia horticulturae*, *78*(1-4), 127-157.

Hashem, A., Abd_Allah, E. F., Alqarawi, A. A., Al-Huqail, A. A., Wirth, S., andEgamberdieva, D. (2016). The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of Acacia gerrardii under salt stress. *Frontiers in microbiology*, *7*, 1089.

Jackson, K.A., 1962. On the origin of dislocations. Philos. Mag. A 7, 1615–1616.

Kim JM., Roh AS., Choi SC., Kim EJ., Choi MT., Ahn BK., Kim SK., Lee YH., Joa JH., Kang SS., Lee SA., Ahn JH., Song J., Weon HY. (2016): Soil pH and electrical conductivity are key edaphic factors shaping bacterial communities of greenhouse soils in Korea. Journal of Microbiology, 54, 838–845.

Kour D., Rana K.L., Yadav A.N., Yadav N., Kumar M., Kumar V., Vyas P., Dhaliwal

ISSN PRINT 2319 1775 Online 2320 7876

Research paper **© 2012 IJFANS. All Rights Reserved,** UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

H.S., Saxena A.K. (2020): Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatalysis and Agricultural Biotechnology, 23:101487

Krishnamurti, K., and S. R. Kate. "Changes in electrical conductivity during bacterial growth." *Nature* 168.4265 (1951): 170-170.

Kumar Srivastava, P., Singh, P.C., Gupta, M., Sinha, A., Vaish, A., Shukla, A., Singh, N., Krishna Tewari, S., 2011. Influence of earthworm culture on fertilization potential and biological activities of vermicompost's prepared from different plant wastes. J. Plant Nutr. Soil Sci. 174, 420–429.

Liu, S., Guo, X., Feng, G., Maimaitiaili, B., Fan, J., and He, X. (2016). Indigenous arbuscular mycorrhizal fungi can alleviate salt stress and promote growth of cotton and maize in saline fields. *Plant and Soil*, *398*(1-2), 195-206.

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., andTribedi, P. (2017). Biofertilizers: a potential approach for sustainable agriculture development. *Environmental Science and Pollution Research*, *24*(4), 3315-3335.

Majeed A., Abbasi M.K., Hameed S., Imran A., Rahim N. (2015): Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology, 6: 198.

Makoi J.H.J.R., Ndakidemi P.A. (2008): Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, 7: 181-191.

Malusà, E., Pinzari, F., and Canfora, L. (2016). Efficacy of biofertilizers: challenges to improve crop production. In *Microbial inoculants in sustainable agricultural productivity* (pp. 17-40). Springer, New Delhi.

Martinez, V., Bernstein, N., andLäuchli, A. (1996). Salt-induced inhibition of phosphorus transport in lettuce plants. *Physiologia Plantarum*, *97*(1), 118-122.

Masunaka, A., Hyakumachi, M., andTakenaka, S. (2009). Plant growth-promoting fungus, Trichoderma koningi suppresses isoflavonoid phytoalexin vestitol production for colonization on/in the roots of Lotus japonicus. *Microbes and environments*, 1102230277-1102230277.

Medina A., Vassilev N., Alguacil M.M., Roldan A., Azcon R. (2004): Increased plant growth, nutrient uptake, and soil enzymatic activities in a desertified mediterranean soil amended with treated residues and inoculated with native mycorrhizal fungi and a plant growth-promoting yeast. Soil Science, 169: 260–270.

Msimbira L.A., Smith D.L. (2020): The Roles of Plant Growth Promoting Microbes in Enhancing Plant Tolerance to Acidity and Alkalinity Stresses. Frontiers in Sustainable Food Systems, 4: 106.

Nadeem, S.M., Zahir, Z.A., Naveed, M., Arshad, M., 2009. Rhizobacteria containing ACC-deaminase confer salt tolerance in

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11,S Iss 3, Dec 2022

maize grown on salt-affected fields. Can. J. Microbiol. 55 (11), 1302–1309.

Naseem, Hafsa, and AsghariBano. "Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize." *Journal of Plant Interactions* 9.1 (2014): 689-701.

Noji M., Saito K. (2003)**:** Sulfur amino acids: biosynthesis of cysteine, methionine. In: Abrol YP, Ahmad A (eds) Sulphur in Plants, Kluwer Academic Publishers, Dordrecht, 135-144

Owen, D., Williams, A.P., Griffith, G.W., Withers, P.J.A., 2015. Use of commercial bio-inoculants to increase agricultural production through improved phosphrous acquisition. Appl. Soil Ecol. 86 (0), 41–54.

Parham J.A., Deng S.P., Raun W.R., Johnson G.V. (2002): Long- term cattle manure application in soil. I.effect on soil phosphorus levels, microbial biomass C and dehydrogenase and phosphatase activities. Biology and Fertility of Soils, 35: 328–337.

Pepper, I.L., Gerba, C.P., Brendecke, J.W., 1995. Environmental Microbiology: a Laboratory Manual. Academic Press.

Reid, J., and Goss, M., 2006. Interactions between soil drying due to plant water use and decreases in aggregate stability caused by maize.. Journal of Soil Science. 33. 47 - 53. 10.1111/j.1365-2389.1982.tb01746.

Shukla S.K., Yadav R.L., Suman A., Singh P.N. (2008): Improving rhizospheric environment and sugarcane ratoon yield through bioagents amended farm yard manure in udic ustochrept soil. Soil and Tillage Research, 99: 158–168.

Simpson, R. J., Oberson, A., Culvenor, R. A., Ryan, M. H., Veneklaas, E. J., Lambers, H., ... and Richardson, A. E. (2011). Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. *Plant and Soil*, *349*(1), 89-120.

Singh T., Purohit S.S. (2011): Biofertilizers Technology, Agrobios., (India). ISBN.13:978-81- 7754-382-7.

Srivastava P.K., Shenoy B.D., Gupta M., Vaish A., Mannan S., Singh N., Tewari S.K., Tripathi R.D. (2012): Stimulatory effects of arsenic-tolerant soil fungi on plant growth promotion and soil properties. Microbes and Environments, 27: 477–482.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Wang, Q., Wang, Z., Awasthi, M. K., Jiang, Y., Li, R., Ren, X., ... and Zhang, Z. (2016). Evaluation of medical stone amendment for the reduction of nitrogen loss and bioavailability of heavy metals during pig manure composting. *Bioresource Technology*, *220*, 297-304.

Wichern, Jannike, Florian Wichern, and Rainer Georg Joergensen. "Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils." *Geoderma* 137.1-2 (2006): 100-108.

Zhongyi Li., Zhenjiang Jin., Qiang Li. (2017): Changes in Land Use and their Effects on Soil Properties in Huixian Karst Wetland System. Polish Journal of Environmental Studies, 26: 699–707.