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Effect of microbial inoculants with low dose of inorganic fertilizers on microbial growth, soil enzymes, plant growth and yield of rice

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ABSTRACT

Continuous application of chemical fertilizers alters the soil biological properties and in situ causes residual effect on the crop plants. Field experiments were conducted with microbial inoculants and reduced inorganic fertilizers. The results of the experiments showed that application of 50 % recommended dose of nitrogen (N), phosphorous (P) and potassium (K) + azolla+ azophos + K releasing bacteria (KRB) + zinc solubilizing bacteria (ZSB) was found to have maximum soil microbial population viz., azospirillum (5.4 MPN/g), phosphobacteria (54 x 104 cfu/g) and diazotrophs (56 x104 cfu/g) under system of rice intensification (SRI) ecosystem, while 25 % recommended dose of NPK + azolla + azophos + KRB + ZSB was found to have maximum soil alkaline phosphatase (538 µg of P nitrophenol/g/h), acid phosphatase (248 µg of P nitrophenol/g/h), urease (79 µg of NH_4/g/24 hrs) and dehydrogenase (110 µg of TPF/g/24 h) enzyme activity under SRI ecosystem. Among the treatments, maximum N (357 kg/ha), P (93 kg/ha), K (315 kg/ha) and yield (6800 kg/ha) was found in the treatment 100% recommended dose of NPK which was found to be on par with the yield (6850 kg/ha) under 50% recommended dose of NPK + azolla+ azophos + KRB + ZSB under SRI ecosystem.

Key words: *Azophos, potassium releasing bacteria, zinc solubilising bacteria, plant growth, yield*

INTRODUCTION

Biological properties are the most sensitive indicators of changes in the soil quality of rice production systems due to their rapid responses to environmental changes (Lima et al., 2013). As an important component in regulating below ground ecological processes, the soil microbial populations are facilitators of nutrient availability, particularly soil nitrogen (N) availability (Coleman and Crossley, 1996). Thus, any change in the availability of soil N may in turn affect the soil microbial community and hence obviates their role in the turnover of soil organic matter. Additionally, changes in soil microbial function and community composition may trigger a series of responses, such as impacting litter and organic matter decomposition rates (Carreiro et al., 2000), humus formation (Magill and Aber, 1998), nutrient transformation and cycling (Fisk and Fahey, 2001) and then alter the interaction between soil microbes and plant communities. Consequently, these biological properties might be useful for characterizing soil fertility and quality changes in short-term experiments. Studies have suggested that nutrient additions can significantly impact the population, composition, and function of soil microorganisms (Mandal et al., 2007; Hopkins et al., 2008; Geisseler and Scow, 2014) and that mineral fertilizer amendments can result in increase in soil microbial activity in subtropical forests (Cao et al., 2010; Geisseler and Scow, 2014). However, other studies have demonstrated that mineral fertilizers have either no effect or a negative effect on soil microbial diversity and activities (Moore-Kucera and Dick, 2008; Feng et al., 2009). Frey et al. (2004) found that active fungal biomass was lower in the fertilized plots compared to control plots in pine stands.

External nutrients including P fertilization supplied to soil would result in significantly lower fungal abundance compared with the unfertilized plot. Zhang et al. (2006) observed significant increase of soil microbial biomass to two years N fertilization in deteriorated grassland in China, however, on the other hand Sarathchandra et al. (2001) reported significant decrease of soil microbial biomass in a perennial pasture of New Zealand due to two years N fertilization. Meanwhile, Johnson et al. (2005) have found that two years application of N did not affect soil microbial biomass in upland grassland of Scotland. The mechanisms behind the variations may depend on other soil features such as soil moisture, soil organic matter, total N, pH, the rate of N addition etc. (Compton et al., 2004, Drenovsky et al., 2004 and Williams and rice, 2007), but the specific drivers are still not completely identified (Arnebrant, 1990 and Zhang and Zak, 1998).

Addition of N through mineral fertilizers has been found to influence the above ground biological processes both directly and indirectly. High N application reduced the soil microbial biomass significantly and also the functional diversity of the microflora. High level of N application in the form of ammonia fertilisers like urea, diammonium phosphate increased the toxicity of ammonia to the bacterial population. Increasing levels of N significantly suppressing the population of Azotobacter in soil is well documented (Bagyaraj and Patil, 1975). Application of calcium nitrate did not alter the microbial biomass N while repeated application of ammonium sulphate reduced the microbial biomass N (Wessen et al., 2010). Long-term fertiliser applications significantly affect soil microbial communities throughout the soil profile; in fact the relative abundance of ammonia oxidizing archaea at 0-40 cm depth was noticed (Li et al., 2014). Application of the ammonium nitrate resulted in relatively lower proportion of gram-negative bacteria while there was an abundance of gram-positive bacteria (Peacock et al., 2001). Long-term continuous application of ammoniacal fertilisers without any supplementation of the organic fertilisers leads to acidification of soils.

Repeated applications of P fertilisers from chemical source leads to disturbance in the microbial diversity. In long-term, continuous use of chemical fertilisers through different sources of phosphate fertilisers inhibits substrate induced respiration and microbial biomass C production ability of the microbes (Bolan et al., 1996). Phosphate solubilising microorganisms (PSM) of the soil/rhizosphere aids in reducing the effect of P deficiency in soils. Phosphate solubilizing microorganism solubilise tri-calcium phosphate by binding free P in the medium and also by release of organic acids.

Microbial inoculants refer to formulations composed of beneficial microorganisms that play an important role in soil ecosystems for sustainable agriculture. Microbial inoculants are environmentally friendly and are a potential alternative to chemical fertilizers and pesticides (Babalola and Glick, 2012). They are composed of active strains of microorganisms which directly or indirectly stimulate microbial activity and hence improve mobility of nutrients from soil (Suyal et al., 2016). They could be phyto-stimulants, bio-fertilizers or microbial biocontrol agents (Alori et al., 2017b). They provide protection against a range of different pathogens and they are effective bio-herbicides (Babalola, 2010b).

It is well proved that heavy dose of chemical fertilisers, in general, have a deleterious effect on beneficial soil microorganisms like N-fixing bacteria and P solubilising and mobilizing organisms. With this background the current research was focused to examine the effect of microbial inoculants alone as a fertilizer,and microbial inoculants with reduced dose of inorganic fertilizers on soil microbial and enzyme activities, plant growth and yield of rice.

MATERIALS AND METHODS

Soil samples

The rhizospheric soil samples for this study were collected from the rice field in the wet land of Tamil Nadu Rice Research Institute, Aduthurai. The soils were collected in sterile polybags and stored in a incubator at $5^{o}C$ for further studies.

Source of biofertilizer inoculum

Azospirillum (*Azospirillum lipoferum*) and phosphobacteria (*Bacillus megaterium* var. *phosphoticum*), K releasing bacteria (*Bacillus mucilagenesis*) and zinc solubilising bacteria (*Enterobacter cloacae*) cultures used in this study were obtained from the biofertilizer production and quality control unit of the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The azolla and blue green algae used in this study were the resource availability of Tamil Nadu

Rice Research Institute, Aduthurai -612101.

Mass culturing of *Azospirillum*, phosphobacteria, K releasing and Zn solubilizing bacteria

Azospirillum, phosphobacteria, K releasing and zinc solubilizing bacterial cultures were mass multiplied in Okon's, nutrient, Alexandro, Bunt and Rovira broth respectively. A loopful of 48 h old azospirillum, phosphobacteria, K releasing and zinc solubilizing bacterial culture was taken from slants and inoculated in the respective broth of 250 ml quantities in 500 ml Erlenmeyer flasks. The flasks were placed in a mechanical shaker for uniform mixing and aeration of the broth. One per cent of the grown cultures were transferred into the fermentor containing sterilized broth separately for each organism

Mass multiplication of azolla and BGA

The azolla and BGA cultures were mass multiplied (agritech. Tnau. ac. in)

Field experiment

Field experiments were laid out to assess the effect of microbial inoculation, microbial inoculation with reduced dose of inorganic fertilizers on soil microbial population and enzyme activities in rice field at Tamil Nadu Rice Research Institute, Aduthurai during Kuruvai and Samba Season (2017-18). The physico-chemical parameters and microbiological populations of the experimental field soil are presented in Table 1. Experiments were carried out in a randomized block design with three replications. The size of the experimental plots were 4 x 3 m and 20 x 15 cm spacing followed at 2 seedlings / hill for normal planting, where as in SRI the spacing were 22.5 x 22.5 cm and single seedling / hill. Fertilizers were applied at 150:50:50 kg N: P: K / ha. When microbial inoculants are included as treatment 75%, 50% and 25% of NPK fertilizers were applied. Azospirillum, phosphobacteria, K releasing and zinc solubilizing bacterial cultures were inoculated by seed treatment, seedling dipping and main field broadcasting. Four hundred gram of each inoculant was used for seed treatment, 1 kg for seedling dipping and 2 kg of each inoculant for soil application/ha.

Details of observation made

Rhizospheric soil samples were collected and analysed for the enumeration of *Azospirillum* (Okon et al., 1977), phosphobacteria (Sperber, 1958) and diazotroph (Watanabe and Barraquio, 1980). Soil enzymes *viz*., acid phosphatase (Eivazi and Tabatabai, 1977), alkaline phosphatase (Eivazi and Tabatabai, 1977), urease (Frankenberger and Tabatabai, 1991a and 1991b) and dehydrogenase (Casida et al., 1965) activities were measured. Organic carbon content were estimated in the soil samples by employing the wet digestion method of Walkley and Black (1934) as described by Piper (1950). The soil samples were collected for available N (Subbiah and Asija, 1956), P (Olsen et al., 1954), K (Standford and English, 1949) and Zinc (atomic absorption spectrophotometer) content analysis and yield were calculated and expressed as kg / ha. Plant samples were collected at periodic interval for

Table 1. The physico-chemical and microbiological populations of the experimental field soil.

Properties	Mean ± SE
pH	7.8± 0.09
EC (d Sm^{-1})	0.04± 0.15
Organic carbon (%)	0.50 ± 0.01
Available N (%)	162± 2.88
Available P (%)	35± 0.57
Available K (%)	250± 1.45
Total bacteria (cfu x 10^5 /gram dry weight of soil)[a]	46 ± 1.15
Fungi (cfu x 10^3 /gram dry weight of soil)[b]	2 ± 0.16
Diazotrophs (cfu x 10^4 /gram dry weight of soil)[c]	21 ± 1.15

[a] - Total bacteria were enumerated by serial dilution plating method on soil extract agar medium (James, 1958), [b] - Total culturable fungi were enumerated by serial dilution plating method as described by Parkinson et al. (1971) and [c] - Total diazotrophs were enumerated by the procedure as described by Rennie (1981).

recording plant growth parameters.

RESULT AND DISCUSSION

Effect of microbial inoculants and microbial inoculants with reduced fertilizers on soil microbial population under SRI and low land rice ecosystem

The maximum Azospirillum (5.4 x104 MPN/g), phosphobacteria (56 x104cfu/g) and diazotrophs (89 x104 cfu/g) population were observed in the treatment 50 % RDF of NPK + Azolla+ Azophos + KRB + ZSB followed by 25 % RDF of NPK +Azolla+ Azophos + KRB + ZSB for *Azospirillum* (2.4 x 104 MPN/g), phosphobacteria (56 x 104 cfu/g) and diazotrophs (64 x 104 cfu/g) under SRI rice system (Table 2). The result revealed that 100% RDF of inorganic fertilizers reduces the microbial activity when compared to 50 % and 25 % RDF of NPK. Among the two method of planting SRI is found to have maximum microbial population than low land rice ecosystem.

Effect of microbial inoculants and microbial inoculants with fertilizers on soil enzyme activities under SRI and low land rice ecosystem

The maximum soil enzymes *viz*., urease (79 µg of NH_4 /g / 24 hrs), dehydrogenase (110 µg of TPF / g / 24 hrs), acid phosphatase (248 µg of P nitrophenol/g/hr) and alkaline phosphatase (538 µg of P nitrophenol/g/hr) enzyme activities were found in the treatment 25 % RDF of NPK + BGA+ Azophos + KRB + ZSB followed by FYM + BGA + Azophos + KRB + ZSB for urease (73 µg of NH_4 / g / 24 hrs), dehydrogenase (100 µg of TPF / g / 24 hrs), acid phosphatase (218 µg of P nitrophenol / g / hr) and alkaline phosphatase (508 µg of P nitrophenol/g/hr) under SRI rice ecosystem. 25 % RDF of NPK acted as a food for microbes and induced the enzyme activity. The enzyme activities were decreased with 100 % RDF of NPK due to the higher substrate addition (Table 3). The enzyme activities of the FYM + BGA + azophos + KRB + ZSB were found to be onpar with 25 % RDF of NPK + BGA+ azophos + KRB + ZSB.

Effect of microbial inoculants and microbial inoculants with low dose of fertilizers on soil N, P, K, Zn and organic carbon under SRI and low land rice ecosystem

Among the treatments, maximum N (315 kg / ha), P (167 kg / ha) and K (684 kg / ha) were found in plot applied with 100 % RDF of NPK followed by 50 % RDF of NPK + *Azolla* + azophos + KRB + ZSB for N(303 kg / ha), P (128 kg / ha) and K (497 kg / ha). Maximum soil organic carbon (0.4 %) was found in the treatment FYM + *Azolla* + azophos + KRB + ZSB followed by 25 % RDF of NPK + Azolla+ KRB + ZSB for organic carbon (0.3%). Maximum available zinc (19 Kg / ha) was found in the treatment 50 % RDF of NPK followed by 100 % RDF of NPK for zinc (4.6 kg / ha) (Table 4).

Table 2. Effect of microbial inoculants on soil microbial population of the soil cropped with rice (ADT 49) under low land and SRI ecosystem.

Treatments	Diazotrophs (30 DAT) ($x10^4$ cfu/g)		Phosphobacteria (30 DAT) ($x10^4$ cfu/g)		Azospirillum (30 DAT) ($x10^4$ MPN/g)	
	SRI	Low land	SRI	Low land	SRI	Low land
T_1 - Un inoculated control	43	37	32	28	0.95	0.76
T_2 - FYM + *Azolla* + Azophos + KRB + ZSB	51	41	25	30	1.40	1.70
T_3 - 50 % RDF of NPK + *Azolla* + KRB + ZSB	47	32	40	32	2.10	2.2
T_4 - 50 % RDF of NPK + *Azolla*+ Azophos + KRB + ZSB	89	42	56	47	5.40	4.3
T_5 - 25 % RDF of NPK + *Azolla* + KRB + ZSB	62	31	40	36	0.64	2.7
T_6 - 25 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB	64	37	56	38	2.40	2.2
T_7 - 100 % RDF of NPK	41	31	24	27	1.5	0.81
SEd	3.88	2.07	2.66	2.24	0.15	0.175
CD (P = 0.05%)	8.46	4.50	5.81	4.87	0.33	0.381

Data are the mean of three replications

Table 3. Effect of microbial inoculants on soil enzyme activities of the soil cropped with rice (ADT 49) under low land and SRI cosystem.

Treatments	Alkaline Phosphatase (60 DAT) (µg of P nitrophenol/g/hr)		Acid phosphatase (60 DAT) (µg of P nitrophenol/g/hr)		Urease (60 DAT) (µg of NH4/g /24 hrs		Dehydrogenase (60 DAT) (µg of TPF /g/24 hrs)	
	SRI	Low land	SRI	Low land	SRI	Low land	SRI	Low land
T_1 - Un inoculated control	492	476	201	171	62	54	65	49
T_2 - FYM + Azolla+ Azophos + KRB + ZSB	505	485	232	202	67	61	77	71
T_3 - 50 % RDF of NPK + *Azolla* + KRB + ZSB	472	462	155	125	82	77	88	82
T_4 - 50 % RDF of NPK + *Azolla*+ Azophos + KRB + ZSB	474	456	174	144	91	85	111	105
T_5 - 25 % RDF of NPK + *Azolla* + KRB + ZSB	538	508	248	218	75	69	88	81
T_6 - 25 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB	482	462	185	155	68	61	72	66
T_7 - 100 % RDF of NPK	405	380	133	105	71	69	58	51
SEd	29.34	27.91	11.85	9.78	4.68	4.34	4.87	4.87
CD (P =0.05%)	63.94	60.81	25.82	21.32	10.22	9.46	10.6	10.6

Data are the mean of three replications

Effect of microbial inoculant, microbial inoculant with low dose of fertilizers on plant growth and yield parameters under SRI and low land rice ecosystem

Among the treatments maximum root length (89 cm/ plant), shoot length (64 cm / plant), dry weight (84 g/ plant) were observed in the treatment 50% RDF of NPK + *Azolla* + KRB + ZSB followed by 25 % RDF of NPK +Azolla+ Azophos + KRB + ZSB for root length (64 cm/plant), shoot length(60 cm/plant), dry weight (75 g / plant). Addition of 50 % RDF of inorganic fertilizer along with biofertilizers may improve the plant growth parameters. The maximum yield (6850 kg / ha) was observed in the treatment 100% RDF of NPK which was found to be on par with 50% RDF of NPK + *Azolla* + KRB + ZSB for yield (6800 kg / ha) (Table 5). The yield variation between the standard check (100 % RDF of NPK) and 50% RDF of NPK + *Azolla* + KRB + ZSB were negligible.

The microbial populations were recorded more in 50 % RDF of NPK + *Azolla* + KRB + ZSB followed by 25 % RDF of NPK + *Azolla* + KRB + ZSB. This indicates the minimum dose of fertilizer triggers the growth of microbes and higher dose of fertilizers inhibits the microbial growth as revealed from the experiment. Kumari et al. (2011) also reported that continuous application of organic manure alone or in combination with inorganic fertilizer significantly influenced the soil microbial biomass. Hasnabade (1992) reported that the highest microbial population *viz*., bacteria, actinomycetes and fungi were registered in 50 % RDF of NPK + 50 % through FYM. Mishra et al. (1991) found that the microbial population increased with increasing application of farm yard manure upto 20 t ha^{-1}. Biomass was more in treatment receiving FYM. The current findings reported that the increased fertilizer decreased soil microbial activity. This was supported by the finding Zhang et al. (2015) reported that all fertilized treatments had decreased soil actinomycetes population compared with the control. This may be attributed to the enhanced acidity caused by N chemical fertilization, which is unfavorable for actinomycetes development.

Soil enzyme activities were higher at 25 % RDF of NPK + BGA+ Azophos + KRB + ZSB followed by FYM + BGA + Azophos + KRB + ZSB. This indicates reduced amount of fertilizers favours the soil enzyme activity than 100 % and 50 % RDF of NPK. The current results support the finding of Dick (1988) who has reported that long-term application of organic manure increased soil enzyme activity and microbial biomass, but NH_4^+ - N fertilizer caused decrease of amidase and urease activity related to N fertilizer cycle. Wang (1982) and Zhou (1983) had reported different type and amount of fertilizers directly affected soil enzymatic activities and then affected nutrient uptake by plant roots.

Maximum NPK was found in the treatment

Table 4. Effect of microbial inoculants on N, P, K, Zn and organic carbon content of the soil cropped with rice (ADT 49) under low land and SRI ecosystem.

Treatments	N 30 DAT (Kg/ha)		P 30 DAT (Kg/ha)		K 30 DAT (Kg/ha)		Zinc (30 DAT) (Kg/ha)		Organic carbon 90 DAT (%)	
	SRI	Low land	SRI	Low land	SRI	Low land	SRI	Low land	SRI	Low land
T_1 - Un inoculated control	289	277	69	63	479	463	3.9	2.2	0.27	0.24
T_2 - FYM + *Azolla* + Azophos + KRB + ZSB	248	261	105	100	490	475	4.5	4	0.40	0.30
T_3 - 50% RDF of NPK + *Azolla* + KRB + ZSB	280	301	97	100	439	425	3.7	3.9	0.29	0.29
T_4 - 50% RDF of NPK + *Azolla* + Azophos + KRB + ZSB	303	293	128	125	497	475	19	17	0.32	0.28
T_5 - 25 % RDF of NPK + *Azolla* + KRB + ZSB	290	285	110	87	446	425	3.0	3.7	0.30	0.29
T_6 - 25 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB	290	237	107	100	420	400	5	5.3	0.26	0.28
T_7 - 100 % RDF of NPK	315	301	167	156	684	663	4.6	4.9	0.20	0.27
SEd	14.22	12.45	1.98	1.78	28.12	28.85	0.45	0.497	0.017	0.016
CD (P = 0.05%)	30.99	27.12	4.32	3.89	61.42	62.87	0.99	1.083	0.038	0.036

100 % RDF of NPK due to the addition of inorganic fertilizers. Maximum soil organic carbon was found in the treatment FYM + *Azolla* + Azophos + KRB + ZSB. Incorporation of organic matter in the form of FYM has a significant positive effect on the bulk density of soils (Celik et al., 2004), soil aggregation properties (Sarkar and Rathore, 1992), soil structure (Chaudhary and Ghildyal, 1969), the moisture retention capacity of soil (Hudson, 1994) and water infiltration rate (Tiwari et al., 1998).

Plant growth parameters, yield and available zinc were found to be higher in 50 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB. Jagadeesha (2018) reported that the application of RDF + biofertilizers (2.5 kg / ha) is more effective in increasing the crop growth, seed yield and seed quality of tomato followed by the treatment FYM (50 %) + vermicompost (50 %) + biofertilizers (2.5 kg / ha).

The study were supported by the finding Pathak and Kumar, 2016 who has report that biofertilizers include all organisms which supply or make different nutrients available to plants like nitrogen fixers, phosphorus solubilizers, potassium solubilizers, sulfur solubilizers, mycorrhiza, etc. Biofertilizers improve the nutritious properties of fresh vegetables by increasing; the antioxidant activity, the total phenolic compounds and chlorophyll (Khalid et al., 2017). Spinach inoculated with different biofertilizers was found to have 58.72 and 51.43% higher total phenolic content than the un-inoculated control (Khalid et al., 2017). These secondary metabolites play preventive roles in cancer, neurodegenerative, and cardiovascular disorders (Rodriguez-Morato et al., 2015).

From the result of the present experiment, it can be inferred that the reduced amount of fertilizers along with biofertilizers can be a eco-friendly farming

Table 5. Effect of microbial inoculants on root length, shoot length, dry weight and yield of rice (ADT 49) under low land and SRI ecosystem.

Treatments	Root length (60 DAT) (cm/plant)		Shoot length (60 DAT) (cm/plant)		Dry weight (90 DAT) (g//plant)		Yield (Kg/ha)	
	SRI	Low land	SRI	Low land	SRI	Low land	SRI	Low land
T_1 - Un inoculated control	43	31	36	35	67	49	5183	4820
T_2 - FYM + *Azolla* + Azophos + KRB + ZSB	51	34	52	29	72	44	5610	5510
T_3 - 50 % RDF of NPK + *Azolla* + KRB + ZSB	47	32	54	40	65	53	6166	5220
T_4 - 50 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB	89	39	64	56	89	54	6800	5780
T_5 - 25 % RDF of NPK + *Azolla* + KRB + ZSB	62	37	48	40	85	51	6333	5620
T_6 - 25 % RDF of NPK + *Azolla* + Azophos + KRB + ZSB	64	32	60	52	95	52	6755	5300
T_7 -100% RDF of NPK	41	34	50	44	92	54	6850	5960
SEd	0.88	2.12	3.30	2.82	5.00	3.12	384.60	333.86
CD (P =0.05%)	1.92	4.64	7.19	6.16	10.90	6.81	837.98	727.44

Data are the mean of three replications.

practices come increasing plant growth and yield.

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