RESEARCH ARTICLE

Integrated Application of *Trichoderma* Mixture and NPK Enhances the rice Productivity in Sodic Soil

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Abstract

Abiotic stresses due to changing environmental conditions and intensive agriculture are causing soil salinity and sodicity leading to soil deterioration. It directly affects nutrient availability and uptake and reduces plant productivity. The microorganisms are known to improve the availability of nutrients in the soil and therefore, the present work aimed to maximize the use of applied fertilizers and boost the productivity. The effects of the *Trichoderma* mixture (TrichoMix) and NPK on plant growth and rice yield in degraded land under field conditions was studied. The abiotic stress tolerant *Trichoderma* strains comprising of *T. koningiopsis* (NBRI-PR5) and *T. asperellum* (NBRI-K14) were used in the treatments (i) Control (Cont.), (ii) NPK, as per recommended dose ($NPK_{100\%}$) (iii) *Trichoderma* mixture (NBRI-PR5+NBRI-K14) (TrichoMix) (iv) TrichoMix + $NPK_{50\%}$. Significantly higher plant growth and yield (6.57q/h) was obtained in TrichoMix+ $NPK_{50\%}$ treatment. The improvement in rice production in the treatment TrichoMix +$NPK_{50\%}$ was ascribed to the significant changes in soil physiochemical characteristics such as water holding capacity (35%), bulk density (40%), total organic carbon (42%), available N (39%), P (44%), K (48%) and microbial biomass carbon (88%). The results conclude that the mixture of *T. koningiopsis* (NBRI-PR5) and *T. asperellum* (NBRI-K14) may be recommended as sodic soil amendment to increase plant growth and yield in combination with chemical fertilizers to support rice cultivation.

Keywords: Chemical fertilizers, Sodic soil, *Trichoderma* mixture, Rice

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Introduction

The major abiotic stresses that deteriorate soil quality under prolonged conditions include salinity and soil sodicity. It affects the nutrient availability and uptake by the plants, consequently reducing productivity. The adverse effects of salt stress on the soil's ion and osmotic balance, pH, nutrient availability or their combinations (Almeida *et al.,* 2017; Singh *et al.,* 2018; Evelin *et al.,* 2019; Mansour and Soliman, 2022). In India, ~ 26.3 M ha (million hectares) of agricultural land is barren and is prone to nutrient depletion (Tandon *et al.,* 2019). Approximately 1125 M ha of agricultural lands are adversely affected by salinity and exceed salt-affected continental boundaries of North and Central Asia (Hossain, 2019). Besides the primary natural processes leading to soil sodicity, secondary degradation in agricultural fields occur by anthropogenic activities such as irrigation with saline or poor-quality water without sufficient leaching of salts, excessive use of chemical fertilizers containing chlorides, sulfates etc. (Lakhdar *et al.,* 2009; Rengasamy 2010). ICAR-CSSRI has reported that approximately 16.2 M ha (million hectares) or more land area compared to the current 6.74 M ha in India would be affected by sodicity by 2050 (Kumar *et al.,* 2020).

Availability of nutrients is a major concern in these soils since nutrients like Zn, P, Mg etc., get fixed due to increased pH and salt. Therefore, to increase nutrient availability application of organic acid-producing microbes may provide a conducive environment or ambient pH conditions to mobilize the nutrients. Many studies have highlighted the role of microorganisms in the interactions of various factors of the agro-ecosystem (El-Ramady, 2022). The application of microorganisms and organic matter has encouraged sustainable, eco-friendly strategies. *Trichoderma* is one such potential microbe that has been extensively used

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in agriculture, mostly as a bio-control agent. In the present study, we have used *T. koningiopsis* (NBRI-PR5) in the mixture, which has been earlier reported for producing organic acid in abiotic stress conditions and P availability (Tandon *et al.,* 2020). The *T. asperellum* (NBRI-K14) other mixture constituents were used because of their stress tolerance and PGPR activity which complements *T. koningiopsis* (NBRI-PR5*)*. The mixture of these two *Trichoderma* has been earlier reported to enhance wheat production in sodic soil (Tandon *et al.,* 2022).

In the present study, we have used rice as the host plant, which is reported to be highly susceptible to sodic stress (Zeng *et al.,* 2000; Upadhya *et al.,* 2020; Huang *et al.,* 2022). Sodium accumulation in carbonate and bicarbonate forms at the seedling stage causes low tillering, leading to significantly high plant mortality and yield losses (Chunthaburee *et al.,* 2016; Kamran *et al.,* 2020). In the soil, microbial interactions improve the long-term nutrient content and reserve by regulating the

C, N and S nutrient cycles (Kaiser *et al,* 2015). Therefore, the present study was taken up with the hypothesis to improve the availability of nutrients in the soil to increase productivity and maximize the use of applied fertilizers. Thus, the study aimed to reduce the use of chemical fertilizer to support the farmers by enhancing grain yield at a low cost and increase their income.

Materials And Methods

Microbial Cultures and Growth

In the present study, two *Trichoderma* strains were used (i) *T. koningiopsis* strain NBRI-PR5 (MTCC 25372) and *T. asperellum* strain NBRI-K14 (MTCC 25373). The cultures were grown on PDA at 28–30°C for 5–7 days.

Field Experiment

The field experiment was done in plots of size 5m×5m during kharif at the seed farm, Sandila, Agriculture Dept., U.P., Lucknow (27.0765°N, 80.5179°E). The fields were prepared in RBD (randomized block designs) with 3 replicates each. Spacing between two rows was 30 cm and spacing between plants was 25 cm each. The initial pH 9.94, EC 2.5 dS/m the temperature ranged from 21°C to 30°C throughout the growing season. Twenty-one-day-old seedlings were dipped in the *Trichoderma* spore suspension for 30 minutes before transplanting. The spore's suspension was prepared by harvesting spores from 5 to 7 days old PDA culture plates in distilled water with CFU 10^6 Log_{10} ml^{-1} (Singh and Nautiyal *et al.,* 2012). The spore suspensions of both cultures were mixed in equal proportion for the treatment. The treatments included (i) Control (Cont.), (ii)NPK as per recommended dose ($NPK_{100\%}$) (iii)*Trichoderma* mixture (TrichoMix.), (iv) (TrichoMix + $NPK_{50\%}$). Watering was done as and when required. The observations for plant growth and yield were taken on maturity

Soil Microbial Population

The soil microbes were estimated on the selective medium by plating the dilutions (Srivastava *et al.,* 2011). Briefly, in 10 ml of sterile distilled water (sdw) 1 g of the soil sample was suspended and vortexed thoroughly. The dilutions were spread on specific media in three replicates. Nutrient agar, Actinomycetes isolation agar, Rose Bengal agar and *Trichoderma* selective media (TSM) were used to quantify heterogenous bacteria, actinomycetes, total fungi and *Trichoderma* (Elad and Chet, 1983). Colony forming units (CFU) per gram of soil sample were calculated.

Soil Enzymatic Activity

Freshly collected samples were used for enzyme assays after finely grinding and sieving (2mm) to remove the debris. DHA (dehydrogenase) activity was determined as reported by Pepper *et al.* (1995). The triphenyl formazan (TPF) formed after 24h incubation was extracted with acetone and measured by a spectrophotometer at 485nm. Fluorescein diacetate activity (FDA) was determined according to Stubberfield and Shaw (1990). The soil samples were incubated with diacetyl-fluorescein solution to estimate the FDA activity.

Analysis of Physico-chemical Properties of Soil

The soil physicochemical analysis was done using finely ground, air dried and sieved soil samples. The processed soil sample was stored at 10 °C for further use. Keen's box method was used to calculate the soil samples' water-holding capacity (WHC). Bulk density was measured by pycnometer according to (Blake, 1965). Total organic carbon was measured in according to (Walkley and Black, 1934). The chloroform-fumigation-extraction method was used for determining the microbial biomass carbon (MBC) (Johnansson *et al.,* 2004). The soil available P was determined by the method described by Olsen *et al.* (1954). The soil available K and Ca were measured using flame photometer (Systronix-128, USA). Kjeldahl's method was used to determine available N in the soil samples. The soil water suspension in 1:2.5 (W/v) ratio prepared in double distilled water was used to determine the soil pH and electrical conductance.

Statistical analysis: The data represented are means of at least three biological replicates in all the experiments. The standard deviation (SD) or standard errors (se) were calculated using Microsoft Excel and presented with the mean values (mean±SE/SD). One way Analysis of variance (ANOVA) and Duncan's post hoc test (DMRT) at p-value 0.05 was applied using SPSS16.0 on the data to determine the statistical significance.

Results And Discussion

The integrated application of the *T. koningiopsis* (NBRI-PR5) and *T. asperellum* (NBRI-K14) and reduced NPK dose enhanced rice productivity in sodic soil. The compatibility of the two *Trichoderma* isolates has been earlier reported where they showed enhanced wheat growth in sodic soil and improved soil properties when applied in sodic soil along with rice straw (Tandon *et al.,* 2022).

Plant growth promotion (PGP)

The present study showed the affectivity of *Trichoderma* mixture to optimally use the NPK in integrated applications and reduce the chemical inputs. As compared to the control

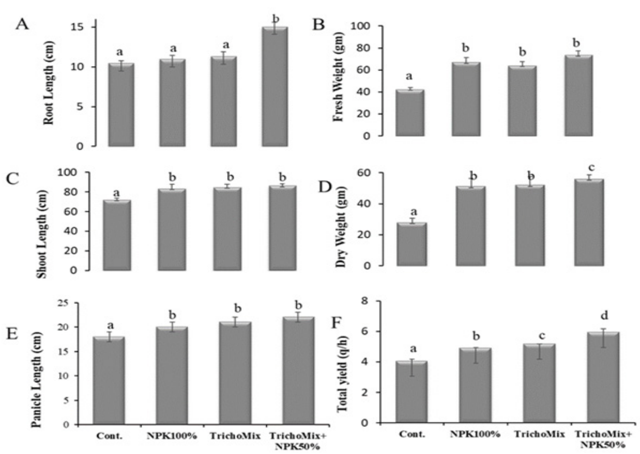

Fig 1: Effect of TrichoMix on vegetative parameters related to biomass of rice grown under sodic field condition. All the values are means of 3 replicates with ±S.E. bars. Different letters indicate significance among the treatments (DMRT, p≤0.05).

Table 1: Heterogeneous microbial population in rhizosphere soil of paddy grown under sodic soil in field conditions, and their correlation with soil microbial enzymes (Log_{10} CFU g^{-1} soil).

	Log_{10} CFU/g soil				*Soil Microbial Enzymes*	
Treatments	*Bacteria*	*Actinomycetes*	*Fungus*	*Trichoderma*	*DHA [μg TPF $g^{-1}h^{-1}$]*	*FDA [μg βNP g^{-1}-h^{-1}]*
Cont.	5.04	4.91	2.98	1.84	2.020	282.850
NPK$_{100\%}$	5.15	5.03	3.01	2.32	2.326	397.116
TrichoMix	5.07	4.83	2.97	2.41	3.370	226.943
TrichoMix +NPK$_{50\%}$	5.27	5.07	2.86	2.64	3.994	326.878

	Correlation coefficient among the soil microbes and soil microbial enzymes					
	Bacteria	Actinomycetes	Fungus	Trichoderma	DHA	FDA
Bacteria	1					
Actinomycetes	0.85265	1				
Fungus	-0.74373	-0.44054	1			
Trichoderma	0.79776	0.42045	-0.58773	1		
DHA	0.68320	0.20268	-0.8156	0.88313	1	
FDA	0.51916	0.84944	0.09854	0.14738	-0.23546	1

the TrichoMix+NPK$_{50\%}$ treatment showed improved parameter as root length was increased by 36%, shoot length improved by 20%, fresh weight was enhanced by 70% and dry weight was improved by 99%. In another study, *T. asperellum* improved the maize root growth parameters under saline-alkaline stress conditions (Fu *et al.,* 2021). Kapadia *et al.* (2022) used halotolerant bacteria to ameliorate salinity stress in rice. The flag leaf parameter is an important indicator of growth as it is the primary source of photosynthetic energy during grain filling. Its length and numbers were increased by 24% and 65% in TrichoMix+NPK$_{50\%}$ as compared to the control (Fig.1). The grain yield parameters like panicle number and length and seed weight were also observed to have increased in the TrichoMix+NPK$_{50\%}$ treatment in comparison to the control. Increased panicle number (98%) and length up to 22% and 76% increase in seed weight were observed in TrichoMix+ NPK50$_{\%}$. The tiller and spikelet increased by 36% and 49% respectively. Overall yield increased by 27% in presence of TrichoMix, 21% in presence of NPK$_{100\%}$ and 46% in presence of TrichoMix+NPK$_{50\%}$ in comparison to control (Fig. 2). As compared to NPK$_{100\%,}$ the TrichoMix+NPK$_{50\%}$ treatment increased the yield by 20 and 14% compared to the TrichoMix alone. It shows that the TrichoMix+NPK$_{50\%}$ treatment microbial mixture helped in utilizing the nutrients more efficiently even when applied in half the recommended dose. Although many results have shown increased growth and productivity by *Trichoderma* there are scanty reports of its application in sodic soil to reduce NPK application and increase productivity.

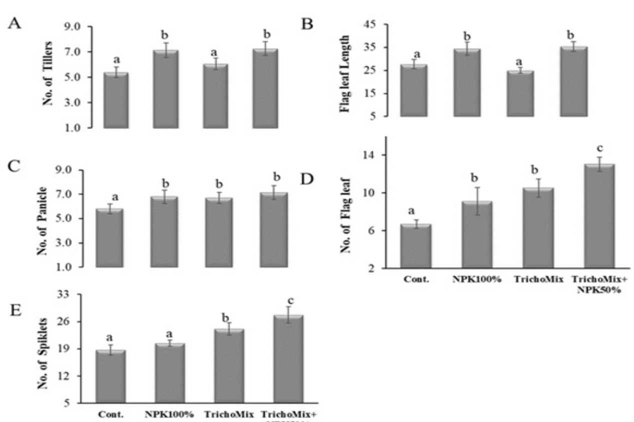

Fig 2: Effect of TrichoMix on vegetative parameters related to yield of rice grown under sodic field condition. All the values are means of 3 replicates with ±S.E. bars. Different letters indicate significance among the treatments (DMRT, p≤0.05).

Rhizospheric Microbes

Many differences were observed in the heterogenous microbial population of bacteria, actinomycetes, total fungi and *Trichoderma* in response to NPK$_{100\%}$, TrichoMix and TrichoMix+NPK$_{50\%}$ application (Table 1). Increased bacterial and actinomycetes population was observed in the TrichoMix+NPK$_{50\%}$ compared to control. Significant correlation of *Trichoderma* with bacterial population (r^2=0.79) and soil microbial enzyme activities DHA (r^2=0.88) shows the role of *Trichoderma* in supporting natural microflora population and activity. On the other hand, the indigenous fungal population was observed to affect the bacterial population (r^2= -0.74) and activity (r^2= -0.81). The NPK treatment resulted in the accumulation of inorganic salts in the rhizosphere, which are source of salinity and thus results in reduced microflora. Such effects have been shown earlier in the presence of NPK (Tandon *et al.,* 2018). Earlier studies report that the abundance of actinomycetes in soil was improved by applying *Trichoderma hamatum* strain MHT 1134 in continuous pepper cropping fields (Mao *et al.,* 2021). In TrichoMix+NPK$_{50\%}$, the reduction in 50% NPK decreased the chemical effect on natural rhizosphere microflora while the *Trichoderma* helped in utilizing the nutrients efficiently, as reported earlier in chickpea rhizosphere (Tandon *et al.,* 2018). Thus, our results showed the ability of both *Trichoderma* to maintain its population in compliance with rhizospheric property and also reshape the rhizosphere microbiome differentially in different conditions.

Physicochemical Properties of Soil

In various studies, *Trichoderma spp.* has improved soil texture and fertility. (Petrisor *et al.,* 2019; Cataldo *et al.,* 2021). The application of microbial mixture in sodic soil improved the soil

Table 2: Physicochemical parameters of Rhizosphere soil of paddy grown under sodic soil in field conditions.

Parameters	*Cont.*	*$NPK_{100\%}$*	*TrichoMix*	*TrichoMix+$NPK_{50\%}$*
pH	9.91±0.04	9.08±0.14	8.87±0.23	8.28±0.72
EC (µS/cm)	159.53±4.04	217.90±3.42	263.96±12.42	189.66±3.04
Bulk density(gm/cc^3)	1.18±0.06	1.22±0.07	1.35±0.08	1.59±0.03
WHC (%)	29.95±8.88	48.870.41	55.83±13.75	79.49±0.69
TOC (%)	0.90±0.28	1.68±0.22±	1.66±0.20	2.15±0.30
SOM (%)	1.55±0.12	1.71±0.01	2.86±0.19	2.89±0.22
MBC (µg/g)	884.5±184	1012.01±222	1238.53±442	1284.5±22
Avail. N (gm/kg)	1.01±0.15	1.59±0.14	1.29±0.20	2.65±0.25
Avail. P mg/g	2.00±0.02	2.05±0.01	2.87±0.02	2.95±0.04
Avail. K(mg/g)	156.69±0.39	185.52±0.24	257.15±0.12	353.09±0.26
Avail.Ca(mg/g)	1241.28±146	2289.52±147	2001.00±152	2269.88±122
Avail. S (mg/g)	1.05±0.11	1.32±0.10	1.56±0.08	3.29±0.04

properties by decreasing the pH from 9.9 to 8.2 and increasing the SOC from 0.90% to 2.15% in Cons.+$NPK_{50\%}$treated soil. In the TrichoMix+$NPK_{50\%}$ treatment soil, the soil parameters were higher than in $NPK_{100\%}$ treatment. This suggests that in $NPK_{100\%}$ having 39, 48, 44% available N, P and K became fixed and were not available. On the other hand, in TrichoMix+$NPK_{50\%}$, the mixture assisted in nutrient availability. Microbial mixture thus reduces the amount of chemical fertilizer used in fields, further reducing the fertilizer cost and increasing the farmer's overall income. The TrichoMix+$NPK_{50\%}$ treatment also improved the soil bulk density, water holding capacity (WHC), and the soil microbial enzymes showing increased microbial activity and microbial biomass carbon (MBC) (Table 2). Biochar of agri-waste is another reported alternative to improve soil characteristics. *T. harzianum* and biochar were reported to reduce the deleterious effects of salt stress on the crop plants (Sofy *et al.,* 2019). When applied, the combination of biochar, manure and NPK improved the soil physicochemical parameters such as soil available phosphorus and total nitrogen, soil pH, total organic carbon, in cucumber (Apori *et al.,* 2021; Agbede *et al.,* 2019; Huang *et al.*, 2022). The role of nutrients such as Ca^+ application and farmyard manure has been reported to improve sodic-saline soils and grain yield in rice (Mansour, and Soliman 2022). Thus, our results imply that the soil's fertility can be improved at low costs by microbial intervention in integrated farming and can be helpful in converting the intensively cultivated deteriorating fields into more productive soil.

Conclusion

An integrated approach is required to tap the plant's optimum yield in the current abiotic stress conditions. The integrated management includes scheduled irrigation, fertilization, and use of salt-resistant varieties. Apart from this the bio-stimulants based on microorganisms are an excellent source of stress-alleviating agents. On application, they can induce the plant stress response system and maximize the plant's stress tolerance and yield capacity. The present study concluded that the application of TrichoMix with NPK50% can improve soil quality by increasing nutrient availability with reduced inputs and support better growth and crop yield in marginally sodic soil. The integrated approach is also recommended to shift intensively cultivated fields to the organic field without compromising the soil fertility and plant yield capacity.

Conflict Of Interest

The authors declare no conflict of interest.

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