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BIO-FERTILIZER ACTIVITY OF *TRICHODERMA VIRIDE* AND *PSEUDOMONAS FLUORESCENS* AS GROWTH AND YIELD PROMOTER FOR MAIZE

Bikram Nepali[1], Subash Subedi[2], Sabin Bhattarai[1], Santhosh Marahatta[1], Devashish Bhandari[1], Jiban Shrestha[3]

[1]Agriculture and Forestry University, Rampur, 15, Bharatpur Metropolitan City, Chitwan, 44209, Nepal
[2]National Maize Research Program, Rampur, 15, Bharatpur Metropolitan City, Chitwan, 44209, Nepal
[3]National Plant Breeding and Genetics Research Centre, Khumaltar, 15, Lalitpur Metropolitan City, 44700, Nepal

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Vastutav autor: Corresponding author: Bikram Nepali
E-mail: bikramn25@gmail.com

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ABSTRACT. The bio-fertilizer potential of *Trichoderma viride* and *Pseudomonas fluorescens* on growth and yield performance of open-pollinated maize variety Rampur Composite was studied at the research farm of Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal during the winter season of 2018/19. The experiment was laid out in randomized complete block design with seven treatments (T1: recommended dose of NPK (120:60:40 kg NPK ha^{-1}), T2: *T. viride* only, T3: *P. fluorescens* only, T4: *T. viride* + 50% NPK; T5: *P. fluorescens* + 50% NPK; T6: *T. viride* + *P. fluorescens* + 100 % NPK, T7: control) and replicated thrice. The package of agronomic practices was followed as per national recommendation. The result revealed that *T. viride* + 50% NPK enhanced most of the growth components like plant height (103%), leaf number (9.77%), stem diameter (73.98%), root length (40.57%), leaf area index (173.28%), leaf biomass (83.36%) and stem biomass (127.72%) of maize compared to the control. Similarly, the higher cob biomass (641 g), yield (5708 kg ha^{-1}) and thousand kernel weight (295 g) were recorded in the plot applied to *P. fluorescens* + 50% NPK. The use of *Trichoderma viride* and *Pseudomonas fluorescens* with a half-dose of recommended fertilizers may increase the vegetative growth and yield of maize and may also help to reduce the rate of chemical fertilizers in maize.

Introduction

Maize (*Zea mays* L.) is the second important staple food crop in terms of area (954 158 ha) and production (2 555 847 t) with a productivity of 2 679 kg ha^{-1} in Nepal (MoALD, 2018). The productivity of maize in Nepal is very low compared to global yield with wide yield gap due to various biotic and abiotic factors (Subedi, 2015). Among yield-limiting factors, the management of fertilizers, particularly nitrogen, is crucial to increase the yield of maize. Chemical fertilizers have been used indiscriminately and have contributed to severe disequilibrium in agroecosystems due to pollution of soil, water, air and food, causing soil degradation and pest resistance, and a threat to human health (NMRP, 2016). Today, it is of great interest to reduce synthetic fertilization and to recover soil microflora utilizing strategies that make it possible to improve agricultural productivity in a non-polluting manner. Bio-fertilization with microorganisms (like *Trichoderma* and *Pseudomonas*) is a common method used in various crops in different parts of the world to improve the growth and yield components. *Trichoderma* and *Pseudomonas* are fungi and bacteria, respectively, considered very potent biological control agents against a wide range of pathogens of different crops (Subedi *et al*., 2015), using different control mechanisms such as antibiosis, lysis and mycoparasitism (Subedi *et al*., 2019). The strength of *Trichoderma and Pseudomonas* spp. as opportunistic microflora are well-known bio-stimulants (Candelero *et al*., 2015), characterized by their rapid growth, the ability to assimilate a wide range of substrates (Chen *et al*., 2006) and the production of a variety of microbial compounds, which stimulate the growth rate, yield and vegetal development of the crop. The maize roots colonized by microflora like *Trichoderma* and *Pseudomonas* spp. require 40% less nitrogen fertilizer in comparison to non-colonized roots, reducing fertilizers' application costs and improving the environment (Páez *et al*., 2006;

Harman, 2006). Bhusal *et al.* (2018) recorded that the application of *Trichoderma viride* was effective for leaf blast management in rice. Mahato *et al.* (2018) found that the application of *Trichoderma viride* as biofertilizer increased the grain yield in wheat. In this study, the effect of commercial formulation with *T. viride* and *P. fluorescens* applications alone or in combination with different doses of chemical fertilizer on maize growth and yield components were investigated. The study aimed to evaluate *T. viride* and *P. fluorescens* as biofertilizers for their ability to promote growth and yield of maize crop.

Materials and methods

The experiment was conducted at the research farm of Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal during the winter season of 2018/19. The latitude, longitude and altitude of the experimental site was 27° 40'N 84° 19' E, and 228 masl respectively. The experiment was laid out in randomized complete block design (RCBD) with seven treatments and replicated thrice. The treatment combinations were T1: Recommended dose of NPK (120:60:40 kg NPK ha^{-1}), T2: *Trichoderma viride* only, T3: *Pseudomonas fluorescens* only, T4: *Trichoderma viride* + 50% NPK; T5: *Pseudomonas fluorescens* + 50% NPK; T6: *Trichoderma viride* + *Pseudomonas fluorescens* + 100 % NPK and T7: control. The micro-floras used in the experiment were the commercial formulation of Biocide Trivi (*Trichoderma viride* – 1×10^9 cfu ml^{-1}) and Guard (*Pseudomonas fluorescens* – 1×10^9 cfu ml^{-1}) received from Agricare Nepal Private Ltd, Bharatpur, Chitwan. The commercial formulation of micro-floras was prepared separately at 2.5 ml each in 5% sugar solution on 1 litre of water and inoculated with the seed of open-pollinated variety Rampur Composite at 2.5 ml kg^{-1} of seed. The unit plot size was 6 rows of 6.5 m length with a row spacing of 0.60 m and plant spacing of 0.25 m. All agronomic practices were followed as per standard of National Maize Research Program, Rampur (NMRP, 2016). In the case of fertilizer, Di-ammonium phosphate (DAP) and Murate of potash (MoP) were applied as basal whereas urea was top-dressed in three splits to the doses mentioned in the treatments. Five plants from each experimental plot were randomly selected for data collection. Data on growth and yield attributing components like plant height (cm), leaf number, stem diameter (cm), root length (cm), leaf area index, leaf biomass per plant (g), stem biomass per plant (g), cob biomass per plant (g), grain yield (kg ha^{-1}) and thousand kernel weight (g) were recorded (CIMMYT, 1985). Leaf area index was calculated dividing leaf area (m^2) by ground coverage of maize (m^2). Grain yield was adjusted to 80% shelling recovery and 15% moisture level. The growth and yield parameters increased over the control was calculated. The soil samples were collected from two different depths (15 and 30 cm) from the experimental field and analyzed in the laboratory of soil science division, NARC, Khumaltar, Lalitpur. The pH, organic matter, nitrogen, phosphorus, and potash content of the soil samples were analyzed (Motsara, Roy, 2008). Microsoft Excel was used for tabulation of data and simple calculation. Data were analyzed statistically by performing analysis of variance (Steel, Torrie, 1980) by using GenStat software (18th edition) and the means were separated by using Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results

The growth parameters like plant height, stem diameter, root length and leaf area index differed significantly among the treatments (Table 1). The higher plant height (250 cm), stem diameter (2 cm) root length (28 cm) and leaf area index (4) of maize were recorded in the plot with *Trichoderma viride* + 50% NPK whereas the lower plant height (123 cm), stem diameter (1 cm) root length (20 cm) and leaf area index (2) of maize were recorded in control plot (Table 1).

Significant differences were revealed in the biomass and yield variables of maize among the treatments (Table 2). The higher leaf biomass/plant (845 g) and stem biomass/ plant (2637 g) of maize were recorded in the plot with *Trichoderma viride* + 50% NPK whereas higher cob biomass/plant (641 g), grain yield (5708 kg ha^{-1}) and thousand kernel weight (295 g) was recorded in the plot with *Pseudomonas fluorescens* + 50% NPK. The lower leaf biomass per plant (461 g), stem biomass per plant (1158 g), cob biomass per plant (150 g), grain yield (4965 kg ha^{-1}) and thousand kernel weight (267 g) of maize were recorded in control plot (Table 2).

Table 1. Effect of treatments on growth traits of maize at Rampur, Chitwan during winter 2018

Treatment	Plant height, cm	Leaf number per plant	Stem diameter, cm	Root length, cm	Leaf area index
Recommended dose of NPK (120:60:40 kg NPK ha^{-1})	175^b	11	2^d	26^b	3^b
Trichoderma viride (1×10^9 cfu ml^{-1}) only	177^b	10	2^b	26^c	2^d
Pseudomonas fluorescens (1×10^9 cfu ml^{-1}) only	169^b	11	2^c	24^d	3^c
Trichoderma viride + 50% NPK	250^a	11	2^a	28^a	4^a
Pseudomonas fluorescens + 50% NPK	172^b	10	2^d	24^d	3^{cd}
Trichoderma viride + *Pseudomonas fluorescens* + 100% NPK	132^{bc}	10	2^e	23^e	2^e
Control	123^c	10	1^f	20^f	2^f
Grand mean	171	10	2	24	3
Significance with F-test	**	NS	**	**	**
LSD (0.05)	41.97	1.57	0.03	0.76	0.20
CV, %	13.80	8.50	1.00	1.70	4.40

Means in the column with the same superscript are not significantly different by DMRT $_{(P<0.01)}$, ** – significant at 0.01 level, NS – not significant

Table 2. Effect of treatments on biomass and yield of maize at Rampur, Chitwan during winter 2018

Treatment	Leaf biomass per plant, g	Stem biomass per plant, g	Cob biomass per plant, g	Yield, kg ha^{-1}	Thousand kernel weight, g
Recommended dose of NPK (120:60:40 kg NPK ha^{-1})	828^{a}	1568^{e}	621^{a}	5451^{a}	285^{b}
Trichoderma viride (1×10^{9} cfu ml^{-1}) only	663^{c}	1860^{b}	537^{b}	5547^{a}	274^{d}
Pseudomonas fluorescens (1×10^{9} cfu ml^{-1}) only	643^{c}	1759^{c}	428^{c}	5539^{a}	278^{c}
Trichoderma viride + 50% NPK	845^{a}	2637^{a}	451^{c}	5667^{a}	283^{b}
Pseudomonas fluorescens + 50% NPK	696^{b}	1749^{c}	641^{a}	5708^{a}	295^{a}
Trichoderma viride + *Pseudomonas fluorescens* + 100% NPK	562^{d}	1636^{d}	430^{c}	5496^{a}	284^{b}
Control	461^{e}	1158^{f}	150^{d}	4965^{b}	267^{e}
Grand mean	671	1767	466	5482	281
Significance with F-test	**	**	**	*	**
LSD (0.05)	22.48	47.75	39.99	356.80	2.27
CV, %	1.90	1.50	4.80	3.70	0.50

Means in the column with the same superscript are not significantly different by DMRT $_{(P<0.01)}$, * – significant at 0.05 level, ** – significant at 0.01 level

Growth parameters increased over the control

The result revealed that *T. viride* +50% NPK enhanced most of the growth components like plant height (103%), leaf number (10%), stem diameter (74%), root length (41%) and leaf area index (173%) of maize crop compared to control plot (Fig. 1).

Biomass and yield components increased over the control

The plot treated with *T. viride* + 50% NPK enhanced biomass like leaf biomass (83%) and stem biomass (128%) of maize compared to control. Similarly, the increased cob biomass (329%), grain yield (15%) and thousand kernel weight (11%) over the control was recorded in the plot applied to *P. fluorescens* +50% NPK (Fig. 2).

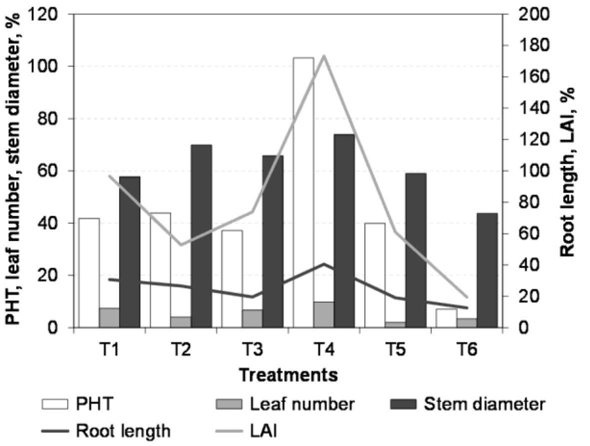

Figure 1. Effect of treatments on growth components increased over control plot at Rampur, Chitwan during winter 2018. (T1: recommended dose of NPK (120:60:40 kg NPK ha^{-1}), T2: *T. viride* only, T3: *P. fluorescens* only, T4: *T. viride* + 50% NPK; T5: *P. fluorescens* + 50% NPK; T6: *T. viride* + *P. fluorescens* + 100% NPK)

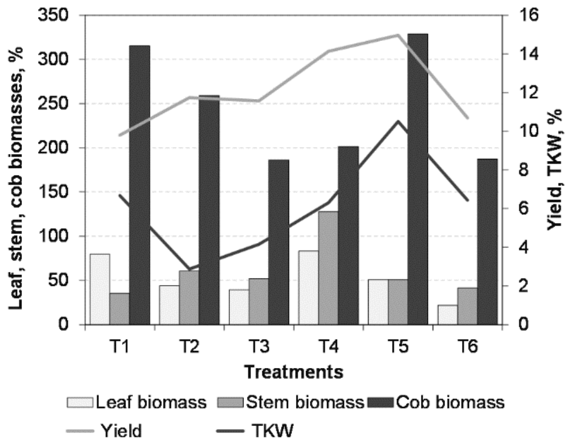

Figure 2. Effect of treatments on biomass and yield components increased over control plot at Rampur, Chitwan during winter 2018. (T1: recommended dose of NPK (120:60:40 kg NPK ha^{-1}), T2: *T. viride* only, T3: *P. fluorescens* only, T4: *T. viride* + 50% NPK; T5: *P. fluorescens* + 50% NPK; T6: *T. viride* + *P. fluorescens* + 100% NPK)

Soil analysis

The laboratory analysis of soil sample up to 15 cm depth revealed that the increased organic matter (23.25%), nitrogen (25%) and phosphorous (50%) over the control was recorded in the plot applied to *T. viride* + 50% NPK and *P. fluorescens* + 50% NPK (Table 3). Similarly, in 30 cm soil depth, the plot treated with *T. viride* + 50% NPK increased the amount of organic matter (35.99%), nitrogen (35.29%) and phosphorous (67.80%) over the control (Table 3). The amount of potassium was found higher in control plot both in 15 and 30 cm soil depth.

Table 3. Analysis of soil sample from the plots treated with *Trichoderma viride* and *Pseudomonas fluorescens* in combination with chemical fertilizer at 15 and 30 cm soil depths during winter 2018

Treatment	pH	Organic matter	Nitrogen	Phosphorus	Potassium
Soil depth (15 cm)					
Recommended dose of NPK (120:60:40 kg NPK ha^{-1})	4.7	2.78	0.14	51.75	39.65
Trichoderma viride + 50% NPK	5.2	3.14	0.16	94.35	28.32
Pseudomonas fluorescens + 50% NPK	4.8	3.14	0.16	94.81	22.66
Control	4.8	2.41	0.12	48.09	90.62
Soil depth (30 cm)					
Recommended dose of NPK (120:60:40 kg NPK ha^{-1})	4.5	2.84	0.14	39.85	28.32
Trichoderma viride + 50% NPK	5.2	3.39	0.17	54.04	28.32
Pseudomonas fluorescens + 50% NPK	4.9	2.29	0.16	50.84	22.66
Control	4.8	2.17	0.11	17.4	90.62

Discussion

The results obtained in this study are in agreement with López-Valenzuela *et al.* (2019) and García-Reyna *et al*. (2005), both of whom reported that, when inoculating maize seeds with soil micro-flora as growth and yield promoters, the average dry weight of maize foliage increased by 30% on an individual basis compared to the control plot and also reduced the nitrogen fertilization rate to 50%. Harman (2006) reported that maize roots colonized by *Trichoderma* spp. need 40% less nitrogen fertilizer in comparison to non-colonized roots. The findings of this study are also consistent with Páez *et al.* (2006) on inoculation with *Trichoderma* spp., which reduced the rate of nitrogen required by maize plants and, as a result, the cost of achieving appreciable environmental improvement. The result of the experiment is also in line with the findings of many studies conducted with various bacterial promoters of vegetable growth and yield in different soils, environments and crops of agronomic significance showed a 5 to 30% increase in yields, as well as a 25 to 50% decrease in the dosage of chemical fertilizers, such as nitrogen and phosphorus (Hernández-Escareño *et al*., 2015; García Crespo *et al*., 2012; Adesemoye *et al*., 2009; Aguado, Moreno, 2008). The findings achieved with soil microflora in this study can be attributed to the participation of these microorganisms in the biotransformation of cellulose, the acceleration of cell reproduction, the mineralization of nitrogen and the solubilization of phosphorus present in the soil, but also the increase in the volume of roots, the enhancement of their space for exploration and, as a result, obtaining more nutrients and water present in the soil favouring both growth and yield of the crops (Subedi *et al*., 2019; Santana *et al*., 2003).

Conclusions

Both microflora *Trichoderma viride* and *Pseudomonas fluorescens*, fertilized with a half dose (50%) of recommended fertilizers showed the effectiveness in growth and yield promotion of maize crop and proved as potential biofertilizers. The use of such microorganisms as biofertilizers may be an alternative source of biofertilizers because they minimize the usage of chemical fertilizers, the cost of production and soil pollution from the unnecessary use of synthetic fertilizers.

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Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions
BN – acquisition of data, drafting of the manuscript;
SS – analysis and interpretation of data, critical revision and approval of the final manuscript;
SB – study conception and design;
SM – drafting of the manuscript;
DB – study conception and design;
JS – analysis and interpretation of data, critical revision and approval of the final manuscript.

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