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Seed biopriming with *Trichoderma* improves yield of Rajmash (*Phaseolus vulgaris* C.V. HUR-137) in Varanasi region of Uttar Pradesh

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Abstract

Plants of red kidney bean (*Phaseolus vulgaris*) were treated with different grades of recommended dose of fertilizer along with biopriming with *Trichoderma harzianum*. The treatment along with the inorganic fertilizer treatment was applied as an attempt to enhance the yield through the microbial agent *Trichoderma*. Treatment of red kidney bean seeds were treated with the *Trichoderma* isolates using powder (talca) formulation to promote growth and development of the bean plants in greenhouse conditions. Among the treatments the plants treated with full dose of fertilizer without any bio-treatments was found the best as per growth and development but the bean plants treated with inorganic fertilizers at 90% dose combined with biopriming was comparable and suggests that the use of bio-agents can be used significantly to improve the yield and growth of the crops which is reduced as a part of the treatments. Also, the bean plants treated solely with the bio-agent represented a very good rhizospheric growth without the use of any kind of inorganic inputs. The treatments bioprimed with *Trichoderma* showed a good growth despite a lower dose of nutrient applied.

Keywords: Bio-priming, yield, dry matter production, height, red kidney bean

Introduction

Common bean (*Phaseolus vulgaris* L.; 2n=2x=22) is a predominantly self-pollinated crop originated mainly in Latin America and spread to other parts of world and now it is widely cultivated in the tropics and subtropics as well as in temperate regions of the world (Gepts and Bliss 1988; Zeven 1997, 1999; Kumar *et al*, 2008) ^[4, 15, 6]. In India, common bean is known by the names like 'Rajmash' and 'Frash bean (green bean)' and grows in certain parts of the country. Common bean production in India is 4,340 million tones as compared 18,943 million tons in the world. (Anonymous, 2003; Kumar *et al*, 2008) ^[1, 6].

Bio-priming is a new technique of seed treatment that integrates biological (inoculation of seed with beneficial organism to protect seed) and physiological aspects (seed hydration) of disease control. Biopriming using naturally occurring soil microorganisms is a safe, non-polluting, and environmentally sound disease control measure and an effective pre-sowing seed treatment. There are some known beneficial bacteria and fungi which, if applied to seed prior to planting, may enhance plant growth and development. Biopriming allows rapid colonization of the beneficial organism on the seed, and often results in a more uniform coverage of the seed surface compared to other priming techniques (Smith, 1996; Warren and Bennett, 1997) ^[10, 12]. Biopriming is a common practice in the seed industry worldwide. During the seed coating process, inoculants, fungicides, or insecticides are added together with other substances, which provide a protective barrier on the seed. Biopriming has been shown to be more effective than regular chemical treatment for disease control (Ashraf and Foolad, 2005) ^[2]. Seed are coated with bacteria and soaked in warm water until their moisture content rises to 35-40%, the term "biopriming" is used.

The stem diameter, root and foliar dry weight parameters suggested a positive effect of *T. koningiopsis* Th003 on tomato growth response after transplant as well. These results are in agreement with an early report in cucumber plants inoculated with *T. harzianum* T-203 (Moreno *et al*, 2009). The shoot fresh and dry weight as well as yield was enhanced by non-autoclaved *Trichoderma* WT inocula applied at 1% and produced with a fermentation time of

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6 or 12 days in lettuce (Ousley *et al*, 2013). Bioagents *Trichoderma* enhance the seed treatment on germination and vigour in chick pea (Kumar *et al*, 2014) [5]. Single inoculations of common dry bean with selected *Trichoderma* species have been reported to increase plant dry growth and height (Yobo *et al*, 2009) [14]. Biopriming of chickpea and rajma seeds with *Trichoderma*, *Pseudomonas* and *Rhizobium* showed increased seed germination and accumulation of biomass in both the crops under both pot and field conditions (Yadav *et al*, 2013) [13]. *Trichoderma* isolates increases the height, dry weight and yield of plants as it is known to produce plant hormones like auxin, gibberellins and other volatile compounds (Contreras-Cornejo *et al*, 2009; Vinale *et al*, 2008) [3, 11].

Materials and Methods

The pot experiment was conducted during Rabi season of 2016-2017 in the net house of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, B.H.U. Varanasi, U.P.

To conduct the pot experiment, bulk surface (0-15cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, and Varanasi. The soils of Varanasi are formed on alluvium deposited by the river Ganges and have predominance of illite, quartz and feldspars minerals. The alluvial soil is characterized by higher silt content. Illicit nature of alluvial sediments is clearly demonstrated by higher K₂O content. The cropping history of the site from where the soil was collected is the rice-wheat. The fertility status of the soil can be included as low to medium with a slightly alkaline reaction.

After collecting the soil, it was sieved and 10kg of soil was filled in each polythene lined earthen pot.

Crop and variety

Crop: Kidney Bean

Scientific name: *Phaseolus vulgaris*

Family: Fabaceae

Variety: HUR-137

Seeds of red kidney beans were first soaked in sodium hypochlorite solution for one minute. Rinse with sterilized water 4-5 times and kept for air drying or in laminar air flow. Spores of *Trichoderma harzianum* were collected in vials by adding 4-5ml of 0.85% saline water in the petri plate. In petri plate, autoclaved filter paper were spread and dried. Per treatment 50 seeds were taken in the petri plates. Then the microbial suspension were spread uniformly over seeds and kept for drying.

Experimental Details

The experiment has been under taken on alluvial soil at the Institute of Agricultural Sciences, Banaras Hindu University, during November 2016 to March 2017, under net-house conditions, bioprimed seed of kidney bean with microbial consortium along with different levels recommended fertilizer doses was grown. Weeding was done as soon as weeds emerge. Irrigation is given as per requirement of crop that keep soil moist throughout growth period. Fertilizers applied in recommended dose N: P: K @ 120: 60: 60 kg/ha, 1/2th N, Full P & K given at time of sowing and rest N given in 2 split dose at 25 DAS and 45 DAS. Pods were harvested after they were fully matured. The experimental design was an experiment under completely randomized block design with three replications (CRD).

Table 1: Details of treatment

Treatment	Fertilizer dose	Microbe used in seed treatment
T1	Control N: P: K @ 0:0:0 kg/ha	No
T2	100% of RDF N: P: K @ 180: 60: 60 kg/ha	No
T3	70% OF RDF	<i>Trichoderma harzianum</i>
T4	80% OF RDF	<i>Trichoderma harzianum</i>
T5	90% OF RDF	<i>Trichoderma harzianum</i>
T6	N: P: K @ 0:0:0 kg/ha	<i>Trichoderma harzianum</i>

First of all the total experimental pots were divided into three blocks. Each block was divided into 3 units containing 9 pots and each having 2 plants. So there were total 54 experimental units.

Physicochemical properties of soil

Physico-chemical properties of soil determined for alluvial soil. From the table it is evident that bulk density of soil was 1.39 Mg m⁻³. The experimental soil was sandy loam in texture. The soil is almost neutral in reaction. The soil has low organic matter content. EC varied from 0.44 dSm⁻¹, CEC from 28.98 C mol (p⁺)/kg, available N 229 kg/ha, P 17 kg/ha, K 230 kg/ha.

Table 2: Physicochemical properties of experimental soil

Physical Parameter	Alluvial Soil
Bulk Density (Mg m ⁻³)	1.39
Particle density (Mg m ⁻³)	2.56
Water holding capacity (%)	39.9
Sand (%)	48.78
Silt (%)	30.48
Clay (%)	20.44
Soil Texture	Sandy loam
pH (1:2.5)	7.2
EC (dS/m)	0.44
CEC (C mol (p ⁺)/kg)	28.98
Organic carbon (%)	0.37
Available N (kg/ha)	229
Available P (kg/ha)	17
Available K (kg/ha)	230
Available Zn(ppm)	0.15

For Dry weight of plants, after harvesting plant samples were kept in paper bags and dried in hot air oven at 60 ± 2 °C till the constant weight. For Yield attribute each pod was selected from every pot were weighed by electronic balance. Yield/pot was recorded after harvesting of crop. Regarding Statistical analysis Duncan multiple range analysis was done through software STAR.

Results and Discussion

Plant Height

Data on plant height as affected by different treatments, recorded at 30, 60 and 90 days after sowing are presented in table-3 and depicted graphically in figure-1. Observations reveal that shoot elongation continued to increase with the advancement of crop growth stages. Slow increase in plant height was noted during early stages of growth up to 30 DAS. Rate of elongation was higher in between 60 to 90 DAS. Among the treatments T₂ gave the maximum plant height of red kidney bean. Increasing levels of NPK fertilizers up to RDF of N: P: K @ 100: 60: 25 kg/ha (T₂) gave maximum enhancement of plant height at all the stages of crop growth as compared to other treatments. Reduction in NPK levels i.e. 90%, 80% and 70% RDF significantly decreased the plant

height. It is clearly evident that application of RDF of N: P: K @ 100: 60: 25 kg/ha (T_2) significantly produced highest plant height at all growth stages of red kidney bean in comparison to lower doses of N:P:K addition. Among the treatments at 30 DAS, T_2 caused significantly higher plant height and this treatment was significantly superior over rest of the treatments. At 60 DAS significantly higher plant height was recorded with treatment T_2 (36.825cm, RDF of N: P: K @100: 60: 25 kg/ha) which is at par with T_5 and T_6 followed by T_5 (seed treatment with *T. harzianum* +90% RDF of N:P: K), T_4 (seed treatment with *T. harzianum* +80% RDF of N:P: K), T_3 (seed treatment with *T. harzianum* +70% RDF of N:P: K), T_6 (seed treatment with *T. harzianum* +RDF of N: P: K @ 0:0:0 kg/ha) and T_1 (control N: P: K @ 0:0:0 kg/ha). However, at 90 DAS significantly plant height was recorded with the treatment T_2 (35.675 cm) which is at par with T_5 , this was significantly superior than rest of the treatments. Significantly lower plant height was recorded with T_1 (control N: P: K @0:0:0 kg/ha).

At 90 DAS plant height ranged between, 36.33 to 63.00 and 44.50 to 47.12 cm in different treatments. In case of effect of *T. harzianum* and different graded dose of NPK application, plant height was recorded in following order: $T_2 > T_5 > T_4 > T_3 > T_6 > T_1$ in all the growth stages of red kidney bean.

Result clearly illustrated that plant height of red kidney bean was boosted by the combined use of *Trichoderma harzianum* and N: P: K. The increase in plant height may be due to increased nutrient availability by root elongation and plant growth promoting activities by *Trichoderma harzianum*. It may also be ascribed due to adequate supply of nutrients due to mineralization of nutrients by increased population of *Trichoderma harzianum*. Enhanced plant height by *Trichoderma* may be due to production of secondary metabolites which may act as an auxin compound and other secondary metabolites such as harzianolide and, anthraquinone. Similar results also reported by Vinale *et al.* (2008) [11] in wheat, Molla *et al.* (2012) [7].

Table 3: Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on plant height (cm) of red kidney bean at different growth stages and yield (g pot⁻¹)

Treatment	Vegetative stage	Pod formation	Maturation stage	Yield/pot(g)
T1:Control	9.875a	29.175a	31a	23.98c
T2:RDF	13.675c	36.825c	35.675b	86.48a
T3:70% RDF+ Biopriming	12.525bc	31.95ab	32.675a	40.92bc
T4: 80% RDF+ Biopriming	12.95bc	33.675abc	33a	49.31b
T5:90RDF+ Biopriming	13.525c	36.175bc	33.675ab	55.39b
T6:Control+ Biopriming	11.575ab	29.575a	31.75a	32.87bc

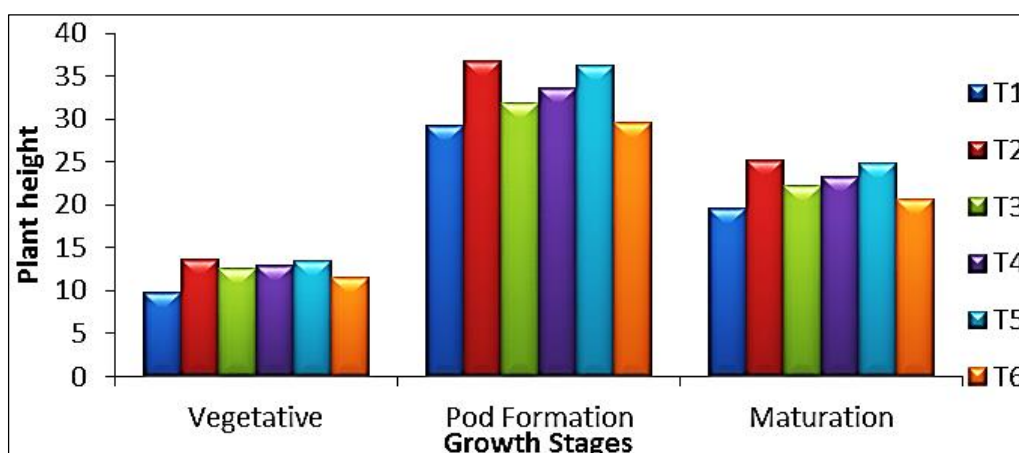


Fig 1: Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on plant height (cm) of red kidney bean at different growth stage

(T_1 : Control N: P: K @ 0:0:0 kg/ha, T_2 : RDF of N: P: K @100: 60: 25 kg/ha, T_3 : Seed treatment with *T. harzianum* + 70% N and RDF of N: P: K, T_4 : Seed treatment with *T. harzianum* +80% RDF of N: P: K, T_5 : Seed treatment with *T. harzianum* +90% RDF of N:P: K and T_6 : Seed treatment with *T. harzianum* ; DAS-Days after Sowing; RDF-Recommended dose of fertilizer)

Yield of red kidney bean

The effects of *Trichoderma harzianum* and soils type with graded dose of nitrogen application significantly influenced grain yield and are shown in table-4 and graphically represented in figure-2. Significant yield increases due to different treatment combinations were observed. All the treatments with graded NPK application along with seed

biopriming with *Trichoderma harzianum* significantly increased grain yield of red kidney bean. Significantly maximum grain yield was recorded with T_2 (86.48 g pot⁻¹) (RDF of N: P: K @ 100: 60: 25 kg/ha) followed by the treatments T_5 (55.39 g pot⁻¹), T_4 (49.31 g pot⁻¹), T_3 (40.92 g pot⁻¹), T_6 (32.87 g pot⁻¹) and T_1 (23.98 g pot⁻¹) i.e. control.

Grain yield of rajmash ranged from 23.98 to 86.48 g plant⁻¹. Increase in grain yield by 62.50 g plant⁻¹ in T_2 (RDF of N: P: K @ 100: 60: 25 kg/ha) over T_1 (Control 0:0:0 N and RDF of P: K) have been demonstrated. T_2 with maximum yield is statistically different from rest all the treatments. One of the good yielding treatment i.e. T_5 is statistically different from T_1 , similar to T_4 while at par with T_3 and T_6 . The increase in grain yield may be due to steady and slow release of nutrients to the plants (Figure 2).

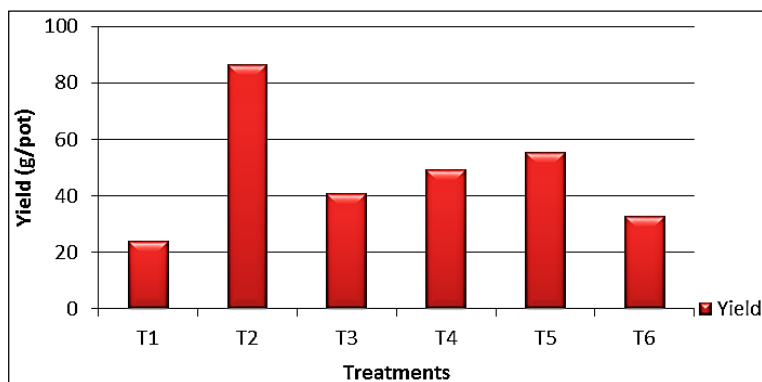


Fig 2: Effect of biopriming with *T. harzianum* and graded levels of N application on yield of red kidney bean

The overall result revealed that priming of red kidney bean seeds with inoculants of *Trichoderma* along with 90% RDF application gave better performance in all respect of yield and bio-control potential as compared to control and others and emerged as an alternative to full dose of RDF. These findings are in accordance with number of researchers (Geraldine *et al.*, 2013; Verma *et al.*, 2014) [16, 17] observations: under varied agro-ecological niche.

Dry matter production

The data on dry matter production (DMP) are given in table 5 and depicted graphically in figure-3. The experimental results revealed that almost a consistent increase in the DMP occurred with the advancement of the crop growth stages and reaching the maximum at the maturity.

The rate of increase in DMP was enhanced rapidly between 30 to 60 DAS. It is clearly evident that application of RDF of N: P: K @ 100: 60: 25 kg/ha (T₂) produced lucidly higher DMP/plant than lower doses at all the growth stages. Among the treatments at 30 DAS, T₂ caused significantly higher dry matter production (0.225g plant⁻¹) and this was significant over rest other treatments. T₂ had maximum dry matter production which is significantly different from T₆ and at par with treatments T₅, T₄ and T₃. T₁ had minimum dry matter production with 0.122 g plant⁻¹.

At 60 DAS significantly higher dry matter production was recorded in treatment T₂ (0.3067g plant⁻¹, RDF of N: P: K @ 100: 50: 25 kg/ha) which is significantly different from T₆ and T₃ while at par with T₄ and T₅. Dry matter production followed by T₅ (seed treatment with *T. harzianum* +90% N and RDF of P: K), T₄ (seed treatment with *T. harzianum* +80%N and RDF of P: K), T₃(seed treatment with *T. harzianum* +70% N and RDF of P: K), T₆ seed treatment with *T. harzianum*+ N: P: K @ 0:0:0 kg/ha and T₁ (control N: P: K @ 0:0:0 kg/ha). At 90 DAS the dry matter production increased as compared to 30 to 60 DAS and the significantly higher dry matter production was recorded with the treatment T₂ (1.425 g plant⁻¹) which is statistically different from all other treatments but at par with T₅ (1.277 g plant⁻¹) and was significantly higher than rest of the treatments. Lowest dry matter production was recorded with T₁ (control N: P: K @ 0:0:0 kg/ha). At 90 DAS dry matter production ranged between 1.0175, 1.425, 1.1275, 1.21, 1.2775 and 1.21 g plant⁻¹ respectively in different treatments.

Result clearly illustrated that dry matter production of red kidney bean plant was boosted by the combined use of *Trichoderma harzianum* and N: P: K. The increase in dry matter of shoot may be due to increased root dry matter enabling large volume of soil exploitation of the plant which could increase the chance for nutrients uptake through maximum access to use mineral nutrients.

Table 4: Effect of biopriming with *T. harzianum* and graded levels of NPK application on dry matter production (g plant⁻¹) of red kidney bean

Treatment	Vegetative stage	Pod formation	Maturation stage
T1:Control	0.1225a	0.123333c	1.0175c
T2:RDF	0.225a	0.306667a	1.425a
T3: 0%RDF+Biopriming	0.1675abc	0.206667bc	1.1275bc
T4: 80% RDF+ Biopriming	0.1875ab	0.236667ab	1.21b
T5: 90%RDF+Biopriming	0.1925ab	0.266667a	1.2775ab
T6:Control+Biopriming	0.1575bc	0.19bc	1.21b

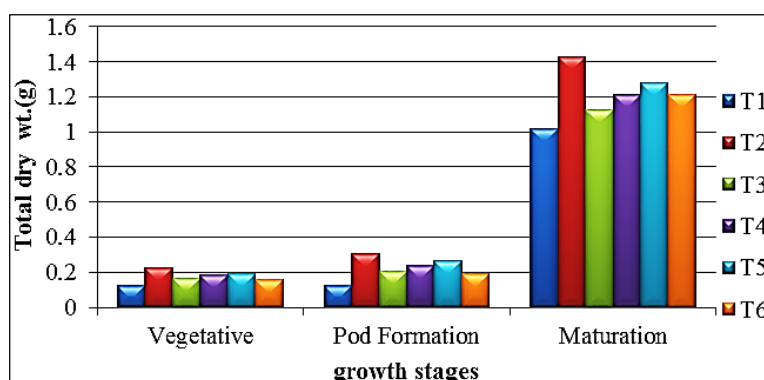


Fig 3: Effects of biopriming with *T. harzianum* and graded levels of N application on DMP (g plant⁻¹) of red kidney bean at different growth stages

It may also be ascribed due to adequate supply of nutrients due to mineralization of nutrients by increased population of *Trichoderma harzianum*. Significant increase in dry matter of tomato plant by the combined use of biofertilizer and N: P: K (Molla *et al.*, 2012) [7]. None of the interaction was found significant in this respect during the present experimentation. *Trichoderma* spp. enhancing plant growth has been reported in several crop plants and has been attributed to auxin (Contreras-Cornejo *et al.*, 2009) [3]. Besides, nutrient acquisition is improved enhancing indirect growth promotion.

Conclusion

The research data supports the points that biopriming of the crop with *Trichoderma harzianum* improved the yield, plant height and dry matter production of red kidney bean. This happens because of the fact that the fungus produces plant growth regulators such as auxin and other volatile organic compounds which results in.

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