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Seed Biopriming With *Trichoderma* Improves Nutrient Uptake in Rajmash (*Phaseolus vulgaris* cv HUR-137) in Varanasi Region of Uttar Pradesh

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ABSTRACT

Red kidney bean plants were treated with different grades of recommended dose of fertilizer (RDF) along with biopriming with *Trichoderma harzia-num* in greenhouse. Results represented that T_2 showed maximum growth attributes while T_5 showed comparable growth followed by T_4 , T_3 , T_6 and T_1 . Biopriming improved nutrient uptake with lesser fertilizer doses comparable to RDF in relation to

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nitrogen, phosphorus and potassium. Plants treated with RDF without bio-treatments was found the best as per growth but the plants treated with 90% RDF combined with biopriming was comparable and suggests that the use of bio-agents can be used significantly to supplement the nutritional needs of the crop which is reduced as a part of the nutrients. Also, the plants treated solely with the bio-agent represented good rhizospheric growth without the use of inorganic inputs suggesting the role of biopriming in development of healthy root growth and increase in root biomass.

Keywords Biopriming, Nutrient uptake, Rajmash, Dry bean.

INTRODUCTION

Incorporation of pulse crop in the cropping system is getting momentum after the new initiative, resolutions made in 2016 as FAO nominated the year as International Year of Pulses to heighten public awareness towards the nutritional benefits of pulses. India is the largest producer and consumer of pulses in the world. Latest reported acreage in India under pulses is 25.26 Mha as per 2015-16 (DAC and FW 2016-17). The Indian production contribution of dry beans is 34% (IISc 2016). The domestic production is often less than the estimated demand i.e., 23-24 million tons. Thus the average gap of 5MT is met through

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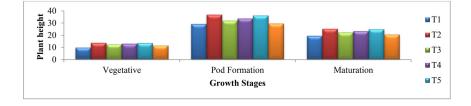


Fig. 1. Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on plant height (cm) of red kidney bean at different growth stages. (T_1 : Control N: P: K @ 0:0:0 kg/ha, T_2 : RDF of N: P: K @ 100: 60: 25 kg/ha, T_3 : Seed treatment with *T. harzianum* + 70% N and RDF of N: P: 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).

imports. Due to the low productivity-low input nature, pulses are grown as residual/alternate crops on marginal lands after taking care of food/income needs from high productivity high input crops like paddy and wheat by most farmers. We can go for the organic treatments including such microbial agents which increase the pulse production sustainably and serving the purpose (Meena and Meena 2017). So, the best way to increase in the production without causing an ecological alarm is to integrate both of the organic methods and fertilizers in a judicious way.

Further, *Trichoderma harzianum* is a cosmopolitan and ubiquitous species found on a wide variety of substrates and in the last decade gained momentum in its use and popularity due to a continuous advocacy as it has the potential to heal biotic and abiotic stresses. It has been isolated from soil, rotting plant material, other fungi and recently as one of the

Table 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 stages. (T_1 : Control, T_2 : RDF, T_3 : 70%RDF+Biopriming, T_4 : 80% RDF + Biopriming, T_5 : 90% RDF+Biopriming, T_6 : Control+Biopriming). Means with the same letter are not significantly different.

Treat- ments	Vegetative stage	Pod formation	Maturation stage
T,	9.875ª	29.175ª	31ª
$\begin{array}{c} T_1 \\ T_2 \\ T_3 \\ T_4 \\ T_5 \end{array}$	13.675°	36.825°	35.675 ^b
T,	12.525 ^{bc}	31.95 ^{ab}	32.675ª
Τ,	12.95 ^{bc}	33.675 ^{abc}	33 ^a
T,	13.525°	36.175 ^{bc}	33.675 ^{ab}
T_6^3	11.575 ^{ab}	29.575ª	31.75 ^a

species most commonly isolated as endophytes in the sapwood of tropical trees (Chaverri et al. 2015). Efficient soil microorganisms pose a great beneficial impact on soil-plant system and plant growth and uptake which help in environmental sustainability (Meena et al. 2014, Meena et al. 2015). By improving the plant height, chlorophyll, leaf area and dry matter production growth of plants can be made better and higher uptake of nutrients ensure this through combined use of biofertilizer and inorganic nutrients in a proportion which is both profitable and sustaining. Growth parameters and nutrient uptake will ultimately contribute to the production and productivity at a lower cost of cultivation. The biopriming is a process of biological seed treatment that refers to combination of seed hydration and inoculation of seed with a biological agent to protect seed, improves seed germination, seedling establishment and vegetative growth (Rakshit et al. 2014, Babu et al. 2014, Kumar et al. 2017). Keeping these in mind, an experiment was conducted on red kidney beans (HUR-137, Malviya Rajma) following biopriming with the microbial fungal agent Trichoderma harzianum.

MATERIALS AND METHODS

The pot experiment was conducted during *rabi* season of 2016-2017 in the net house on alluvial soil of Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, BHU, Varanasi, UP bulk surface (0–15 cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University,

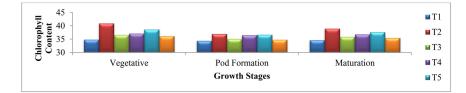


Fig. 2. Effect of biopriming with *T. harzianum* and graded levels of N: P: K application on chlorophyll content of red kidney bean at different growth stages.

Varanasi. It had available N (229 kg/ha), Available P (17 kg/ha) and Available K (230 kg/ha). For each treatment fifty seeds were taken in the petri plates. Then the microbial suspension were spread uniformly over seeds and kept for drying.

Fertilizers applied in recommended dose N : P : K @ 120:60:60 kg/ha, 1/2th N, Full P and 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). Leaf area of the plants was measured using leaf area meter (LI-300°C) in cm² units at 60 and 90 days after sowing. After harvesting, plant samples were kept in paper bags and dried in hot air oven at $60 \pm 2^{\circ}$ C till the constant weight. The chlorophyll content was estimated using SPAD meter. This instrument enables non-destructive and nearly instantaneous chlorophyll measurements. It directs a beam of light corresponding to the wave length absorbed by the chlorophyll molecule through a plant leaf. Nitrogen content in plant and grain sample was determined by modified Kjeldahl

 Table 2. Effect of biopriming with *T. harzianum* and graded levels of N:P:K application on chlorophyll content of red kidney bean at different growth stages.

Treat- ments	Vegetative stage	Pod formation	Maturation stage
T,	34.825ª	34.4ª	18.125ª
1	40.925 ^b	36.95ª	24.625 ^b
T,	36.6ª	35.125ª	22.825 ^{ab}
T_2 T_3 T_4 T_5	37.125ª	36.5ª	23.325 ^{ab}
T,	38.625 ^{ab}	36.675ª	23.925 ^{ab}
T_6^3	36.15 ^a	34.8ª	20.075 ^{ab}

method as per procedure outlined by Gupta (2007). Uptake of nutrient was calculated, to study the effect of increase/ decrease in nutrients application to soil and that different soil fertility levels on uptake of that particular nutrient by plant. Following formulae were used to calculate the total uptake of each nutrient.

Uptake (kg ha⁻¹)= percent nutrient in grain \times yield grain or straw (q ha⁻¹)

The analysis of data was carried out using STAR. One way ANOVA for CRD was performed to compare the means of different treatments and significant differences. Duncan Multiple Range Test (DMRT) to differentiate the treatment means at $p \le 0.5$.

RESULTS AND DISCUSSION

Plant height

Among the treatments T_2 gave the maximum plant height (Table 1, Fig. 1). It was followed by T_5 , T_4 , T_3 , T_6 and T_1 . However, at 90 DAS significant increase in plant height was recorded with the treatment T_2

Table 3. Effect of biopriming with *T. harzianum* and graded levels of N: P: K application on leaf area (cm⁻²) in red kidney bean at 30 and 60 DAS.

Treatments	Vegetative stage	Pod formation	
Τ,	71.725ª	249.6ª	
T,	85.1 ^b	397.5 ^b	
T ₂	78.5 ^{ab}	293.4 ^{ab}	
T,	80.225 ^{ab}	338 ^{ab}	
T,	80.425 ^{ab}	390.725 ^b	
$\begin{array}{c} T_2\\T_3\\T_4\\T_5\\T_6\end{array}$	73.575 ^{ab}	267.325ª	

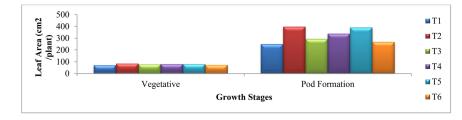


Fig. 3. Effect of biopriming with T. harzianum and graded levels of N application on leaf area of red kidney bean at 30 and 60 DAS.

(35.675 cm). 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 indicated in following order : $T_2 > T_5 > T_4 > T_3 > T_5 > T_1$ in all the growth stages of red kidney bean. Enhanced plant height by Trichoderma inoculation can be attributed to production of secondary metabolites which may act as an auxin compound and other secondary metabolites such as harzianolide and anthroquinoues. Similar results were also reported by Vinale et al. (2008) in wheat, Molla et al. (2012), Azarmi et al. (2011), Rudresh et al. (2005), Inbar et al. (1994), Bjorkman et al. (1998). However, Mwangi et al. (2009) in also found increment in plant height in tomato and napier but increment was found to be non-significant and attributed the increase in plant height to production of growth hormones (Mwangi et al. 2009, Windham et al. 1986, Reddy et al. 1996). A 10.55% increase was observed in plant height on inoculation with the fungus over control (Khan et al. 2001). Phytohormones like auxin and gibberellins are produced by Trichoderma and solubilization of nutrients like phosphorus, iron, manganese, zinc have been found to be responsible for improvement in plant

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.

Treat- ments	Vegetative stage	Pod formation	Maturation stage
T ₁	0.1225ª	0.123333°	1.0175°
	0.225ª	0.306667ª	1.425 ^a
T,	0.1675 ^{abc}	0.206667 ^{bc}	1.1275 ^{bc}
$\begin{array}{c} T_2 \\ T_3 \\ T_4 \end{array}$	0.1875 ^{ab}	0.236667 ^{ab}	1.21 ^b
T ₅	0.1925 ^{ab}	0.266667ª	1.2775 ^{ab}
T_6^{2}	0.1575 ^{bc}	0.19 ^{bc}	1.21 ^b

height (Kakabouki *et al.* 2021, Elkelish *et al.* 2020, Smith and Reddy 2010).

Chlorophyll content

At 30 DAS maximum chlorophyll content was observed in T₂ (40.925) as shown in Table 2, Fig. 2 followed by T_5 (38.625), T_4 (37.125) and T_3 (36.6). At 60 DAS trend of chlorophyll content was in the order i.e., $T_2 > T_5 > T_4 > T_3 > T_6 > T_1$. At 90 DAS maximum chlorophyll content was observed in T₂ (24.625). The chlorophyll content followed the order : $T_2 > T_5 > T_4 > T_3 > T_6 > T_1$. Throughout the growth duration of the crop, chlorophyll content was minimum in T₁ i.e., control with values 34.825, 34.4 and 18.125 at vegetative (30 DAS), pod formation (60 DAS) and maturation stages (90 DAS) respectively. Data of chlorophyll content at 60 DAS of plants was significantly affected due to application of graded doses of fertilizer with combination of seed biopriming by T. harzianum. This happened due to the fact that this soil has better potential with application of different dose of N application with seed biopriming by T. harzianum (Singh and Singh 2011, Meena

 Table 5. Effect of biopriming with T. harzianum and graded levels of NPK application on nutrient uptake of red kidney bean.

	Nitrogen uptake	Phosphorus uptake	Potassium uptake
Τ,	0.74°	0.30°	14.07°
1	1.82ª	0.54ª	31.34 ^a
T,	1.00°	0.39 ^b	20.38 ^{bc}
$T_2 T_3 T_4$	1.13 ^{bc}	0.44 ^b	25.84 ^{ab}
T ₅	1.47^{ab}	0.47^{ab}	28.16 ^{ab}
T_6^{2}	1.03°	0.43 ^b	20.80 ^{abc}

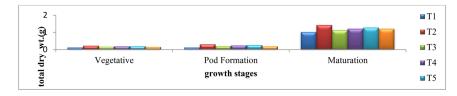


Fig. 4. Effect of biopriming with *T. harzianum* and graded levels of N application on dry matter production of red kidney bean at different growth stages.

et al. 2016). Higher chlorophyll content leads to higher photosynthetic efficiency as a result of higher nutrient uptake and translocation from roots to aerial parts with some growth regulators (Azarmi *et al.* 2011) and due to production of VOCs (Hung *et al.* 2013). It also suppresses chlorophyll losses in drought conditions (Shukla *et al.* 2012). Hexon *et al.* (2009) demonstrated increase in photosynthetic pigments in *Trichoderma* inoculated *Arabidopsis thaliana* based on the fact that *Trichoderma* increased root biomass leading to better nutrient acquisition and in turn more photosynthetic pigments. Similar results were encountered in maize plant by Akladious and Abbas (2014).

Leaf area of red kidney bean at different growth stages

A gradual and consistent increase in leaf area was observed up to 60 DAS and there after it decreased. Maximum leaf area was recorded with T₂ in both growth stages of red kidney bean.Maximum leaf area increase was recorded between 30 DAS to 60 DAS. At all growth stages maximum leaf area was recorded in treatment T_2 . At 30 DAS, treatment T_2 has maximum leaf area (85.1 cm² plant⁻¹) and minimum was recorded in T₁. T₆ is significantly different from T₂ with leaf area 73.575 cm² plant⁻¹ (Table 3, Fig. 3). At 60 DAS treatment T₂ has maximum leaf area (397.5 cm² plant⁻¹. Increase in leaf area of T_2 over control was 13.375 and 147.9 cm² plant⁻¹ at 30 and 60 DAS respectively. Similar extent of increase in leaf area by the application of Trichoderma harzianum with T₃ and T₂ suggested role of Trichoderma harzianum in solubilizing several plant nutrients (Altomare et al. 1999) improving the plant root system which have been manifested in above ground biomass. *Trichoderma* inoculation leads to increase in rate of leaf photosynthesis (Vargas *et al.* 2009, Inbar *et al.* 1994, Yedidia *et al.* 2001, Azarmi *et al.* 2011). Bader and co-workers in a recent study showed that seed treatment with *Trichoderma harzianum* increased leaf area with effect from phytohormone production (Bader *et al.* 2020). Khan and co-workers in observed and increment in number of leaves in tomato plants inoculated with *Trichoderma* (Khan *et al.* 2001).

Dry matter production

The rate of increase in DMP was enhanced rapidly between 30 to 60 DAS. At 30 DAS, T₂ caused significantly higher dry matter production (0.225 g plant⁻¹) and this was significant over rest other treatments. 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 $(0.3067 \text{ g plant}^{-1})$ followed by T₅, T₄, T₃, T₆ and T₁. 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). Lowest dry matter production was recorded with T₁ (Table 4, Fig. 4). 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. 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 plant by the combined use of biofertilizer and N: P: K in experiments conducted in Bangladesh

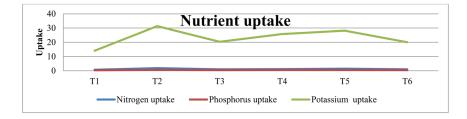


Fig. 5. Effect of biopriming with T. harzianum and graded levels of N application on nutrient of red kidney bean at different growth stages.

(Molla *et al.* 2012, Haque *et al.* 2012). *Trichoderma* spp. enhancing plant growth has been reported in several crop plants and has been attributed to auxin (Conteras-Cornejo *et al.* 2009). Besides, nutrient acquisition is improved enhancing indirect growth promotion due to better nutrient supply and uptake (Bjorkman *et al.* 1998, Rudresh *et al.* 2005). Some workers affirmed the improvement in root and shoot growth production in corn inoculated with *Tricho-derma* backed by the fact that it can produce auxin (IAA) which initiate and promote root elongation and shoot growth reflected in the dry matter production of the crop (Zhang *et al.* 2012).

Nutrient uptake

At 30 DAS, T_2 caused significantly higher nitrogen uptake (1.82) and T_1 had minimum nitrogen uptake with 0.74. For phosphorus uptake it was higher recorded in treatment T_2 followed by T_5 , T_4 , T_3 and T_1 (Table 5, Fig. 5). For potassium uptake was recorded with the treatment T_{2} (0.54). Potassium uptake ranged between 0.30, 0.54, 0.39, 0.44, 0.47 and 0.43 respectively in different treatments.Result clearly illustrated that nutrient uptake of red kidney bean plant was boosted by the combined use of Trichoderma harzianum and N: P: K. The increase in nutrient uptake of shoot may be due to increased volume of root biomass enabling large volume of soil exploitation of the plant which could increase the chance for nutrients uptake through maximum access to use mineral nutrients. Higher N uptake may be due to the microbial inoculants which is an ensuring unit with the capacity to promote the plant growth, enhance nutrient availability and uptake and support the health of plant due to production of growth promoting substances (Adesemoye *et al.* 2009, Singh and Singh 2011, Azarmi *et al.* 2011, Rudresh *et al.* 2005).

CONCLUSION

The research data supports the points that :

a) Biopriming of the crop with *Trichoderma harzia-num* improved the plant height, leaf area, chlorophyll content, dry matter production and nutrient uptake.

b) Full doze of fertilizer was best for the productivity of dry bean followed by 90% RDF+biopriming with *T. harzianum*.

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