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Seed Biopriming with *Trichoderma* Enhances Yield attributing Characters of Rajmash (*Phaseolus vulgaris* c.v. 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 harzianum* in greenhouse to study the combined effect of fertilizers and microbial inoculant in lack of such studies and especially in red kidney bean in which N-fixation (nodulation) is either absent or meagre. 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 different yield attributing characters with lesser fertilizer doses comparable to RDF in relation to nitrogen, phosphorus and potassium. Plants treated with RDF without bio-treatments was found to be 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: Bio-priming, yield attributes, seed index, 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 25.26Mha as per 2015-16 (DAC&FW 2016-17). The Indian production contribution of dry beans is 34%.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 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 *et al.*, 2016). 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.

Phaseolus vulgaris L. the common bean is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit (both commonly called beans) (Sarhan *et al.*, 2018). The main categories of common beans, based on use, are dry beans (seeds harvested at complete maturity) and snap beans (tender pods with reduced fiber harvested before the seed development phase). *Phaseolus* is a highly nutritious grain legume crop, including a good source of carbohydrates and protein (Sarhan *et al.*, 2018). It also helps in the improvement of soil fertility by biological N_2 fixation

(Singh, 1999). In 2016, world production of green beans was 23.6 million metric tons as well as the world dried bean production in 2016 was 26.8 million metric tons, which are produced worldwide (FAOSTAT, 2017).

While using inorganic fertilizers any longer not only exhausting our productive lands rendering them towards low fertility but also they are provoking many environmental issues as they get into the food chain through bio magnification, fresh water sources by runoff making them unfit for any kind of further usage, disturbing the biological balance of the soil-rhizosphere system and getting accumulating in the soil itself making it a toxic reserve of heavy metals.

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. This will be going to fulfill the nutrient need of the crop production along with harness of the biota of the soil-plant-atmosphere continuum

Naming the microbial agents that are in modern agricultural use includes bacterial genera *Azospirillum* and *Rhizobium* are well-studied examples for plant growth promotion, *Bacillus, Pseudomonas, Serratia, Stenotrophomonas*, and *Streptomyces* and the fungal genera *Ampelomyces*, *Coniothyrium* and *Trichoderma*.

Trichoderma harzianum has been popularly used as a biocontrol agent in different crops against variety of pathogens as well as an agent to mitigate a variety of biotic and abiotic stresses as it is found in all kinds of soils (Sharma et al, 2012). But in recent decades its growth promoting activities has been discovered and explored in growth promotion of various crops. Phytohormones like auxin and other hormones have been known to be produced by *Trichoderma* sp. which enhances different plant growth parameters leading to increased production with lesser amount of inorganic fertilizers like any other biofertilizer (Carvalho *et al*., 2011). Further, enhancement in root growth and proliferation due to *Trichoderma* inoculation leads to more efficient uptake of nutrients and water contributing to higher production (Singh *et al.,* 2017).

By improving number of trifoliate branches, number of pods, pod weight, number of seeds in pods, seed index etc. are different yield related attributes of red kidney bean plants can be made better and higher biological yield, economic yield and harvesting index owed to the uptake of nutrients ensure this through combined use of biofertilizer and inorganic nutrients in a proportion which is both profitable and sustaining.

Growth parameters will ultimately contribute to the production and productivity at a lower cost of cultivation. The bio-priming 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 using alluvial soil in Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, B.H.U.,Varanasi, U.P. Bulk surface (0-15 cm) soil was collected from the Agricultural Research Farm, IAS, Varanasi which had 229 kg/ha available N, 17 kg/ha available P and 230 kg/ha of available K. Seeds were treated with microbial suspension and dried while fertilizers were applied as per recommended dose (N: P: K @ 120: 60: 60 kg/ha) as per treatments. The experimental design was under completely randomized block design with three replications (CRD).

Yield attributing characteristics like number of trifoliate branches/plant, Pod weight, number of Pods per plant, number of seeds per pod, pod length, seed index were taken manually in the crop at the time of harvesting followed by calculation of yield expressed per pot and kg/ha. Seed index (g) and other yield related features like Economic Yield, Biological Yield and Harvest index were also observed at the time of harvesting. Harvest index was calculated by using the formula:

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

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) was also performed to differentiate the treatment means from each other at $p \leq 0.5$.

RESULTS AND DISCUSSION

Number of trifoliate branches/plant. At harvesting, among all the treatments T_2 (pots applied with full dose of RDF without any biopriming) (6) gave maximum number of trifoliate branches/plant (Table 1 and Fig. 1). It was followed by T_5 (5.75), T_4 (4.75), T_3 (4.5), T_6 (3.75) and T_1 (2.5). Enhanced number of trifoliate branches can be attributed to colonization of plant roots with *T. harzianum* resulting in increased nutrient uptake, improved germination and increased plant stand (Singh *et al.,* 2017). PGPR activities of *T. harzianum* producing auxins and other growth hormones increased number of trifoliate branches in common bean or snap bean (Sarhan *et al.,* 2018). Similar results involving improvement in number of trifoliate branches were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al*., 2021). Also, *Trichoderma* sp. enhanced the yield related growth in other crops as well like number of spikes in wheat crop owing to the improved root growth, plant growth promoting activities and enhanced nutrient uptake in wheat, Arabidopsis (*Sharma et al.*, 2012; El-Gizawy, 2009; Harman *et al.,* 2004; Contreras-Cornejo *et al*., 2009). Trifoliate branches enhanced by *Trichoderma* inoculation can also 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). 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 trifoliate branches (Kakabouki *et al*. 2021; Elkelish *et al*. 2020; Smith and Read 2010).

Pod weight. A gradual and consistent increase in weight of pods on each plant was observed in the pod formation stage i.e. 60 DAS. At the time of harvesting of common bean crop maximum pod weight was observed in T_2 (2.35 g) as shown in Table 1 and Fig. 1 followed by T_5 (2.18 g), T_4 (2.17 g), T_3 (2.13 g), T_6 (1.93 g) and T_1 (1.85 g).

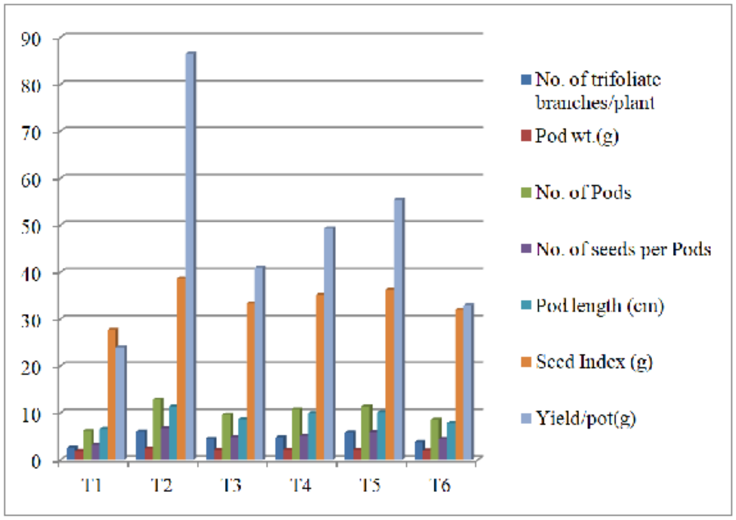
PGPR activities of *T. harzianum* producing auxins and other growth hormones increased pod weight in common bean or snap bean (Sarhan *et al*., 2018). Osmolytes accumulation, enhanced water use efficiency and other rhizospheric effects have been attributed for the increment in yield related parameters like pod weight in different crops (Petropoulos *et al.*, 2020). Data of pod weight at 60 DAS of plants was significantly affected due to application of graded doses of fertilizer with combination of seed biopriming by *T. harzianum*. Higher pod weight is 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 pod weight.

Number of Pods. A gradual and consistent increase in number of pods on each plant in the pod formation stage i.e. 60 DAS was observed. Highest number of pods was recorded in treatment T_2 (12.8 plant^{-1}) and minimum was recorded in T_1 (6.1 plant^{-1}) (Table 1, Fig. 1). Increase in number of pods of T_2 over control was 6.7.

Enhanced number of pods can be attributed to colonization of plant roots with *T. harzianum* resulting in increased nutrient uptake, improved germination and increased plant stand (Singh *et al.,* 2017). Similar results involving improvement in number of pods per plant were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al.*, 2021). Also, *Trichoderma* sp. enhanced the yield related growth in other crops as well like number of spikes in wheat crop owing to the improved root growth, plant growth promoting activities and enhanced nutrient uptake in wheat, Arabidopsis (Sharma *et al*., 2012; El-Gizawy, 2009; Harman *et al*., 2004; Contreras-Cornejo *et al.*, 2009). Likewise increase in number of pods per plant due to *T. harzianum* was also observed by other workers in common bean in addition to disease control (Carvalho *et al.,* 2015). In another experiment per plant number of pods increased in faba bean when treated with *T. harzianum* (Kumari *et al*., 2017). Osmolytes accumulation, enhanced water use efficiency and other rhizospheric effects have been attributed for the increment in yield related parameters like number of pods in different crops (Petropoulos *et al.*, 2020). Similar extent of increase in number of pods by the application of *Trichoderma harzianum* with T_3 and T_2 suggested role of *Trichoderma harzianum* in solubilizing several plant nutrients (Altomere *et al.*, 1999) improving the plant root system which have been manifested in above ground biomass.

Table 1: Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on yield attributing characters of red kidney bean at harvesting growth stage.

Treatment	No. of trifoliate branches/plant	Pod wt.(g)	No. of Pods	No. of seeds per Pods	Pod length (cm)	Seed Index (g)	Yield/pot(g)
T1	2.53	1.85	6.13	3.24	6.54	27.63	23.98
T2	6.01	2.35	12.82	6.85	11.35	38.64	86.48
T3	4.5	2.14	9.66	4.92	8.67	33.27	40.92
T4	4.75	2.17	10.75	5.17	9.87	35.18	49.31
T5	5.75	2.18	11.46	5.98	10.15	36.25	55.39
T6	3.75	1.93	8.56	4.50	7.85	31.89	32.87
Sem ±	0.43	0.19	1.13	0.48	0.87	3.21	6.04
LSD (*P*=0.05)	0.12	0.06	0.35	0.17	0.29	1.14	1.92



(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)

Fig. 1. Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on yield attributing characteristics of Rajmash at harvesting stage.

Number of seeds per Pods. At harvesting significantly higher number of seeds per pods was recorded in treatment T_2 (12.8 g pod^{-1}) followed by T_5 (5.9), T_4 (5.1), T_3 (4.9), T_6 (4.5) and T_1. Result clearly illustrated that number of seeds per pod 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 and nutrient uptake by increased population of *Trichoderma harzianum* in the crop rhizosphere (Singh *et al.*, 2017; Masunaka *et al.*, 2011). Similar results involving improvement in number of seeds per pod were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al.*, 2021). Also, *Trichoderma* sp. enhanced the yield related growth in other crops as well like number of spikes in wheat crop owing to the improved root growth, plant growth promoting activities and enhanced nutrient uptake in wheat, Arabidopsis (Sharma *et al.*, 2012; El-Gizawy, 2009; Harman *et al.*, 2004; Contreras-Cornejo *et al.*, 2009). Likewise increase in number of seeds per pods due to *T. harzianum* was also observed by other workers in common bean in addition to disease control (Carvalho *et al.*, 2015). *Trichoderma* spp. enhancing plant growth has been reported in several crop plants and has been attributed to auxin (Contreras-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).

Pod length. At harvesting T_2 caused significantly higher pod length (11.35 cm) and further followed by T_5, T_4, T_3, and T_1 (Table 1, Fig. 1). Pod length ranged between 11.35, 10.15, 9.87, 8.67, 7.85 and 6.54 respectively in different treatments. T_5, T_4 were found to be at par with each other while rest of the treatments were different from each other significantly. Result clearly illustrated that pod length of red kidney bean plant was boosted by the combined use of *Trichoderma harzianum* and N: P: K.

The increase in pod length 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 pod length 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).

Seed Index. At harvesting T_2 caused significantly higher seed index of 38.64 g and was further followed by T_5, T_4, T_3, and T_1 (Table 1, Fig. 1). Seed Index ranged between 38.64 g, 36.25 g, 35.18 g, 33.27 g, 31.89 g and 27.63 g respectively in different treatments while T_5 and T_4 were found to be at par with each other. Result clearly illustrated that seed index 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 and nutrient uptake by increased population of *Trichoderma harzianum* in the crop rhizosphere (Singh *et al.*, 2017; Masunaka *et al.*, 2011). Similar results involving improvement in seed index were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al*., 2021). Also, *Trichoderma* sp. enhanced the yield related growth in other crops as well like number of spikes in wheat crop owing to the improved root growth, plant growth promoting activities and enhanced nutrient uptake in wheat, Arabidopsis (Sharma *et al.,* 2012; El-Gizawy, 2009; Harman *et al.,* 2004; Contreras-Cornejo *et al*., 2009). Likewise increase in 100 seed weight due to *T. harzianum* was also observed by other workers in common bean in addition to disease control (Carvalho *et al.,* 2015). Osmolytes accumulation, enhanced water use efficiency and other rhizospheric effects have been attributed for the increment in yield related parameters like in different crops (Petropoulos *et al.,* 2020).

Yield/pot. At harvesting significantly higher yield per pot was recorded in treatment T_2 (86.48 g pot^{-1}) followed by 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. Result clearly illustrated that number of seeds per pod of red kidney bean plant was boosted by the combined use of *Trichoderma harzianum* and N: P: K. All the treatments were found to be statistically different from other treatments.

Yield enhancement by *Trichoderma harzianum* along with disease control in cowpea bean was found to be improved as presented by Pan and Das in 2011 and in other crops as well (Liu & Hunary 2000; El-Mohamedy 2004). Improved plant growth, supplying favourable conditions in the crop rhizosphere, increased root growth and nutrient uptake lead to increment in yield of crop (Singh *et al.,* 2017; Harman *et al.,* 2004; Sallam *et al.,* 2008). PGPR activities of *T. harzianum* producing auxins and other growth hormones increased yield in common bean or snap bean (Sarhan *et al.,* 2018). Similar results involving improvement in yield were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta et al, 2021). Similar results were obtained by other workers in red kidney bean (Liton *et al.,* 2019). Osmolytes accumulation, enhanced water use efficiency and other rhizospheric effects have been attributed for the increment in yield related parameters like yield in different crops (Petropoulos *et al.,* 2020).

Economic Yield. At harvesting, among all the treatments T_2 (16.86 q/ha) gave highest economic yield (Table 2 and Fig. 2). It was followed by T_5 (14.81 q/ha), T_4 (13.03 q/ha), T_3 (12.23 q/ha), T_6 (11.83 q/ha) and T_1 (9.85 q/ha). However T_3 and T_6 were found to be statistically at par with each other while rest of the treatments were observed to be significantly different from each other.

Yield enhancement by *Trichoderma harzianum* along with disease control in cowpea bean was found to be improved as presented by Pan and Das in 2011 and in other crops as well (Liu & Hunary 2000; El-Mohamedy 2004). Improved plant growth, supplying favourable conditions in the crop rhizosphere, increased root growth and nutrient uptake lead to increment in yield of crop (Singh *et al.,* 2017; Harman *et al.,* 2004; Sallam *et al.,* 2008). PGPR activities of *T. harzianum* producing auxins and other growth hormones increased yield in common bean or snap bean (Sarhan et al, 2018). Similar results involving improvement in seed production were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al.,* 2021). Similar results were obtained by other workers in red kidney bean (Liton *et al.,* 2019).

Biological Yield. At harvesting, significantly higher yield was recorded in treatment T_2 (25.78 q/ha) followed by T_5 (23.64 q/ha), T_4 (22.13 q/ha), T_3 (20.89 q/ha), T_6 (19.25 q/ha) and T_1. Result clearly illustrated that biological yield of red kidney bean plant was boosted by the combined use of *Trichoderma harzianum* and N: P: K. All the treatments were found to be statistically different from other treatments. Likewise increase in grain production due to *T. harzianum* was also observed by other workers in common bean in addition to disease control (Carvalho *et al.,* 2015).

Yield enhancement by *Trichoderma harzianum* along with disease control in cowpea bean was found to be improved as presented by Pan and Das in 2011 and in other crops as well (Liu & Hunary 2000; El-Mohamedy 2004). Improved plant growth, supplying favourable conditions in the crop rhizosphere, increased root growth and nutrient uptake lead to increment in yield of crop (Singh *et al*., 2017; Harman *et al.,* 2004; Sallam *et al.,* 2008). PGPR activities of *T. harzianum* producing auxins and other growth hormones increased yield in common bean or snap bean (Sarhan *et al.,* 2018). Similar results involving improvement in seed production were obtained by treating groundnut seeds with *T. harzianum* in other regions of country (Dutta *et al.,* 2021). Similar results were obtained by other workers in red kidney bean (Liton *et al.,* 2019).

Harvesting Index. At harvesting T_2 caused significantly higher Harvesting index (65%) and was further followed by T_5, T_4, T_3, and T_1 (Table 2, Fig. 2). Harvesting index ranged between 65%, 63%, 61%, 60%, 59% and 58% respectively in different treatments. All the treatments were different from each other significantly. Result clearly illustrated that Harvesting index of red kidney bean plant was boosted by the combined use of *Trichoderma harzianum* and N: P: K.

Table 2: Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on yield characteristics of red kidney bean at harvesting growth stage.

Treatment	Economic Yield (q/ha)	Biological Yield (kg/ha)	Harvesting Index (%)
T1	9.8525	16.43	57
T2	16.855	25.78	65
T3	12.2225	20.89	60
T4	13.0325	22.13	61
T5	14.81	23.64	63
T6	11.8275	19.25	59
Sem ±	1.24	2.14	5.73
LSD (*P*=0.05)	0.41	0.69	1.93

(T1: Control; T2: RDF; T3: 70%RDF+Biopriming; T4: 80% RDF+Biopriming; T5: 90%RDF+Biopriming; T6: Control+Biopriming); Means with the same letter are not significantly different.

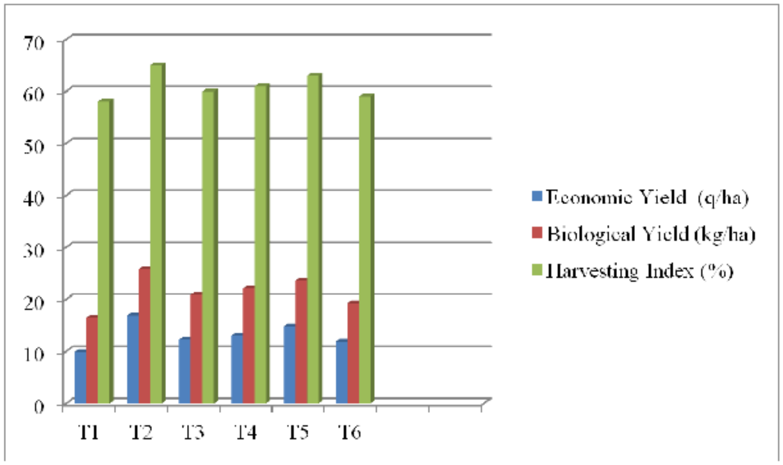


Fig. 2. Effect of biopriming with *T. harzianum* and graded dose of N: P: K application on yield characteristics of red kidney bean at harvesting growth stage.

CONCLUSION

The research data supports the points that Biopriming of the crop with *Trichoderma harzianum* improved different yield attributes like number of trifoliate branches/plant, weight of pods, number of pods, number of seeds per pods, pod length, seed index,Yield/pot in red kidney bean. Improvement in these traits of the crops resulted in increased economic as well as biological produce of red kidney and ultimately harvesting index for the crop.

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Conflict of Interest. None.

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