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Trichoderma improves photosynthetic features 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 (talc) 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 leaf area and chlorophyll content of the plant and ultimately the 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

1. Introduction
Pulses such as common beans (*Phaseolus vulgaris*) have contributed to human nourishment since ancient time and even in recent times, common beans are recognized as part of healthy diet due to their high composition of protein, resistant starch and dietary fiber (Ampofo and Ngadi, 2020) [1]. In world, common bean is grown over an area of 18 million hectares with 12 million tons of production per year (Raggi *et al.* 2019) [2]. As common bean is a poor nodulating legume its nutrient requirements are huge as compared to other legume which are good nodulators and hence their nutrient management have to be met with the use of fertilizers be it inorganic or biofertilizers. Use of non-biofertilizers is debatable as its nutrient use is inefficient. Additionally, it has other side effects *viz.* ground water pollution, acidification, eutrophication, destruction of soil structure and aggregation etc.
Use of microorganisms for the nutrient management of heavy feeder crops like common bean can be availed and used efficiently without devising the ill effects of inorganic fertilizers. Side by side, it has additional benefits like healthy soil microflora maintenance, organic matter balance, buffering capacity and aggregation etc. these microorganisms can be used maybe N-fixer, P-mobilizer or solubilizer, organic matter decomposer, cellulolytic, PGPR and so on. These microorganisms can be used in seed or soil treatment, seedling treatment etc. of all the inoculant applications seed treatment is considered best as it provides and enhance the population of effective microorganisms and their establishment in the rhizosphere with ease and therefore give more efficient results.
One such technique of seed treatment is seed biopriming in which seed hydropriming is an additional process and ensures better establishment and cover or film of effective microbes on the seeds. For mycorrhizal inoculants it promotes advanced germination of spores which ensures better mycelial cover on seed material and ultimately improves the plant growth and developments and shows effects on the economic production.
The plants of mung bean bioprimed with *Trichoderma* sp. (BHU B13-398 and BHU M) showed a significant increase in leaf surface area (87.06% and 62.28%) and chlorophyll content (16.21% and 6.00%) as compared to Control (Kumari *et al.* 2018). There was increase in total chlorophyll by 104% in treated plants compared to the control in different vegetable plants (Singh *et al.* 2016) [4].
At most of the stages, number of leaves, a rapid and progressive increase in total leaf area, commencing from 3 months after planting to harvest was visualized statistically significant in all the treatments as compared to control in banana crop biohardened using PGPR (Harish *et*

al, 2008) [5]. Total chlorophyll content increased in all seedlings under salinity conditions in bioprimed maize seedlings (Panuccio *et al*. 2018) [6].

2. 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.

Seeds of 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. 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 @120:60:60kg/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 no.	Fertilizer dose	Microbe used in seed treatment
T1	Control N: P: K @0:0:0kg/ha	No
T2	100% of RDF N: P: K @100: 60: 25kg/ha	No
T3	70% OF RDF	*Trichoderma harzianum*
T4	80% OF RDF	*Trichoderma harzianum*
T5	90% OF RDF	*Trichoderma harzianum*
T6	N: P: K @0:0:0kg/ha	*Trichoderma harzianum*

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. Leaf area of the plants was measured by the use of Leaf Area Meter incm2 units LI-3000C at 60 and 90 days after sowing. The chlorophyll content was estimated at SPAD meter. Regarding Statistical analysis Duncan multiple range analysis was done through software STAR.

3. Results and Discussion

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

Chlorophyll content of the crop was recorded at 30, 60 and 90 days after sowing. Chlorophyll content in leaves was significantly affected by combination of NPK-fertilizer levels with seed biopriming by *T. harzianum* are presented in table 1 and depicted in figure 1. At 30 DAS maximum chlorophyll content was in T_2 (40.925) followed by T_5 (38.625). After this T_4 (37.125) and T_3 (36.6). At 60 DAS trend of chlorophyll was $T_2>T_5 >T_4>T_3>T_6>T_1$. There was no significant difference in between the treatments and are statistically similar. At 90 DAS maximum chlorophyll content was observed in T_2 (24.625) which is at par with T_5, T_4, T_3 and T_6 than in T_5 (23.925). The chlorophyll content followed the order $T_2> T_5 > T_4 >T_3>T_6 >T_1$.

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.

Treatment	Vegetative stage	Pod formation	Maturation stage
T1:Control	34.825a	34.4a	18.125a
T2:RDF	40.925b	36.95a	24.625b
T3: 70%RDF +Biopriming	36.6a	35.125a	22.825ab
T4: 80% RDF +Biopriming	37.125a	36.5a	23.325ab
T5: 90%RDF +Biopriming	38.625ab	36.675a	23.925ab
T6:Control+Biopriming	36.15a	34.8a	20.075ab

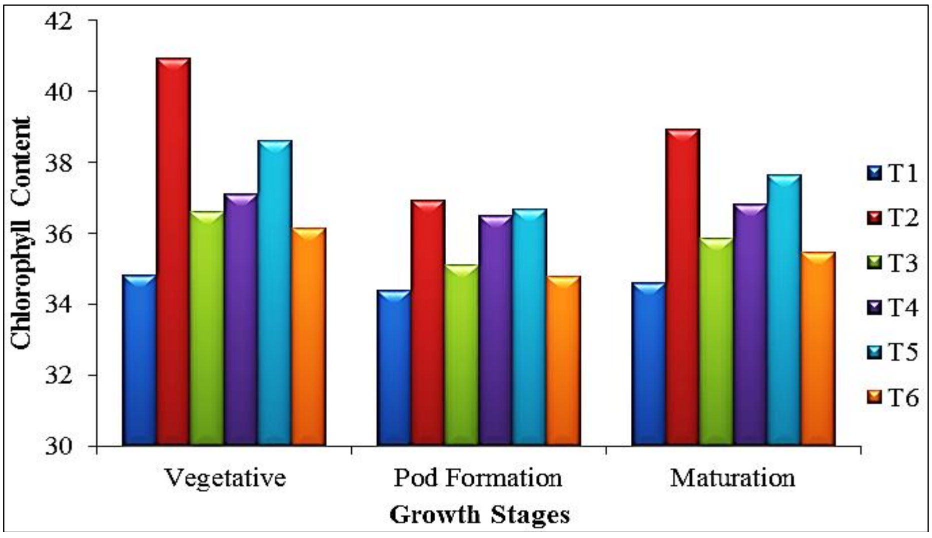

Fig 1: Effect of biopriming with *T. harzianum* and graded levels of N: P: K application on chlorophyll content of red kidney bean at different growth stages

(T_1: Control N: P: K @0:0:0kg/ha, T_2: RDF of N: P: K @100: 60: 25kg/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)

Throughout the growing stage chlorophyll content was minimum in T_1 i.e. control with values 34.825, 34.4 and18.125 at vegetative (30 DAS), pod formation (60DAS) and maturation stages (90DAS) respectively. Further gradual increment of chlorophyll content is frequently observed along the successive growth stage of crop. During the initial growth stage of red kidney bean from vegetative stage to pod formation stage chlorophyll content increased slowly due to uptake of N and P as facilitated by microbe inoculation. Data of chlorophyll content at 60 DAS of plants significantly affected due to application of graded doses of N application with combination of seed biopriming by *T. harzianum*.

Results (Table-2) clearly indicate that leaf area was significantly influenced with sole application as well as combined treatment of *Trichoderma harzianum* with graded dose of NPK application. A gradual and consistent increase in leaf area was observed up to 60 DAS and there after it was decreased. Maximum leaf area was recorded with T_2 (RDF of N: P: K @100: 60: 25kg/ha) in both growth stages of red kidney bean.

Table 2: 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

Treatment	Vegetative stage	Pod formation
T1:Control	71.725a	249.6a
T2:RDF	85.1b	397.5b
T3: 70%RDF +Biopriming	78.5ab	293.4ab
T4: 80% RDF +Biopriming	80.225ab	338ab
T5: 90%RDF+Biopriming	80.425ab	390.725b
T6:Control+Biopriming	73.575ab	267.325a

Data of red kidney bean at 30 DAS revealed that leaf area increased with the advancement of crop growth stages. 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 which is at par with T_3, T_4 and T_5 and least area was in T_2 i.e. control. At 30 DAS treatment T_2 has maximum leaf area (85.1cm^2 $plant^{-1}$) and minimum was recorded in T_1. T_6 is significantly different from T_2 with leaf area 73.575cm^2 $plant^{-1}$ statistically at par with T_2, T_3, T_4 and T_5. At 60 DAS treatment T_2 has maximum leaf area (397.5cm^2 $plant^{-1}$) which is at par with T_5 (390.725cm^2 $plant^{-1}$) and next to these is T_4 (338cm^2 $plant^{-1}$) which is at par with T_3 (293.4cm^2 $plant^{-1}$). T_1 is statistically different from T_2 but at par with T_6 with a leaf area of 294.6cm^2 $plant^{-1}$). Increase in leaf are of T_2 over control was 13.375 and 147.9cm^2 $plant^{-1}$ at 30 and 60 DAS respectively. Different treatment combination was always superior over the control. Significant interactions were recorded between different treatments with N-application and seed biopriming with *Trichoderma harzianum* on leaf area at 30 and 60 DAS. Similar extent of increase in leaf area by the application of *Trichoderma harzianum* with T_3 (seed treatment with *T. harzianum* + 70% N and RDF of P: K) and T_2 RDF of N: P: K @100: 60: 25kg/ha suggested role of *Trichoderma harzianum* in solubilizing several plant nutrients (Altomere *et al.* 1999) [7] 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) [8].

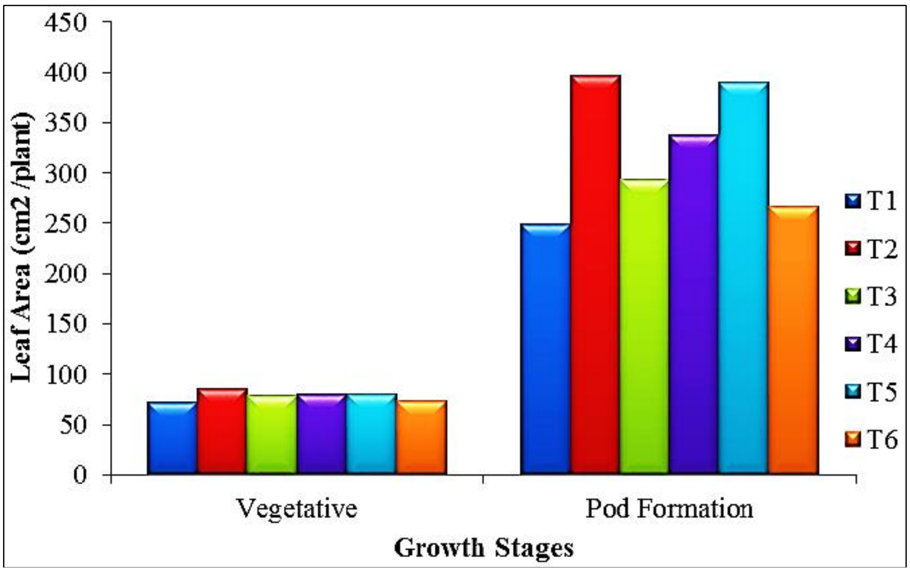

Fig 2: Effect of biopriming with *T. harzianum* and graded levels of N application on leaf area of red kidney bean at 30 and 60 DAS.

(T_1: Control N: P: K @0:0:0kg/ha, T_2: RDF of N: P: K @100: 60: 25kg/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)

4. Conclusion

The research data supports the points that seed biopriming of the red kidney bean crop with *Trichoderma harzianum* improved the chlorophyll content and leaf area of red kidney bean in pot culture.

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