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Impact of *Trichoderma viride* as Bio-stimulator on Nodule Enumeration, Nutrient Quality and Yield of Chickpea (*Cicer arietinum* L.) in Central India

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ABSTRACT

Background: Chickpea (*Cicer arietinum* L.) commonly known as gram or Bengal gram, is an important pulse crop in India. Its productivity is low due to some biotic and abiotic stress, where diseases constitute major biotic constraints. For eco-friendly and sustainable management, *T. viride* was found effective as biocontrol and in enhancing yield.

Methods: An experiment was conducted during *Rabi* season of 2018-19 on nodule enumeration and nutrient quality of chickpea influenced by inoculation of *T. viride* derived from treatment of different carbon and nitrogen sources at research field JNKVV, Jabalpur, India. Trials followed randomized block design (RBD) experimental design, where a total of 16 treatments including fertilized uninoculated (FUI) replicated thrice using chickpea var. JG-16 as test crop.

Result: From the result it can be proposed that seed treatment from *T. viride* isolates derived from peptone + ammonium sulphate was found significantly effective on the nodule enumeration like no. of nodule, nodule biomass, nutrient content in seed and straw and yield of chickpea.

Key words: Carbon, Chickpea, Nitrogen, Nodule, Nutrient, *Trichoderma viride,* Yield.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) a major pulse crop, is cultivated in India on an area of 26.28 Mha, producing around 75% of the world's consumption (Tomar *et al.* 2010) corresponding to the largest area of 3.16 Mha and 3.29 Mt production in Madhya Pradesh. The production of crops like chickpea has been oriented to drastic route for producing more yield ever since the inception of Green Revolution; while 'sustainable' have been thrust out. Owing to the ill effect of chemical inputs, current attentions have been shifted to the use of biofertilizers and biocontrol agents as an important tool. *T. viride* are environment friendly biological agent and have been explored effectively since 1920s (Samuels, 1996), although its first description dated back to 1794 (Persoon, 1794).

T. viride widely known to be plant growth promoter produces molecules, *e.g.* zeatin and gibberellin, which help in seed germination and development (Osiewacz, 2002). Effective nodulation, plant height, biomass and podding are also associated with their special role in root colonization and architecture mediated by *Trichoderma* sp. (Marzani *et al.*, 2017; Rehman *et al.*, 2013). They secrete various organic acids such as glutonic acid, citric acid and fumaric acids (Gomez-Alarcón *et al.* 1994) which are capable of solubilizing nutrients by acidifying their surrounding environment especially phosphates, magnesium and micronutrients like iron and manganese (Benitez *et al.*, 2004; Harman *et al.*, 2004). They are also opportunistic plant symbionts, enhancing systemic resistance of plants (Shoresh *et al.*, 2010). By colonizing plant roots, a cellulose binding functional protein or invading them, they are also carried through soil and occupy new niches. This interaction with plants as well as their rhizosphere competence leads to enhanced root proliferation, better growth and protection of the plants against toxic chemicals, against which *T. viride* themselves show a remarkable resistance.

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MATERIALS AND METHODS

Experimental site, climate and soil properties

The entire experiment was carried out in two components. Studies of component-I and II were performed at the Laboratory of Microbes Research and Production Centre and the Research Farm, Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur India, situated at (latitude of 23° 13′ N and longitude of 79° 57′ E with an altitude of 393 m above mean sea levels). The component I comprised the laboratory works to obtain isolates of *T. viride* with the supplementations of 16 different of media composing of three carbon sources *viz.*, (peptone, glucose and mannitol) and three nitrogen sources (ammonium sulphate, urea and potassium nitrate) and their combinations supplemented into the basal medium of potato dextrose (PD) broth. The commonly used medium alone was considered as control.

Under component II, The isolates of *T. viride* obtained from the component I, performing best were earmarked and specially short-listed for the field trial on chickpea var. JG-16 to observe sustainability of the attributes. Therefore the 16 isolates plus 1 control of fertilized un-inoculated (FUI) were tried under RBD with 3 replications. The experimental soil was deep black soil in texture, having pH, 7.4; organic carbon; 0.53%, available N, 207 kg ha^{-1} and 15 kg ha^{-1} of available P and 227 kg ha^{-1} of available K, was recorded), respectively. Plot size of 2 m × 3 m = 6 m^2 with 40 cm row to row spacing was maintained and recommended dose of fertilizers 20:80:20 (N:P_2O_5:K_2O kg ha^{-1}) was applied through Urea, SSP and MOP. Before sowing, seeds were treated with the *T. viride* isolates @ 10 ml/kg seed.

The climate of the experimental site is characterized by sub-tropical climate with hot dry summers and cool dry winter. The average maximum temperatures during the months of May-June varies between 42.5 to 46.4°C and are the hottest month of the year, while the average minimum temperature varies between 4.2 to 8.7°C during December-January (Fig 1). The average annual seasonal rainfall of this region is about 1200 mm which is mostly received between June to September and a little rainfall (75 to 175 mm) received from October to May. The average humidity of the region is about 73 per cent and average evaporation is 3.93 mm/day.

Plant sampling and analysis

Three plants were randomly uprooted to count the number of nodules at 60, 90 and 120 DAS (days after sowing). After counting the number of nodules, the nodules were oven dried at 60°C and weighed to obtain the nodule dry weight expressed in g plant^{-1}. Seed and stover sample were taken after harvest for analysis of N, P, K and protein content. Nitrogen content was estimated by Kjeldhal's method as described by (Amma, 1989). For determination of P content, the plant sample (1g) was digested with di-acid mixture of HNO_3 and $HClO_4$ (3:1) for development of vanadomolybdo-phosphoric yellow colour and absorbance of the colour intensity was measured with spectrophotometer at 420 nm (Koenig and Johnson, 1942). Potassium content was estimated from acid digest with a flame photometer using the procedure of (Bhargava and Raghupathi, 1984).The protein content in seed was determined by multiplying the N content in seed to 6.25 and expressed in%. After taking the biological/bundle yield, the harvested crop plot wise was kept for sun drying and threshing was done. The seeds were weighed and taken as seed yield (kg). Stover yield was obtained by deducing the seed yield from biological yield of each treatment and expressed in kg ha^{-1}.

Statistical analysis of data

Data were statistically treated by analysis of variance, RBD to test the statistical significance of variance among different treatment means as influenced with the application of the treatments on various attributes of chickpea.

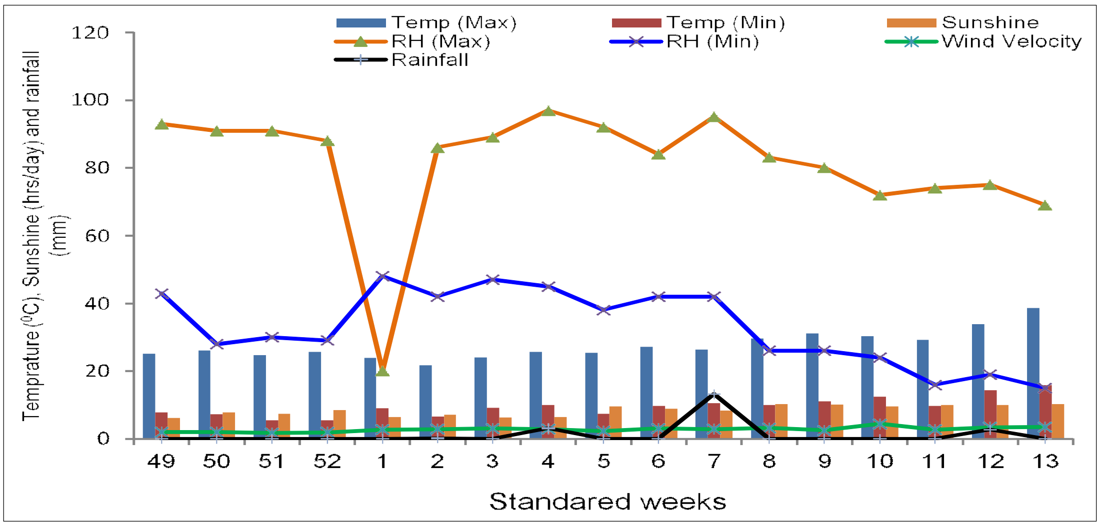

Fig 1: Weekly meteorological data during 2018-19 (December to March).

(Source: Department of Meteorology, College of Agricultural Engineering, JNKVV, Jabalpur (M.P.).

*Temp (Max) - Temperature maximum.

*Temp (Min) - Temperature minimum.

*RH (Max) - Relative humidity maximum.

*RH (Min) - Relative humidity minimum.

RESULTS AND DISCUSSION

Results of field experiments revealed that inoculation with selected *T. viride* isolates significantly promoted the nodule, nutrient quality and yield contributing parameters of chickpea. However, the rate of enhancement varied with *T. viride* isolates.

Number of nodules plant^{-1}

Effect of different *T. viride* isolates inoculation on number of nodule has been presented in Table 1. The maximum number of nodules plant^{-1} at 60 and 120 DAS recorded was 26 and 42 nodules plant^{-1} with the isolate of peptone + ammonium sulphate which significantly increased by 60.4 and 47.1% response over the control FUI (16 and 28 nodules plant^{-1}), respectively, while at 90 DAS the isolate of mannitol+ammonium sulphate increased the number nodules plant^{-1} to maximum by 67 nodules plant^{-1} with 39.9% response over FUI (48 nodules plant^{-1}). The number of nodules plant^{-1} recorded at 60 and 90 DAS period showed a significant increase in number of nodules and thereafter declined.

Significantly higher nodules number plant^{-1} was observed across all isolates except control. These findings were in close proximity with those of (Bhattacharya and Chandra, 2013) reported that the beneficial effects of microorganism on nodule number in chickpea. Khan *et al.,* (2014); Nirmalkar *et al.*, (2017) also observed similar findings with *Trichoderma* sp. showed significantly better and higher number of nodulation. Enhancement in nodulation attributes could possibly be due to competition exclusion by *Trichoderma* sp. over other microorganisms of non-beneficial which favored symbiosis with roots and thereby improve nodulation of legumes (Badar and Qureshi, 2012). The decline in number of nodules plant^{-1} with advancement in growth age is chiefly due to decay of nodular tissues at pod formation due to accumulation of readily degradable albumin reported by (Tagore *et al.* 2013).

Nodule dry biomass

The data in Table 1 elaborate the influence of *T. viride* application on dry weight of nodules at 60, 90 and 120 DAS. The maximum statistically significant nodule biomass was obtained by the isolate from treatment of peptone + ammonium sulphate, with 0.089, 0.271 and 0.154 g plant^{-1} which gave 46.2, 53.8 and 41.2% response over FUI (0.061, 0.176 and 0.109 g plant^{-1}), respectively at 60, 90 and 120 DAS. The similarity findings with (Polo and Mata, 2017) elucidated that peptone contain high amounts of L-α amino acids (85%), free amino acids (17%) and organic-nitrogen content (12%). The nodules also contain molecules that activate the crop signalling mechanism to increase the use of nutrients for productive functions. This possibly explained why peptone as carbon source yielded the highest response. When ammonium treated soil was limed to increase the pH a reduction in the activity of *Trichoderma* sp. occurred (Simon and Sivasithamparam, 1988). Therefore, it seemed obvious that the addition of ammonium sulphate might be a reason for an increased activity of *T. viride* and thereby stimulated plant growth. The superiority of glucose could be attributed to its role as an energy source, an important component of rhizodeposition and the soil sugar pool, which participate in the regulation of plant growth (Ma *et al.,* 2017). This increment in nodule biomass was possibly due to higher enumeration of nodules boosted up with seed inoculation

Table 1: Inoculation effect of different isolates of *T. viride* derived from treatment of different C and N on nodulation of chickpea.

Treatment	No. of nodules plant^{-1}			Nodule biomass (g plant^{-1})		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
Peptone (Pep)	20.7	56.0	35.0	0.070	0.208	0.134
Glucose (Glu)	20.0	56.3	33.7	0.066	0.203	0.127
Mannitol (Mann)	20.3	54.0	32.7	0.064	0.209	0.124
Ammonium sulphate (Amm. Sul.)	21.0	57.0	36.7	0.072	0.215	0.133
Urea	19.0	53.0	33.3	0.064	0.202	0.124
Potassium nitrate (Pot. Nit.)	19.3	54.0	34.0	0.065	0.207	0.125
Pep + Amm. sul.	25.7	65.7	41.7	0.089	0.271	0.154
Pep + Urea	19.3	54.7	35.0	0.065	0.198	0.127
Pep + Pot. nit.	21.0	57.0	37.3	0.074	0.218	0.139
Glu + Amm. sul.	24.3	64.7	40.7	0.087	0.260	0.151
Glu + Urea	19.7	56.3	36.3	0.066	0.192	0.124
Glu + Pot. nit.	21.3	60.3	38.3	0.081	0.222	0.140
Mann + Amm. sul.	22.7	66.7	40.0	0.087	0.254	0.144
Mann + Urea	19.3	55.0	35.3	0.067	0.217	0.123
Mann + Pot. nit.	21.3	57.0	40.0	0.076	0.236	0.145
PD	15.0	47.0	29.7	0.059	0.175	0.108
FUI	16.0	47.7	28.3	0.061	0.176	0.109
$SE_m \pm$	1.283	2.575	1.923	0.003	0.014	0.005
$CD_{5\%}$	3.78	7.59	5.67	0.01	0.04	0.02

of *Trichoderma* (Rudresh *et al*., 2005; Shaban and El-Bramawy, 2011).

Nutrient content in seed and stover

Table 2 represents the data on NPK content in seed and stover at harvest. The N content in seed and stover of chickpea was significantly enhanced by different isolates of *T. viride*. Amongst all, peptone + ammonium sulphate observed highest N content in seed and straw of 3.26 and 1.09% N and increment of 12.4 and 32.9% over FUI (2.90 and 0.82% N), respectively.

Considering the effect on P content in seed and stover, peptone + ammonium sulphate recorded the maximum of 0.552 and 0.185% with 17.9 and 24.0% response over that of control FUI (0.443 and 0.149% P), respectively. The highest performance was followed by peptone+potassium nitrate and glucose + ammonium sulphate.

However, the isolates glucose + potassium nitrate responded the maximum K content in seed for 1.060 and 1.0002% K with an increment of 16.5 and 22.7% over the control FUI (0.910 and 0.817% K), respectively. While, the performance of isolates from the remaining treatments of mannitol+ammonium sulphate, mannitol+potassium nitrate, glucose+potassium nitrate, ammonium sulphate, peptone and glucose were statistically at par with that of control.

The effect of *T. viride* on NPK content was in harmony with the reported of a group researcher where *T. viride* inoculated chickpea plants increased NPK when compared with control. This improvement might possibly be due to better root development and the synergistic effect of microbes which increased the nutrient content. Similar result was also presented by (Mohammadi *et al.,* 2010; Jakhar *et al.*, 2018), whose work elaborated the significant effect of biofertilizers on increasing the quality and nutrient content in crop.

Protein content in seed

Protein content varied from 18.15 to 20.35%, the mean value of which was computed as 19.04%. Based on the observations recorded, the treatment peptone + ammonium sulphate responded with highest protein content of 20.35% exhibiting 12.2% increment over control FUI (18.15% protein), followed by glucose+ammonium sulphate and mannitol + ammonium sulphate for the protein content of 20.25 and 19.88% with 11.6 and 9.5% response, respectively. The ability to utilize nutrients is the resulting capability of *Trichoderma* to acquire ATP from the diverse types of sugars, cellulose, glucan and others, all of them turning into glucose (Chet *et al.* 1997). Increment in protein content could be due to nutrient acquisition by plant roots and the role of *Trichoderma* in producing antibiotics.

Yield

The data pertaining to yields of seed and stover of chickpea are projected in Fig 2. Among all the treatments, the isolate from treatment combination of peptone+ammonium sulphate produced highest seed yield for 1465 kg ha^{-1} with 16.3% more over FUI (1261 kg ha^{-1}), followed by glucose + ammonium sulphate, mannitol+ammonium sulphate and glucose + potassium nitrate for 1453, 1430 and 1419 kg ha^{-1} seed yield with the respective increment of 15.2, 13.4, 12.5 and 11.6%.

Table 2: Inoculation effect of different isolates of *T. viride* derived from treatment of C and N sources on NPK and protein content.

Treatment	Nutrient content (%)						Protein content (%) in seed
	Seed			Stover			
	N	P	K	N	P	K	
Peptone	3.00	0.489	0.962	0.89	0.169	0.876	18.75
Glucose	2.99	0.482	0.956	0.86	0.166	0.855	18.69
Mannitol	2.99	0.480	0.953	0.83	0.163	0.834	18.67
Ammonium sulphate	3.03	0.492	0.963	0.90	0.171	0.881	18.94
Urea	2.99	0.485	0.955	0.87	0.164	0.874	18.69
Potassium nitrate	3.00	0.488	0.979	0.85	0.162	0.860	18.75
Pep + Amm. sul.	3.26	0.522	1.021	1.09	0.185	0.989	20.35
Pep + Urea	3.00	0.483	0.953	0.87	0.165	0.883	18.73
Pep + Pot. nit.	3.06	0.517	1.028	0.94	0.183	0.997	19.15
Glu + Amm. sul.	3.24	0.505	1.033	1.07	0.182	0.975	20.25
Glu + Urea	3.00	0.484	0.959	0.88	0.164	0.885	18.77
Glu + Pot. nit.	3.13	0.484	1.060	0.96	0.175	1.002	19.54
Mann + Amm. sul.	3.18	0.499	1.001	1.02	0.180	0.969	19.88
Mann + Urea	3.00	0.485	0.962	0.88	0.164	0.882	18.77
Mann + Pot. nit.	3.10	0.487	1.008	0.98	0.177	0.982	19.40
Control (PD)	2.92	0.470	0.933	0.81	0.161	0.792	18.27
FUI	2.90	0.443	0.910	0.82	0.149	0.817	18.15
$SE_m\pm$	0.058	0.017	0.027	0.036	0.012	0.030	0.362
$CD_{5\%}$	0.17	0.05	0.07	0.10	0.04	0.09	1.07

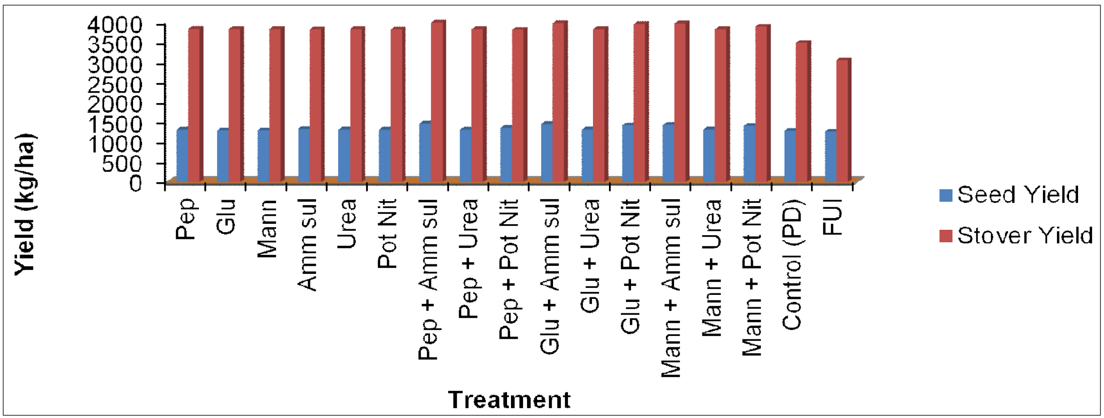

Fig 2: Inoculation effect of different isolates of *T. viride* derived from treatment of different sources of C and N on yield of chickpea.

Similarly, the highest stover yield of chickpea 3984 kg ha^{-1} was recorded with the isolate from treatment peptone + ammonium sulphate by 30.8% response over FUI (3046 kg ha^{-1}). Increased in yield of chickpea with application of *T. viride* might be attributed to better nodulation, N_2 fixation and crop growth. These are in harmony with the earlier findings of (Chalie-U *et al.*, 2018; Jakhar *et al.*, 2018; Jakhar *et al.*, 2021) reported that biofertilizer enriched chickpea production.

CONCLUSION

Trichoderma is considered as plant symbionts playing a significant role in growth promotion. Like any other living being, *T. viride* survival is also affected by nutritional sources, especially carbon and nitrogen. The addition of carbon and nitrogen to the media is therefore an experiment to find out whether it performs equally well in the field condition as it did under laboratory experiment. Enhancement in nodulation, nutrient content both in seed and stover was achieved owing to the application of isolates of *T. viride*.

Conflict of interest: None.

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