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Effect of Bio-Priming with Isolates of Fungal Bio Agents on Morphological Attributes and Growth Parameters of Chickpea

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops of India. Chickpea is one of the major and abundant sources of protein among pulses; production of chickpea is very low all over the worldwide due to several biotic and abiotic stresses. Biotic stress diseases are the major problems. Chickpea wilt caused by *Fusarium oxysporum* f. sp. ciceri has been considered as devastating one to cause up to 10 per cent loss in yield every year. For eco-friendly and sustainable management of the disease, six isolates of two fungal bioagents *Trichoderma*

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harzianum and Trichoderma viride were evaluated for their antagonistic efficacy on colony growth by dual culture plate method. The results showed that the bio-agents suppressed the colony growth ofpathogen which ranged between 70.80-59.29%. Isolates of T1 [*Trichoderma harzianum* (KN)] 70.80% was found very effective antagonistic effect compare to all treatments and control followed by T2 [*Trichoderma harzianum* (VN) which inhibited 68.80%, T3 [*Trichoderma harzianum* (UNN) 66.45% and T4 [*T. viride* (FBD)] 63.58% mycelia growth of pathogen and so on. The result of seed treatment in vivo condition showed that T1 found highest germination percent among all the treatments with a value of 85.71%. In all the treatment, substantially boosting the morphology of chickpea against control, the maximum number of branches was recorded in T1, with value of 12 and maximum root length was 13.33 cm with increase 81.85% over the control. In the yield parameter, the highest number of pods was found in T1 with 41 pods, followed by T2 and T3 with 37 and 36 pods, respectively. Result of the study show that bio-agents significantly reduced the wilt incidence, treated plants subsequently significantly increased germination percentage, morphological parameters and yield parameters over the non-treated plants

Keywords: Chickpea; Fungal bio-agent; inhibition; fusarium; mycelia growth.

1. INTRODUCTION

“Chickpea (*Cicer arietinum* L.), 2n=2x=14, which is a member of the *Papilionaceae* subfamily of the *Leguminoceae* family. Bengal gram or king of pulse crop is another name of chickpea. It is one of the main pulse crops grown during the *Rabi* season in India. Pulses continue to play a significant role in human diets, particularly among the vast majority of vegetarians in the nation. India, Pakistan, Turkey, Iran, Myanmar, Ethiopia, Mexico, Australia, Syria, Spain, Canada, United States, Bangladesh, Algeria, Malawi, Sudan and Portugal are among the major producers of chickpeas in the world. The primary origins of the chickpea are in South-West Asia and the Mediterranean region with Ethiopia serving as the secondary origin. It is a significant pulse crop in the tropical, subtropical and temperate parts of the world” [1-3]. “The main producers of chick pea are India, Australia and Pakistan, contributing 67.32%, 6.19% and 5.72% respectively to global production. Agricultural Organization's (FAO) current global production will be at 16 million metric tonnes in 2021. India is the world's top producer, contributing to about 75% of global output. India is also the world's biggest consumer of chickpeas. As a result, despite accounting for over 75% of global production, the nation came in second. In India, chickpeas make up around 49% of all the pulses that are produced. Major chick pea growing states are Rajasthan, Maharashtra, Uttar Pradesh and Andhra Pradesh contributes about 17%, 16%, 6% and 5% of the total production, respectively” [4,5]. Chickpea now a day affected by various abiotic and biotic stresses. The several biotic and abiotic constraints responsible for low productivity of chickpea, diseases are the most serious constraints causing up to 100 per cent losses of this crop.

“Among the fungal disease Fusarium wilt of chick pea caused by *Fusarium oxysporium* f. sp. *ciceri* causes more damage. Chickpea crop more vulnerable during the flowering and pod forming stage, if the crop is subjected to sudden temperature rise and water stress the wilt incidence level also fluctuate with weather parameter so we can say wilt incidence directly correlated with weather parameters” [6]. “Fusarium wilt results in major economic losses ranging from 10-40% worldwide” [7]. It causes 100% loss under specific conditions [8] and at particular growth stages of crop like vegetative and reproductive [9,10]. “The disease is most devastated, widespread and important throughout the world” [11,12]. “In India, it is estimated that 10% yield losses were caused annually under certain conditions it may go up to 60%. The incidence was varied from 14 to 32 per cent in different states” as reported by Dubey *et al*. [6].

“A successful evaluation of disease is always followed by management strategies to combat the disease. Management of Fusarium wilt of chickpea is difficult to achieve and no single control measure is sufficiently effective, it is because basically they are soil borne in nature. The best way of management of Fusarium wilt through applying cultural and chemical method but, chemical management strategies cause hazardous effect on human and animal health and environment. So, several researchers searching new eco-friendly non-

hazardous ideas for the management of Fusarium wilt of chick pea. In this respect a novel comprehensive sustainable approach for disease management is appreciable" [13-15]. The use of bioagent found to be an effective measure to manage wilt disease of chickpea. Fungal bio-agents, such as non-pathogenic and non-host *Fusarium* species, have been used successfully and resulted in a significant reduction in both pathogenic fungal growth *in vitro* and disease development in the field. Seed bio priming is a technique where seeds are treated with beneficial microorganisms to enhance their germination and growth [16-19]. To manage wilt through seed bio priming, seeds are coated with fungal antagonist that suppresses the growth of the wilt pathogen. The aim of this research to managing wilt through seed bio priming is to enhance the resistance of seeds and seedlings to wilt causing pathogens, ultimately leading to healthier plants and increased crop yields. This approach can help minimize the incidence and severity of wilt disease, thereby improving crop productivity and sustainability.

2. MATERIALS AND METHODS

2.1 Collection of Diseased Materials

The specimens were obtained from the Student Instruction Farm of C.S.A. University of Agriculture & Technology Kanpur. From the field the infected chickpea plant that are exhibiting wilting like symptoms followed by yellowing were gathered and delivered to the lab for initial analysis. For additional investigation, the specimen and diseased sample were placed between the fold of sterilised blotting paper and kept in a refrigerator at 4-6°C. The entire specimen was gathered and tested for the presence of the causative organism and virulence in the lab.

2.2 Isolation and Purification of the Pathogen

Isolation of the fungus was done from the part of the plant showing initial wilt symptoms [Gurha *et al*. 2002 and Kaushal *et al*. 1990]. The affected roots were first washed in tap water to remove dust particles. The affected pieces of root surface were sterilized in 0.1 percent aqueous solution of mercuric chloride for one minute and subsequently washed thoroughly 3-4 times with distilled water. Excess water was removed by putting the pieces in between the folds of sterilized blotting papers. These pieces were inoculated on PDA plates kept in incubator at 25^0 – 27^0C for 24 hours. Single spore isolation technique was done for the purification of the culture and the culture thus obtained was maintained on PDA (Potato Dextrose Agar) medium slants for further studies.

2.2.1 Identification of pathogen

Identification of the pathogen was made by comparing the cultural and morphological characters of the fungus with that of described by C. Booth [Booth 1971] for *Fusarium oxysporum* f. sp. *ciceri* following growth habit, cultural and morphological characters.

2.2.2 Pathogenicity test

Pathogenicity test of *Fusarium oxysporum* f. sp. *ciceris* were carried out in plastic pots. Plastic pots filled with double sterilized soil. The inoculums of *Fusarium oxysporum* f. sp. *ciceris* was multiplied on sterilized sorghum seeds and added to each pot. The inoculums were thoroughly mixed in soil. Similarly 2 pots with sterilized soil without inoculums were used as control. Seeds of chickpea susceptible variety (JG- 62) were sown in each pot.

2.2.3 Laboratory screening of antagonists against the test pathogen

Two bioagents viz. *Trichoderma viride* and *Trichoderma harzianum* were assessed for comparative efficacy against *Fusarium oxysporum* f. sp. *ciceri* by using dual culture plate technique [Morton and stroube 1955]. Five mm disc of test fungus and the antagonistic fungi, cut from the edge of five days old culture were used for inoculation. Test fungus was inoculated before72 hour of bioagent inoculation, on potato dextrose agar medium Petri plates. The test fungus and bioagents were inoculated opposite to each other at a distance of 5 mm from the periphery of the Petri plate. Control without bioagent was prepared for each treatment. Three replicates of each treatment were made. All treatments were incubated at 25 $\pm$1ºC; the data were recorded after inoculation when the inhibition zone was formed, it was expressed as percent inhibition.

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

Whereas,

C= Mycelial growth in control plates, T= Mycelial growth in Treatment plates

2.2.4 Seed priming with different bio agents

The surface of chickpea seeds of the cultivar "JG 62" was sterilized for five minutes with 1% sodium hypochlorite, followed by three rinses with sterilized distilled water and air drying. Seeds were primed by the powder of various strain of *Trichoderma* spp. within the different Petri plates. Just after priming seeds were sown on the next day early morning in a well pulverized plot and kept in the wire house. Three rows were sown for each treatment in a block. Regular observations were taken at 10 days interval from 30 days after sowing. Numbers of plant were counted row wise and fixed for further studies. Number of plants dead was also counted at 20 days interval. After five regular observations, number of plants survived was counted and observations were made for calculating the effectiveness of bioagent in checking the attack of *Fusarium oxysporum* f. sp. *ciceri* on chickpea. The untreated seeds served as control. The completely randomized design (CRD) was kept for the experimental setting. Table list contains the information about the isolates and further treatments that have been used in the study.

2.2.5 Observation recorded

To record observations of several morpho-physiological characteristics of the plant 20-35 days after sowing (DAS), sampling was carried out at random. Three plants were randomly chosen from each three replication of a treatment, and information was gathered on a variety of attributes.

Germination percentage: Ten days after sowing (DAS), the number of seeds that germinated in each treatment was counted and recorded. Germination percent calculated by given formula:

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated in particular treatment}}{\text{Total number of seed treated in particular treatment}} \times 100$$

2.2.6 Shoot and root length (cm)

After stretching the plant with the aid of a metre scale, the shoot and root length was calculated in centimetres (cm) from the plant's base (ground level) to the end of its main axis. Data was taken 40 days after transplanting (DAT).

Disease incidence

Disease incidence was calculated by using the following formula.

Disease incidence = Number of diseased plants / Total number of plants * 100

2.3 Statistical Analysis

Complete randomization design (CRD) was used in this experiment. It was use the analysis of variance technique to draw conclusions from the data. The estimated value of F will be compared to the probability value that has been tabulated for the appropriate degree of freedom (Fisher and Yates, 1968).

List 1. Treatment detail

S.N.	Treatment	Treatment detail (isolates with place)
1	T_1	Seed primed with *Trichoderma harzianum*(KN)
2	T_2	Seed primed with *Trichoderma harzianum* (VAN)
3	T_3	Seed primed with *Trichoderma harzianum* (UNN)
4	T_4	Seed primed with *Trichoderma viride* (FBD)
5	T_5	Seed primed with *Trichoderma harzianum* (KD)
6	T_6	Seed primed with *Trichoderma viride* (BDA)
7	T_7 (control)	Untreated control

KN = Kanpur Nagar, VAN= Varanasi, UNN= Unnao, FBD= Farrukhabad, KD=Kanpur Dehat and BDA= Banda

3. RESULTS AND DISCUSSION

3.1 Isolation of Pathogen

The isolation of pathogen was made on potato dextrose agar medium from infected chickpea plants which showed typical symptoms of wilt. The pathogen (*Fusarium oxysporum* f. sp. *ciceri*) was found to be associated with the infected roots of the chickpea plant samples collected from the field. The pathogenic culture of *F. oxysporum* f. sp. *ciceri* produced white colour mycelium with fluffy growth and smooth margin, yellow to dusky red (pink) colour pigmentation was observed on third day after isolation on PDA. On the basis of morphological characters, the pathogen was identified as *F. oxysporum* f. sp. *ciceri*. Pure culture was obtained by sub-culturing on PDA slants by hyphal tip method and incubated at 25±1^{0}C temperature in BOD incubator for a week and then stored at 4^{0}C temperature in refrigerator and the culture was maintained on PDA slants for further studies.

3.1.1 Evaluation of different isolates of *Trichoderma* against *Fusarium oxysporum* f. sp. *ciceri* through dual culture technique

Trichoderma species were evaluated through dual culture technique for their antagonistic potential against *Fusarium oxysporum* f. sp. *ciceri* (*Foc*) under *in vitro* conditions by adopting dual culture technique. The results are interpreted in terms of per cent inhibition over the fungal growth of control and presented in Table 1. Among the six isolates of *Trichoderma* spp., T_1 [*T. harzianum* (KN)] showed maximum inhibition (70.80%) in the mycelial growth of pathogen followed by T_2 *[T. harzianum* (VAN)] which inhibited 68.80% mycelial growth of pathogen. The next effective antagonist was T_3 [*T. harzianum* (UNN)], which inhibited 66.45% mycelial growth of pathogen followed by T_4 [*T. viride* (FBD) 63.58%]and T_6 [*T. viride* (BDA)](59.29%) in order to superiority.

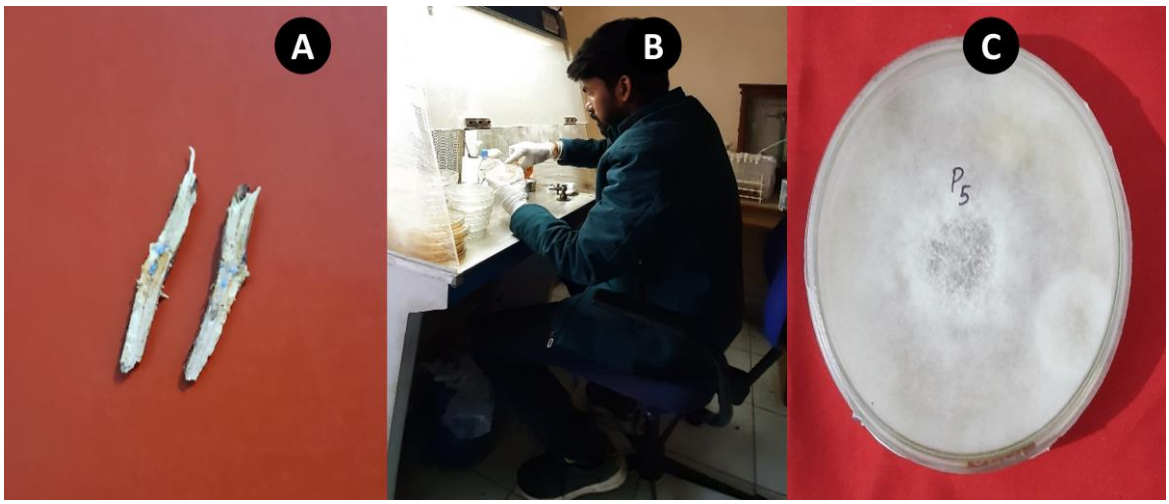

Plate 1. Isolation and purification process A-collection of disease plant sample, B-Isolation of fungus in aseptic condition of Laminar Air Flow and C-Pure culture of pathogen

Table 1. Evaluation of different isolates of *Trichoderma* against *Fusarium oxysporum* f. sp. *Cicero*

Sl. No.	Treatment details	Radial growth of *Foc* (mm)	Percent inhibition
1.	T_1 [*T. harzianum* (KN)]	19.20	70.80
2.	T_2 [*T. harzianum* (VAN)]	21.10	68.80
3.	T_3 [*T. harzianum* (UNN)]	23.55	66.45
4.	T_4 [*T. viride* (FBD)]	26.42	63.58
5.	T_5 [*T. harzianum* (KD)]	36.25	53.75
6.	T_6 [*T. viride* (BDA)]	30.71	59.29
7.	T_7 [Control]	90	
C.D.			4.54

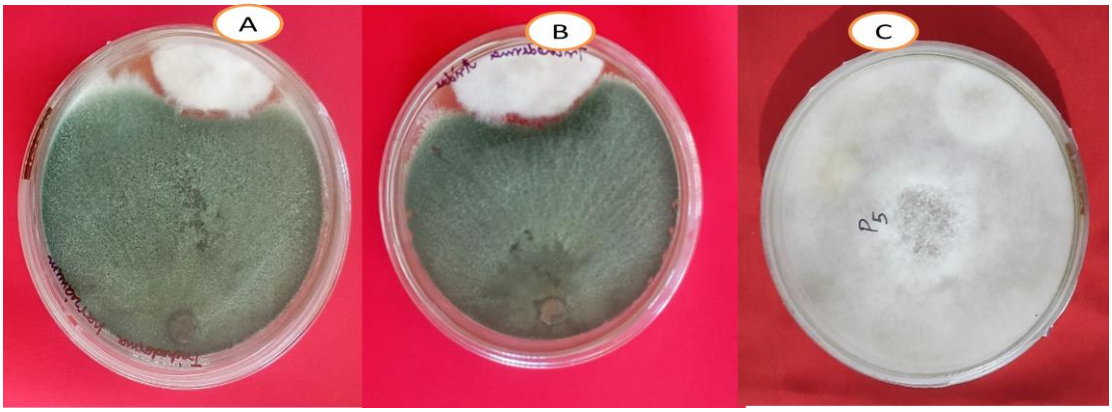

Plate 2. Antagonistic effect of *Trichoderma* Spp. against pathogen
T_1 [T. harzianum (KN)], B= T_2[T. harzianum (VAN)] and C= Fusarium oxysporum f. sp. Cicero

3.1.2 Effect of seed bio-priming with different isolates of *Trichoderma* spp. on seed germination and disease incidence of chickpea under wire house condition

The data summarized in the Table-2 demonstrated that every treatment was capable of substantially boosting the germination percentage of chickpea seed over control. The treatment T_1 which was treated with *Trichoderma harzianum* (KN), seeds were showed that the highest germination percent among all the treatments, with a value of 85.71%, followed by T_2 and T_3 representing 76.19% and 76.19% seed germination respectively. Lowest germination % was recorded from control about 47.61% only in T_7 so, all treated pots significantly increased germination % over control.The highest incidence was recorded from untreated (T_7) pot about 52.38 per cent. While, minimum disease incidence was recorded from T_1 which treated by *T. harzianum* (KN) with the value about 14.28 per cent, Second best result observed from T_2 which, treated with *T. harzianum* (VAN) with the value of 19.04 per cent.

Plate 3. Effects of seed bio-priming on growth parameters of chickpea
A= T_1Trichoderma harzianum (KN), B=T_2Trichoderma harzianum(VAN), C=T_3Trichoderma harzianum(UNN), D=T_4Trichoderma viride(FBD), E=T_5Trichoderma harzianum(KD), E=T_6Trichoderma harzianum(BDA) and F=T_7 Untreated Control

Table 2. Effect of seed bio-priming with different isolates of *Trichoderma* spp. on seed germinationand disease incidence of chickpea

Treatment details			Total No. of plants	No. of diseased plants	Germination (%)	Disease incidence (%)
Sl. No.	Treatment	Bioagents with location				
1.	T_1	*Trichoderma harzianum* (KN)	21	3	85.71	14.28
2.	T_2	*Trichoderma harzianum* (VAN)	21	4	76.19	19.04
3.	T_3	*Trichoderma harzianum* (UNN)	21	6	76.19	28.57
4.	T_4	*Trichoderma viride* (FBD)	21	7	71.42	33.33
5.	T_5	*Trichoderma harzianum* (KD)	21	8	66.66	38.09
6.	T_6	*Trichoderma viride* (BDA)	21	8	57.14	38.09
7.	T_7	**Control**	21	11	47.61	52.38
C.D.					3.268	1.648

3.1.3 Effect of seed bio-priming with different bioagent on morphological attributes of chickpea under wire house condition

The data represented in Table 3, showed that, every treatment was capable of substantially boosting the numbers of chickpea branches against control. In all the treatment, the maximum number of branches were reported from T_1 with value of 12, followed by T_2 and T_3 representing 10 and 10, respectively. The others remain of the treatments were also able to increase number of branches over control.All treated plots recorded higher root length over non treated plants. The maximum root length was recorded from T_1 treatment with the value of 13.33 cm with increase 81.85% over control. The treatment T_2 showed second best result about 12.0 cm with 63.71% increased over control. From the table it is cleared that seed treated with different isolates of *Trichoderma* spp. increased morphological root length against over control. Every treatment was capable to boosting of the chickpea shoot length over control. Among all the treatments, the maximum numbers of branches were observed from T_1 which treated with *Trichoderma harzianum* (KN) value of 37 cm with increase 94.73% over control. The treatment T_2 showed second best resulted value of 35 cm with 84.21% increased over control.

Plate 4. Control and treatment efficacy results
A=Untreated Control, B=T1Trichoderma harzianum (KN), C= All Treatments

Table 3. Effect of seed bio-priming on morphological attributes of chickpea

Treatment details	No. of branches/ plants	Root length (cm)	Per cent increase over control (Root length)	Shoot length (cm)	Per cent increase over control (Shoot length)
T_1 [*T. harzianum* (KN)]	12	13.33	81.85	37	94.73
T_2 [*T. harzianum* (VAN)]	10	12.00	63.71	35	84.21
T_3 [*T. harzianum* (UNN)]	10	11.00	50.06	33	73.68
T_4 [*T. viride* (FBD)]	8.3	9.00	22.78	30	57.89
T_5 [*T. harzianum* (KD)]	8.0	9.00	22.78	29	52.63
T_6 [*T. viride* (BDA)]	8	8.67	18.28	24	26.31
T_7 [Control]	7	7.33		19	
C.D.	0.418			1.269	

Table 4. Effect of seed bio-priming with different isolates of *Trichoderma spp.* on yield attributes in chickpea under wire house condition

Treatment details	Yield attributes		
Seed priming with bioagent	**No. of pods/plant**	**Seed index (gm) (Weight of 100 seeds)**	**% increase over control**
T_1	41	25.5	50.00
T_2	37	23.25	36.76
T_3	36	22.75	29.41
T_4	35	22.05	29.70
T_5	33	21.50	26.47
T_6	31	20.03	17.82
T_7 (Control)	22	17.00	
C.D.	1.481	0.627	3.328

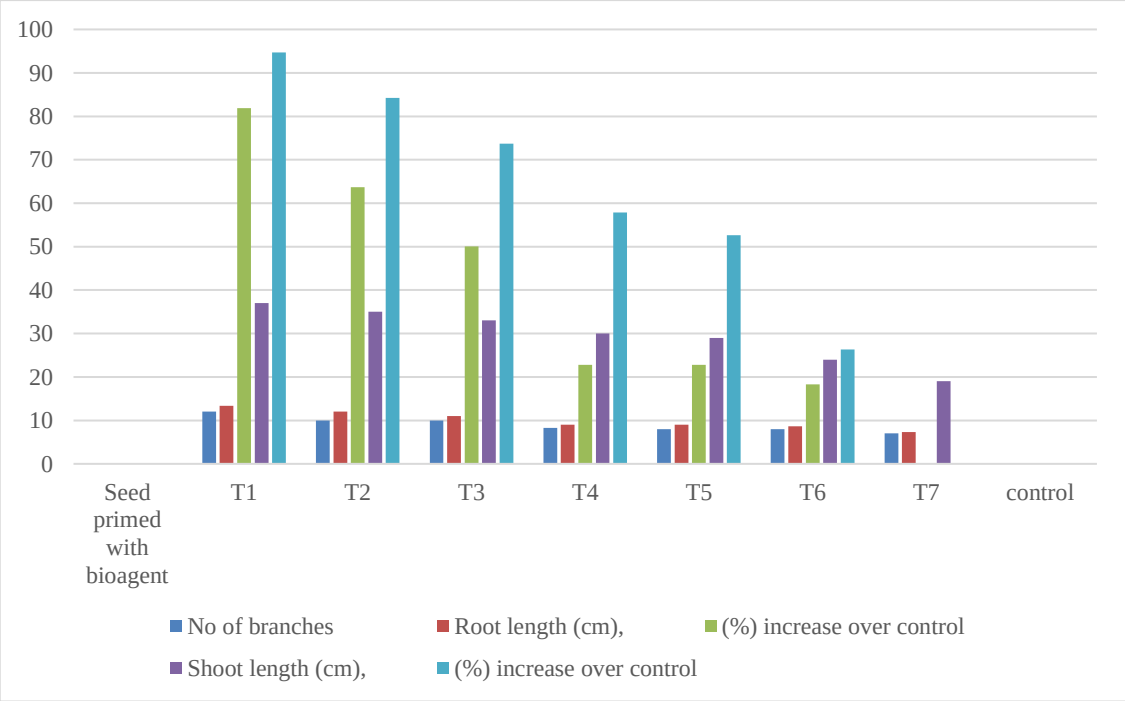

Fig. 1. Effects of seed bio-priming on growth parameters of chickpea

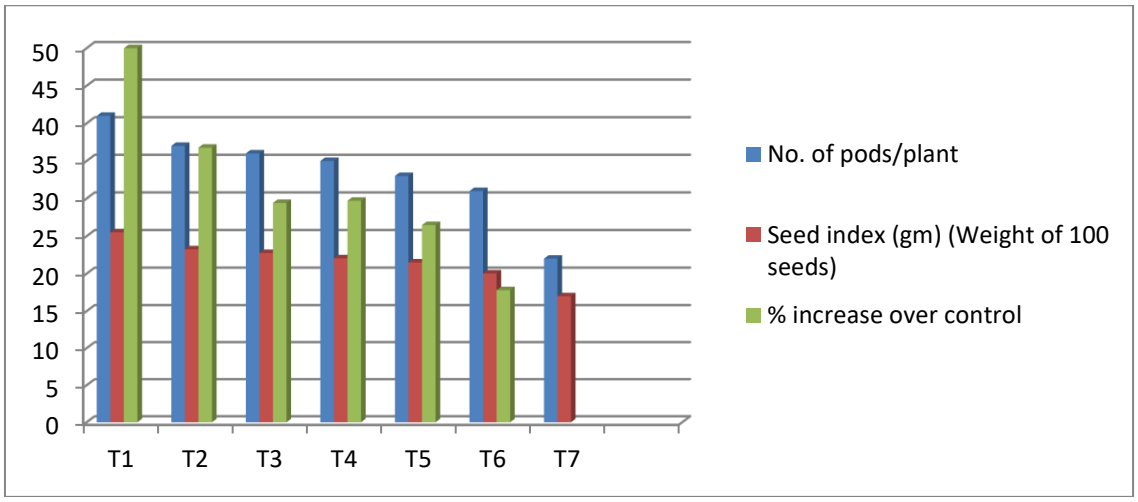

Fig. 2. Effects of seed bio-priming on yield attributes in chickpea

3.1.4 Effect of seed bio-priming on yield attributes in chickpea under wire house condition

The data presented in Table 4 founded that, all treatments were substantially increasing the chickpea yields over control. Among all the treatments, the maximum number of pods 41 was observed from T_1 which increased 50% over control with 25.5 gm seed index. The treatment T_2 was showed second best result as 23.25 gm with increased of 36.76% with 23.25 gm seed index, while non treated plant, harvested the number of pod/plant of 22 pod/plant. It may be concluded from the table that all treated plant harvests more tield than non treated plant.

4. CONCLUSION

In vivo results demonstrated that Trichoderma isolates inhibited pathogen mycelial growth significantly. *T. harzianum* (KN) was the most effective, with 70.80% inhibition, followed by *T. harzianum* (VAN) at 68.80%. *T. harzianum* (UNN), *T. viride* (FBD), and *T. viride* (BDA) also showed inhibition. Treatments enhanced Chickpea seedling germination percentage significantly compared to the control group. T_1 with *Trichoderma harzianum* (KN) had the highest germination percentage at 75%, followed by *T. harzianum* (VAN) and *T. harzianum* (UNN) at 76.19%. Control had the lowest germination at 47.61%, indicating a positive impact of all treatments. The highest disease incidence, 52.38%, was in untreated pots. The lowest, 14.28%, was in T_1 treated with *T. harzianum* (KN), followed by T_2 treated with *T. harzianum* (VAN) at 19.04%. Maximum branches were in T_1 *T. harzianum* KN-treated pots (12), followed by T_2 and T_3 (both 10). KN-treated plants had the most branches (37 cm, 94.73% increase), followed by VAN (35 cm, 84.21%) and UNN (33 cm, 73.68%). T_2 had 35 cm (84.21% increase), while untreated had 19 cm. All treatments increased root length; the highest was T_1 (13.33 cm, 81.85%), then T_2 (12.0 cm, 63.71%). Trichoderma-treated seeds increased root length. T_1 had 41 pods/plant (50% increase, 25.5 gm seed index), T_2 had 37 pods/plant (36.76% increase, 23.25 gm), and untreated had 22 pods/plant. Overall, treated plants yielded more than untreated plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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