



Effect of Seed Biopriming with *Trichoderma viride* based A Bio-formulation for Management of Chickpea Wilt

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ABSTRACT

Background: Chickpea is an important legume crop, which is susceptible to various biotic stresses. Wilt disease severely affect chickpea crop and cause substantial yield reduction. *Trichoderma* species is a prominent bio-control agent, which is applied against soil borne pathogens. *Trichoderma* spp. is available in the market in different formulations. The present study investigated the efficacy of a novel *Trichoderma viride* based seed biopriming method against wilt causing pathogen (*Fusarium oxysporum* f. sp. *ciceri*) in chickpea crop.

Methods: The experiment was conducted *in-vitro* and *in-vivo* for two years to assess the effect of different doses of *T.viride* based bioformulation on chickpea seed quality parameters, plant survival against wilt pathogen and chickpea seed yield.

Result: Significantly chickpea plants survived (68.71%) against the impact of wilt pathogen when seeds were bioprimed with *T. viride* bio-formulation @ 5 g/kg seeds. In contrast, only 51.78% chickpea plants survived from untreated seeds. Comparatively high chickpea yield (7.23 q/ha) was produced from bioprimed seeds than untreated seeds (4.65 q/ha). Bio-formation of *T. viride* (@ 5 g/kg seeds-based seed biopriming is an eco-friendly method to overcome notorious wilt pathogen that lessen the disease incidence and improves the seed quality traits and maximizes the seed yield.

Key words: Bio-formulation, Biopriming, Chickpea, *Trichoderma viride*, Wilt.

INTRODUCTION

Chickpea, scientifically known as *Cicer arietinum* L., holds significant importance as a leguminous crop of *rabi* season (Singh *et al.*, 2024), thriving in a variety of soils and agro-climatic conditions. It encompasses approximately 38% of the pulses cultivation area and contributes to about 50% of India's total pulse production. India leads globally in both production and acreage, followed by Australia. Major chickpea-producing states in India are Madhya Pradesh, Rajasthan, Uttar Pradesh, Haryana, Maharashtra and Punjab. Chickpea cultivation in India spans approximately 9.60 million hectares, yielding an annual grain production of 8.83 million tonnes, with an average yield of 920 kg/ha (FAO STAT, 2013). Chickpeas are high in fiber and contain varying levels of vitamins, minerals and bioactive compounds, which may lower the risk of diseases like diabetes and cardiovascular issues (Gupta *et al.*, 2019). According to Sahu *et al.* (2022), the crude protein content in desi and kabuli chickpeas ranges from 18.2% (Dollar variety) to 26.7% (JG315), while total phenolic content varies from 0.74 to 1.22 mg/g. The total flavonoid content is with an average of 0.47 mg/g. Chickpea wilt, caused by *Fusarium oxysporum* f. sp. *ciceris*, stands as a major biotic constraint in chickpea production (Khanna *et al.*, 2024) and widespread affliction of chickpea across varied agro-climatic conditions. Failure to effectively manage this pathogen can result in yield losses ranging from 80 to 100%. This parasitic organism proliferates extensively affecting various parts of the infected plant, including seeds; leading to significant economic losses in chickpea cultivation (Dhawale *et al.*, 2021).

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Seed biopriming represents a holistic strategy towards agricultural sustainability. It facilitates the swift colonization of seeds by beneficial organisms and ensures a more consistent coverage of the seed surface compared to alternative methods. Additionally, it proves highly efficient in combating numerous diseases caused by both seed

and soil-borne pathogens (Sabalpara, 2015). *Trichoderma* spp. are beneficial microorganisms that enhance soil health and crop development (Awad-Allah *et al.*, 2023). The effectiveness of *Trichoderma* species has been significantly impacted by various physiological parameters like pH, moisture, temperature and nutrients. Temperature plays key role in determining the effectiveness of *Trichoderma* against phytopathogens. *Trichoderma* has been thoroughly researched over the past 55 years, with no documented adverse effects on humans or livestock. Keeping this in view, experiments were conducted involving the seed biopriming of chickpea seeds with *Trichoderma viride*-based bio-formulation to manage the negative impacts of wilt fungus on chickpea yield.

MATERIALS AND METHODS

Collection of diseased samples

Chickpea plants, both healthy and diseased, showing typical wilt symptoms, were collected from Nawabganj Research Farm, C.S. Azad University of Agriculture and Technology, Kanpur and brought to the laboratory for isolation of pathogen.

Isolation and purification of pathogen

The chickpea roots affected by wilt were initially rinsed in tap water to eliminate dust particles. The affected root

pieces were then sterilized in a 0.1 per cent solution of mercuric chloride for one minute, followed by thorough washing with distilled water 3-4 times. Excess water was removed by placing the root pieces between sterilized blotting papers. The PDA (Potato Dextrose Agar) medium, used for isolating the pathogen, had the following composition: 200 g peeled potato, 20 g dextrose, 20 g agar and 1000 ml distilled water, prepared according to the method described by Johnston and Booth (1983). These pieces were then inoculated on PDA plates and placed in an incubator at $25\pm^{\circ}\text{C}$ for 24 hours. Single spore isolation technique was used to purify the culture and the obtained culture was maintained on potato dextrose agar medium slants for further investigation.

Identification of pathogen

The pathogen was identified by examining its cultural and morphological traits, comparing them with the characteristics described by Booth (1971) for *Fusarium oxysporum* f. sp. *ciceri* given in Fig 1. The growth pattern, cultural attributes and morphological features were analyzed on PDA to determine its identity. Growth habit characters of the fungus on seeds in Standard blotter method and cultural and morphological characters of the colony on potato dextrose agar medium in agar plate method (ISTA, 1991) were observed. The morphological characters include mycelium colour, branching pattern, septation and width. The size of

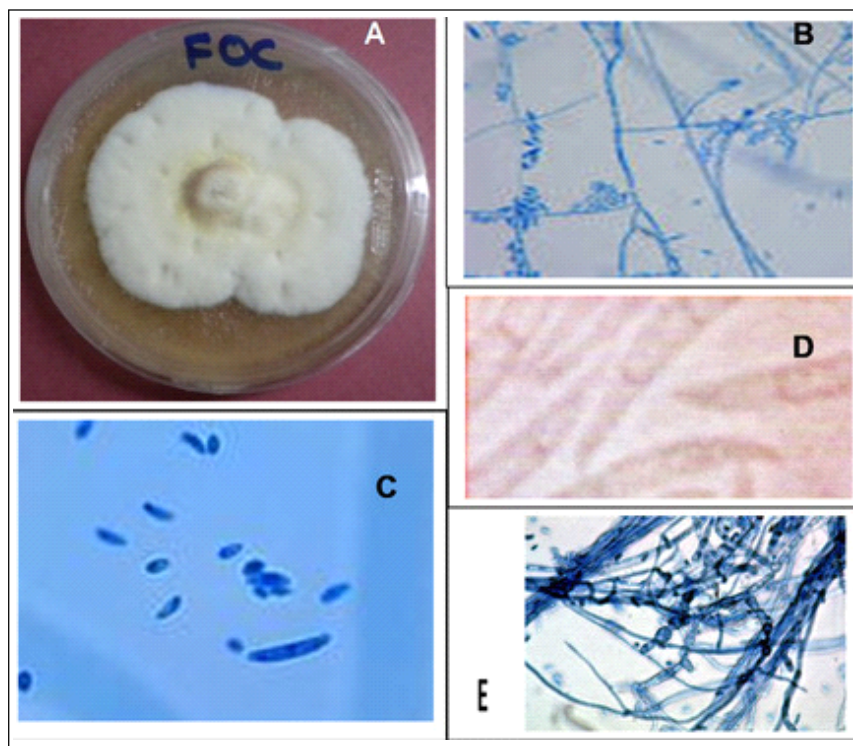


Fig 1: A.) Pathogen (*Fusarium oxysporum* f.sp. *ciceri*) colony growth, B.) Mycelium, C.) Microconidia D.) Macroconidia, E.) Chlamydospores.

the hyphae, micro conidia, macro conidia and chlamydospores was based on the measurement. The nomenclature of the colour was based on the colour standards given by Ridway (1912).

Sample collection, isolation and purification of *Trichoderma viride*

Soil samples were collected from the rhizospheric soils of chickpea fields. These samples were carefully placed in designated polythene bags, clearly labeled with collection date and transported to the laboratory for further analysis. *Trichoderma* selective medium (TSM) containing specific ingredients such as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, K_2HPO_4 , KCl, NH_4NO_3 , Glucose, Chloramphenicol, Apron 35SD, Captan, Rose Bengal, Agar-agar and distilled water was used to isolate *Trichoderma*, this method was described by Elad *et al.* (1981). The soil samples were processed using the serial dilution plate technique as outlined by Johnson and Crul (1972) and the plates were then placed in incubation at $28 \pm 1^\circ\text{C}$ for 5 days. Colonies were observed and recorded between the 3rd and 5th days and individual colonies were selected for further investigation. Purification of *Trichoderma* isolates was accomplished using the single spore isolation method, where sub-culturing was carried out from the growing end of a new colony. A small amount of spores from each isolate was aseptically streaked onto potato dextrose agar (PDA) plates by using a sterilized inoculating needle.

Morphological characterization of *Trichoderma* spp

Isolated *Trichoderma* cultures were grown on PDA media for seven days. Colonies exhibit distinctive features such as growth pattern, rate of growth, odor and coloration, which are indicative of their classification as *Trichoderma* (Gams and Bissett, 1998), were recorded.

Microscopic features

The common methods are applied for species identification typically involves using of a light microscope to examine morphological traits. These traits include the branching pattern of conidiophores, the elongation and shape of conidiophores (whether coiled, straight, or undulate), the morphology and dimensions of phialides and the shape of conidia (Bissett, 1991; Rifai, 1969; Samuels *et al.*, 1996) are given in Fig 2.

In vitro screening of *T. viride* isolates against the chickpea wilt causing pathogen

Trichoderma viride isolates were evaluated for its effectiveness against *Fusarium oxysporum* f. sp. *ciceri* using the dual culture technique. Both the target fungus and the fungal antagonist were cultured on Potato Dextrose Agar (PDA) medium. Bits of 5 mm diameter were cut from the periphery of seven days old cultures of the pathogen and the antagonistic fungi (*T. viride*), then placed diametrically opposite each other at a 5 mm distance from the edge of the Petri dish. Each Petri dish was inoculated with a target pathogen, with one plate serving as a control without an antagonist. They were then incubated at $28 \pm 2^\circ\text{C}$ in a BOD incubator. After 7 days, the presence of inhibition zones between *T. viride* and the pathogen was observed. The radial growth of the pathogens on both the dual culture and control plates was measured and the percentage inhibition of the pathogen was calculated according to the method described by Vincent and Budge (1990).

In vivo effect of seed biopriming with different doses of *Trichoderma viride*

The field experiment was carried out during *rabi* season 2013-14 and 2014-15 over two consecutive years, at Nawabganj Research Farm, C.S. Azad University of

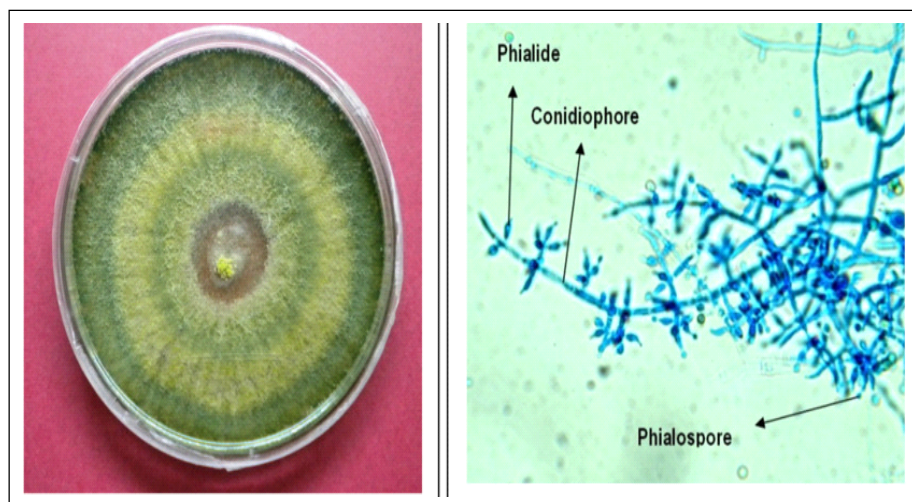


Fig 2: A.) *T. viride* colony growth on PDA medium B.) Conidia under light microscope at 40x.

Agriculture and Technology, Kanpur was aiming to address chickpea wilt management through the biopriming of chickpea seeds using various doses of a bio-formulation containing *Trichoderma viride*. The bioformulations were prepared from isolates of *T. viride* 03 which were found superior during *in vitro* condition, screening against *Fusarium oxysporum* f. sp. *ciceri*. Fungal biomass of *T. viride* 03 isolates were mixed with talcum powder @ 1:2 ratios. This experiment had 7 treatments. Prior to sowing, chickpea seeds underwent soaking (biopriming) in four different concentrations of *T. viride* namely 5, 10, 15, 20 g/kg seeds that acted as four treatments for the experiment, fifth treatment involved application of *T. viride* in the soil furrows at the rate of 2.5 kg/h. In another treatment, seed biopriming was carried out with 0.2% Bavistin per kg of seed while untreated seeds served as the control treatment. Soil was inoculated with *Fusarium oxysporum* f. sp. *ciceri* @ of 5.0 g/m², mixed into the soil before sowing. The experiment involved the following treatment combinations as explained here:

- T₁: Seed biopriming with 5 g of *T. viride* formulation per kg of seed.
 T₂: Seed biopriming with 10 g of *T. viride* formulation per kg of seed.
 T₃: Seed biopriming with 15 g of *T. viride* formulation per kg of seed.
 T₄: Seed biopriming with 20 g of *T. viride* formulation per kg of seed.
 T₅: Soil application of *T. viride* formulation in furrows at a rate of 2.5 kg/h.
 T₆: Seed biopriming with 0.2% Bavistin per kg of seed.
 T₇: Control (without treatment).

Experimental details

- Design : R.B.D.
- Soil : Sandy loam
- Bioagent : *Trichoderma viride*
- Replication : 03
- Treatments : 07
- Variety : Uday
- Plot size : 8 m × 3 m
- Row to row distance : 30 cm
- Plant to plant distance : 25 cm
- Treatment distance : 50 cm
- Replication distance : 1 m

Observations recorded

Following observations such as seed germination%, plants survival%, yield/ha, test weight in g, root length of plant in cm, shoot length of plant in cm were recorded as shown in Fig 3.

Statistical analysis

The data obtained from experiment were analyzed statistically by analysis of variance (ANOVA) according to randomized block design (RBD). The treatments means were subjected to Fisher's least square difference (LSD) post hoc test, which compared treatment means with critical differences (CD) at 5% level of significance as computed by OPSTAT software.

RESULTS AND DISCUSSION

In vitro screening of *T. viride* isolates against chickpea wilt pathogen by dual culture method

The antagonistic effect of different isolates of *Trichoderma viride* against *Fusarium oxysporum* f.sp. *ciceri* (Foc) was assessed through *in vitro* study. The results are summarized in Table 1. The results indicate that all three isolates of *T. viride* exhibited significant antagonistic effects against *Fusarium oxysporum* f. sp. *ciceri*, as evidenced by the reduction in radial growth of the pathogen. The isolate *T. viride* 03 was the most effective, showing the highest inhibition percentage of 61.42%, followed by *T. viride* 01 with 60.00% and *T. viride* 02 with 58.57%. In contrast, the control treatment showed no inhibition of radial growth, with the fungus reaching a full radial growth of 70.0 mm. Similar finding supported with Srivastava *et al.* (2012) evaluated the antagonistic capabilities of different *Trichoderma* species against *F. oxysporum* f. sp. *ciceri*. The highest inhibition of mycelial growth was 62.00% observed in *Fusarium oxysporum* f. sp. *ciceri*. Singh *et al.* (2013) explored the antagonistic effects of *Trichoderma viride* on mycelial proliferation against *Fusarium oxysporum* f. sp. *ciceri*, with maximum inhibition of mycelial growth at 59.25%. These findings suggest that *T. viride* isolates could be potent biocontrol agents against *Fusarium oxysporum* f.sp. *ciceri*, offering an eco-friendly alternative to chemical fungicides.

In vivo effect of seed biopriming with different doses of *T. viride* on growth parameters of chickpea

Under field condition the effect of seed biopriming with different doses of *Trichoderma viride* isolate 03 which was most effective under lab condition was evaluated for the growth parameters of chickpea over two consecutive years. The parameters assessed include germination percentage, root length and shoot length of the plants. The results are presented in Table 2. The data indicate a significant influence of *T. viride* on growth parameters like, germination rate, root length and shoot length of chickpea. Treatment T1 (*T. viride* @ 5 g/kg seeds) exhibited the highest mean germination percentage (70.99%), root length (5.75 cm) and shoot length (34.65 cm) over the two years. In contrast, T7 (Control-untreated seeds) showed the lowest values for all parameters. Similar finding supported

Table 1: *In vitro* antagonistic effect of *T. viride* isolates against *Fusarium oxysporum* f. sp. *ciceri*.

Treatment	<i>Fusarium oxysporum</i> f.sp. <i>ciceri</i>	
	Radial growth (mm)	Inhibition per cent (%)
<i>T. viride</i> 01+ <i>Foc</i>	28.0	60.00
<i>T. viride</i> 02+ <i>Foc</i>	29.0	58.57
<i>T. viride</i> 03+ <i>Foc</i>	27.0	61.42
Control (<i>Foc</i>)	70.0	00
CD@5%	4.861	

with Parmar and Gohel (2024) that observed seed biopriming had a beneficial effect on promoting robust shoot and root development, as well as disease control, in laboratory conditions and in field trials spanning two years, seed biopriming for 10 hours using a suspension of talc-based *Trichoderma viride* or *T. asperellum* (at a concentration of 2×10^8 CFU/g) at a rate of 50 g per 250 ml of water per kilogram of seed, followed by soil application of *T. viride* or *T. asperellum* enriched farmyard manure (10 g/kg FYM) at 100 g/m² of soil, proved to be significantly effective for disease management and increased yield. According to Rehman *et al.* (2013) treating seeds with *T. viride* and *T. harzianum* led to a notable decrease in wilt occurrence and a notable increase in seed germination compared to untreated seeds. Treating seeds with *Trichoderma viride* specifically resulted in significant improvements in various plant growth factors, including germination (89.99%), dry

weight (30.6 g), root length (36.2 cm) and grain yield (17.36 q). On the other hand, treating seeds solely with carbendazim significantly decreased wilt incidence and seed germination (78.88%) and had a negative impact on plant growth parameters compared to the untreated control.

***In vivo* effect of seed biopriming with different doses of *T. viride* on plant survivability and yield attributes of chickpea**

In field condition the effect of seed biopriming with different doses of *Trichoderma viride* isolate 03, on plant survivability and yield attributes of chickpea was assessed over two years. The parameters evaluated including plant survival percentage, test weight (g) and yield per hectare (q). The results are presented in Table 3. The data clearly showed the impact of seed biopriming with *T. viride* on chickpea plant survivability and yield attributes. Treatment T1



Fig 3: Research trial of chickpea at Nawabganj Farm Kanpur.

Table 2: *In vivo* effect of seed biopriming with different doses of *T. viride* on growth parameter of chickpea.

Treatment	Germination %			Root length of plant (cm)			Shoot length of plant (cm)		
	Years		Mean	Years		Mean	Years		Mean
	I	II		I	II		I	II	
T ₁	71.67	70.3	70.99	5.5	6.0	5.75	34.0	35.3	34.65
T ₂	65.67	69.0	67.34	5.2	5.5	5.35	32.5	30.8	31.81
T ₃	63.50	68.6	66.05	5.0	5.2	5.10	33.5	31.0	32.25
T ₄	62.00	68.3	65.15	4.8	5.0	4.85	32.0	32.0	32.00
T ₅	59.00	69.6	64.3	4.6	4.8	4.70	31.5	30.1	30.80
T ₆	56.00	69.0	62.5	4.0	4.3	4.15	30.0	30.2	30.10
T ₇	51.67	61.6	56.64	3.5	4.0	3.75	29.0	28.16	28.58
CD @5%	4.184	2.046	2.143	0.613	0.669	0.413	2.178	1.309	1.170

Table 3: *In vivo* effect of seed biopriming different doses of *T. viride* on plant survivability and yield attributes of chickpea.

Treatment	Plant survival %			Test wt. (g)			Yield/ha (q)		
	Years		Mean	Years		Mean	Years		Mean
	I	II		I	II		I	II	
T ₁	69.49	67.93	68.71	260.0	267.0	263.50	5.13	9.33	7.23
T ₂	63.29	66.59	64.94	258.0	266.0	262.00	4.76	7.59	6.18
T ₃	60.71	66.33	63.46	257.5	264.5	261.00	4.65	6.52	5.59
T ₄	58.81	66.47	62.64	256.0	263.0	259.50	4.56	6.38	5.47
T ₅	55.64	67.35	61.5	255.0	261.5	258.25	4.44	6.44	5.44
T ₆	52.09	66.44	59.26	253.5	260.5	257.00	4.15	6.25	5.20
T ₇	45.34	58.23	51.78	252.0	258.0	255.00	3.86	5.44	4.65
CD @ 5%	4.869	4.631	3.055	3.644	4.086	2.486	0.552	1.088	0.557

(*T. viride* @ 5 g/kg seeds) recorded the highest mean plant survival percentage (68.71%), test weight (263.50 g) and yield per hectare (7.23 q). On the other hand, T₇ (without treatment) had the lowest values for these parameters. The critical difference at 5% indicates significant differences between the treatments, particularly in terms of plant survival, test weight and yield per hectare. According to Nikam *et al.* (2007) the application of Thiram (0.15%) + Carbendazim (0.1%) as a chemical treatment on seeds has been found to be highly effective in combatting *Fusarium oxysporium* f. sp. *ciceri*. Additionally, findings from pot culture experiments indicated that the soil application of *T. viride* at a rate of 2.5 kg/ha demonstrated significant efficacy in minimizing the occurrence of chickpea wilt. Pradhan *et al.* (2022) highlighted the importance of selecting a high-quality formulation in *Trichoderma viride*-based biocontrol strategies against *Fusarium* wilt disease in chickpea. They assessed two types of formulations- powder for seed treatment (TvP) and tablets for direct application (TvT)-containing *T. viride* as the biocontrol agent, at three different dosages: recommended (RD), double the recommended (DD) and half the recommended (1/2 RD). Field evaluations revealed that the TvP formulation exhibited superior efficacy over the TvT formulation in terms of both seed germination and wilt incidence control

in chickpea. Moreover, both the TvP and TvT formulations demonstrated higher bio-efficacy compared to the synthetic fungicide (Carbendazim 50% WP) and conventional talc-based formulation.

CONCLUSION

It is concluded that among the seven treatments tested *in vivo*, including the control, through seed biopriming of *T. viride* isolate03 based bio-formulation, T₁ treatment (*T. viride* @ 5 g/kg seeds) followed by T₂ treatment (*T. viride* @ 10 g/kg seeds) showed superiority in all mentioned seed quality parameters compared to the control (T₇, untreated). The combined results from two years indicated significant improvements in maximum germination, root length, shoot length and plant survivability for disease management and higher yield of chickpea. These treatments, especially T₁, are recommended for enhancing yield, even under challenging conditions, thus aiding in the management of *Fusarium* wilt of chickpea.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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