International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(6): 391-396
www.biochemjournal.com
Received: 02-03-2024
Accepted: 07-04-2024

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Studies on biological management of chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri*

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DOI: https://doi.org/10.33545/26174693.2024.v8.i6e.1342

Abstract
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. Now-a-days several biotic and abiotic stresses cause harm in Chickpea production. Among of them, Fusarium wilt of chick pea is a serious problem in India. In the sense of biological management of Fusarium wilt of chickpea through *Trichoderma* isolates are significantly reduces the fungal pathogen population in the oil.

In order to manage fusarium, wilt the present investigation was carried out on Department of Plant Pathology, Faculty of Agriculture Sciences and Allied Industries, Rama University, Kanpur, UP. (India). In all the treatment, substantially boosting the morphology of chickpea against control, the highest number of branches were highest studies from T_1, with value of 13. The highest root length was found in T_1 [*Trichoderma harzianum* (KN)] treatment with value the representing 14.33 cm with increase 93.12% over the control. Incase of yield parameters highest number of pods was also found in T_1 [*Trichoderma harzianum* (KN)] representing 42 followed by T_2 [*Trichoderma harzianum* (VAN)] and T_3 [*Trichoderma harzianum* (UNN)] with the value about 36 and 37 respectively. All treated plants subsequently significantly increased germination percentage, morphological parameters and yield parameters over the non-treated plants.

Keywords: Biological management, chickpea wilt, *Fusarium oxysporum* f. sp. *ciceri*

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 vital role in human diets, particularly among the vast majority of vegetarians in the nation. Among the countries that produce the most chickpeas worldwide are India, Pakistan, Turkey, Iran, Myanmar, Ethiopia, Mexico, Australia, Syria, Spain, Canada, the United States, Bangladesh, Algeria, Malawi, Sudan, and Portugal. Ethiopia is the secondary origin of the chickpea, which has its primary origins in South-West Asia and the Mediterranean region. In tropical, subtropical, and temperate regions of the world, it is a prominent pulse crop (Yadav *et al.*, 2023) [15]. It is widely grown in semi-arid regions of India and goes by many names, such as Chana, Chole, Cholla, and Boot, among others (Saxena and Johansen, 1997) [10]. It is a very good source of vitamins (B1, B2 and Niacin) as well as minerals (Calcium 282 mg/100 g, Phosphorus 300 mg/100 g and Iron 7 mg /100 g). Compared to animal sources of protein, chickpeas are a cheap source of protein. Compared too the grain legumes, its proteins have a better nutritional value (Gupta and Kapoor, 1980) [3].

Since chickpea proteins contain a unique combination of amino acids, they are also easily digestible. In addition to being plentiful in minerals, vitamins and carbohydrates, grains also contain significant levels of important amino acids like cysteine, methionine and tryptophan (Singh *et al.*, 1991) [11].

Among the fungi that cause disease Serious harm results from chickpea fusarium wilt, which is brought on by *Fusarium oxysporum* f. sp. *ciceri.* Globally, fusarium wilt causes significant economic losses of between 10 and 40 percent (Nene *et al.*, 1984) [6].

Under certain circumstances (Jalali and Chand, 1992) [4] and during specific crop growth phases (vegetative and reproductive), it results in a 100% loss. The disease is most significant, pervasive, and devastating globally (Sontakke *et al*., 2020) [12].

Fusarium wilt is primarily soil-borne, controlling it is challenging and no single control method is 100% effective. Fusarium wilt, a disease that affects chickpeas, is a monocyclic condition where the pathogen's primary inoculums drive its development (Zeyad *et al.,* 2022) [16].

According to Chaudhry *et al*., (2007) [1], wilt incidence is directly related to weather parameters because chickpea crops are more susceptible during the flowering and pod-forming stages. If the crop is subjected to rapid temperature rise and water stress, the wilt incidence level also fluctuates with weather parameter. In India, it is estimated that 10% yield losses were caused annually under certain conditions it may go up to 60%. According to studies by Dubey *et al*., (2010) [2] the frequency varied from 14 to 32% in different states. It may also cause up to 90% losses depending on weather conditions (Venkataramanamma *et al*., 2018) [14]. Crop wilt incidence ranges from 77–94% in the early stages, while chickpea wilt disease was responsible for 24-65% of the late wilting (Sontakke *et al*., 2020) [12].

An integrated disease management plant can readily incorporate biological control as a cost-effective and ecologically acceptable method of disease management. In fact, biological control with bacterial or fungal antagonists may improve the utilisation of natural resistance for the management of fungal infections in chickpeas. Fungal bio-agents, like non-pathogenic and non-host Fusarium species, have been effectively employed and have led to a notable decrease in the development of diseases in the field as well as *in vitro* pathogenic fungal growths.

The biological control of wilt through the application of Trichoderma bioagents is largely dependent on induced resistance caused by the accumulation of different phenolic compounds and phytoalexins, as well as the activation of peroxidases, polyphenol oxidases, and important enzymes in phenylpropanoid and is flavonoid pathways (Singh *et al.*, 2021) [11].

Several bioagents like *Trichoderma, Pseudomonas,* and *Bacillus* genera have been found in suppressive soils, which is the basis for the natural control of a number of phytopathogens. *Trichoderma* is one of the promising bio control agents that have been described as having potential for the biological control of soil-borne plant diseases (Sabalpara *et al.*, 2009) [9].

Materials and Methods

Collection of seeds

Cicer arietinum (L.) Krust. cultivar JG 62 chickpea seeds are a favoured chickpea variety that local farmers in Kanpur frequently utilises. The genotypes have shown to be vulnerable to Fusarium wilt. For conducting various experiments, the seed was obtained from the Vegetable Research Farm at the Faculty of Agricultural Sciences & Allied Industries, Rama University, Kanpur.

Sowing of seeds in pots

Pots were collected for the raising of gram seed. A 1% solution of sodium hypochlorite was used to surface sterilise gram seeds of the JG 62" cultivar for five minutes. The seeds were then three times rinsed with sterilised distilled water before being allowed to air dry. Pots are filled with compost mixed soil and then soils particles are crushed in very fine particles along with sprinkling of water then after that primed tomato seed is sown in the pot. Complete randomness was maintained in the experimental arrangement.

Collection of Diseased Material

The specimens were obtained from the student instruction farm of the Faculty of Agricultural Sciences & Allied Industries, Rama University, Kanpur. From the field, the infected chickpea plants exhibiting wilting-like symptoms followed by yellowing were gathered and delivered to the lab for initial analysis. For additional investigation, the specimens and diseased samples were placed between the folds of sterilized 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.

For pathogen isolation, the infected plant exhibiting typical symptoms was chosen. To remove dirt particles from the surface, the *stem* and *root* of the diseased plant were first carefully rinsed with running water. Then, a sterilized sharp knife was used to cut the diseased area of the *stem* into small pieces, each containing fragments of both diseased and healthy tissues. These pieces were carefully washed three times in distilled water after being submerged in a 0.1% *mercuric chloride* ($HgCl_2$) solution for 5 seconds, followed by three washes in distilled water with the help of sterilized forceps. Excess moisture was absorbed using sterilized blotting paper. The pieces were then placed on sterilized Petri plates containing *potato dextrose agar* (PDA) medium in the inoculation chamber. Each Petri plate contained two parts that were aseptically inserted before being incubated at room temperature (25±1 °C). Daily checks of the Petri plates were made to spot any *mycelial* development around the pieces.

Isolation and Purification of Pathogen

The pathogen was isolated from infected chickpea plants that exhibited characteristic wilt symptoms using PDA medium. The pathogen, *Fusarium oxysporum* f. sp. *ciceri*, was found 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-colored *mycelium* with fluffy growth and smooth margins. Yellow to dusky red (pink) pigmentation was observed on the third day after isolation on PDA. Based on morphological characteristics, the pathogen was identified as *F. oxysporum* f. sp. *ciceri*. Pure culture was obtained by sub-culturing on PDA slants using the hyphal tip method and incubated at 25±1 °C in a BOD incubator for a week, then stored at 4 °C in a refrigerator. The culture was maintained on PDA slants for further studies. A piece of *sporulating* mycelium was mounted on a glass slide in lactophenol cotton blue and observed under a light microscope. The mycelium was found to be *septate*, with *microconidia* being oval and rounded, while *macroconidia* were sickle-shaped, and *chlamydospores* were also present.

Fungal pathogen identification

The pathogen under examination was identified and confirmed as Fusarium oxysporum f. sp. ciceri based on morphological and cultural traits, microscopic observations, and a pathogenicity test.

Pathogenicity Test

The *mycelia* were removed from the Petri dish and placed into the PDA medium in order to obtain *spore suspension*. The spores were collected by filtration and centrifuged after an inoculation period of one week. Prior to the inoculation experiment, centrifuged spores were diluted with sterile distilled water to maintain the spore density at 10^6 spores/ml. The pathogen's spore suspension was injected into the chickpea seedlings by pin stabbing when they were big enough to have seven leaves. Similarly, sterile distilled water inoculations were made to the control plants. After that, the plants were kept in a greenhouse. The emergence of disease symptoms on chickpea leaves was periodically checked in pots.

Collecting and Maintaining Biotic Inducers

Six isolates of *Trichoderma viride* and *Trichoderma harzianum* were used in the experiment. Each isolate of *Trichoderma viride* and *Trichoderma harzianum* was gathered from KN (Kanpur Nagar), VAN (Varanasi), UNN (Unnao), FBD (Farrukhabad), KD (Kanpur Dehat), and BDA (Banda). All of the bioagent cultures were kept alive on PDA by periodically subculturing and keeping them refrigerated at 4 °C.

Seed Bio-priming

Seed bio-priming is a novel, beneficial, and eco-friendly technique that employs bio-stimulating agents like *Trichoderma* to improve the physiological functioning of seeds and stress resilience.

Seed Priming with Different Bioagents

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 with the powder of various strains of *Trichoderma spp.* within different Petri plates. Just after priming, seeds were sown in pots in the greenhouse. The completely randomized design (CRD) was used for the experimental setting. The table contains information about the isolates and further treatments that have been used in the study, where:

T_1 = Seed primed with *Trichoderma harzianum* (KN)
T_2 = Seed primed with *Trichoderma harzianum* (VAN)
T_3 = Seed primed with *Trichoderma harzianum* (UNN)
T_4 = Seed primed with *Trichoderma viride* (FBD)
T_5 = Seed primed with *Trichoderma harzianum* (KD)
T_6 = Seed primed with *Trichoderma viride* (BDA)
T_7 (control) = Control

Observations Recorded

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

Disease Incidence

Disease incidence was calculated using the following formula:

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \text{ X } 100$$

Morphological Parameters

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

Results and Discussion

Effect of seed biopriming on number of branches

The data represented in Table-1, fig.-1 showed that, every treatment was capable of substantially boosting the numbers of chickpea branches against control. In all the treatment, the highest number of branches were Studies from T_1 with value of 13, followed by T_3 and T_2 representing 10 and 9,respectively.The others remain of the treatments were also able to increase number of branches over control.

Effect of seed biopriming on root length

The data presenting in Table-1, fig.1 showed that, all treated plots recorded higher root length over non-treated plants. The highest root length was recorded from T_1 treatment with the value of 14.33 cm with increase 93.12% over control. The treatment T_2 showed second best result about 12.21cm with 64.55% 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.

Effect of seed biopriming on shoot length

The data represented in Table-1, fig.1 showed that every treatment was capable to boosting of the chickpea shoot length over control. Among all the treatments, the highest number of branches were observed from T_1 which treated with *Trichoderma harzianum* (KN) value of 36 cm with increase 100.93% over control. The treatment T_3 showed second best resulted value of 35 cm with 94.63% increased over control. The minimum shoot length record from untreated plants about 18 cm.

Effect of seed biopriming on yield attributes

The data presented in Table-2, fig.2 founded that, all treatments were substantially increasing the chickpea yields over control. Among all the treatments, the highest number of pods 42 was observed from T_1 which increased 36.11% over control with 24.5 gm seed index. The treatment T_2 was showed second best result as 23.51 gm with increased of 30.61% with 23.51 gm seed index, while non-treated plant, harvested the number of pod / plants of 21 pod / plant. It may be concluded from the table that all treated plant harvests more yield than non-treated plant. Treatment with Trichoderma stimulated yield attributing factors of chick pea. It may enhance number of branches, pods, seed index etc. (Kumar *et al*., 2014; Pandey, 2017) [5, 7].

Effect of seed biopriming on disease incidence

As per data showed in table no.3 and fig. 3 the highest incidence was recorded from untreated (T_7) pot about 57.14 percent. While, minimum disease incidence was recorded from T_4 which treated by *T. harzianum* (FBD) with the value about 14.29 per cent, second best result observed from T_1 which, treated with *T. harzianum* (KN) with the value of 19.05 percent.

Amongst them wilt (*Fusarium oxysporum* f. sp. ciceri) has been measured as devastating one causing up to 10% loss in

yield and the damage has been observed to the extent up to 61 per cent and 43 per cent at seedling and adult stages, respectively (Dubey *et al.*, 2010, 65 Thaware *et al.*, 2015 [13] and Patra and Biswas (2017) [2, 8]. Also found similar results reported *Trichoderma* is the effective bioagent that can reduce disease incidence of chickpea wilt.

Table 1. Effect of seed biopriming with different bioagent on morphological attributes of chickpea underwire house condition.

Treatment details	No. of branches/plants	Root length (cm)	Percent increase over control (Root length)	Shoot length (cm)	Per cent increase over control (Shoot length)
T_1 [*T. harzianum* (KN)]	13	14.33	93.12668	36	100.93
T_2 [*T. harzianum* (VAN)]	9	12.21	64.55526	34	88.89
T_3 [*T. harzianum* (UNN)]	10	11.03	48.65229	35	94.63
T_4 [*T. viride* (FBD)]	8.5	9.01	21.42857	30	66.67
T_5 [*T. harzianum* (KD)]	8.1	9.00	21.2938	28	55.56
T_6 [*T. viride* (BDA)]	8.3	8.93	20.3504	23	27.78
T_7 [Control]	7.2	7.42		18	
C.D.	0.856	0.441596		0.80	
SE(d)	0.399	0.947129		1.71	
C.V.	5.339	5.263305		3.36	

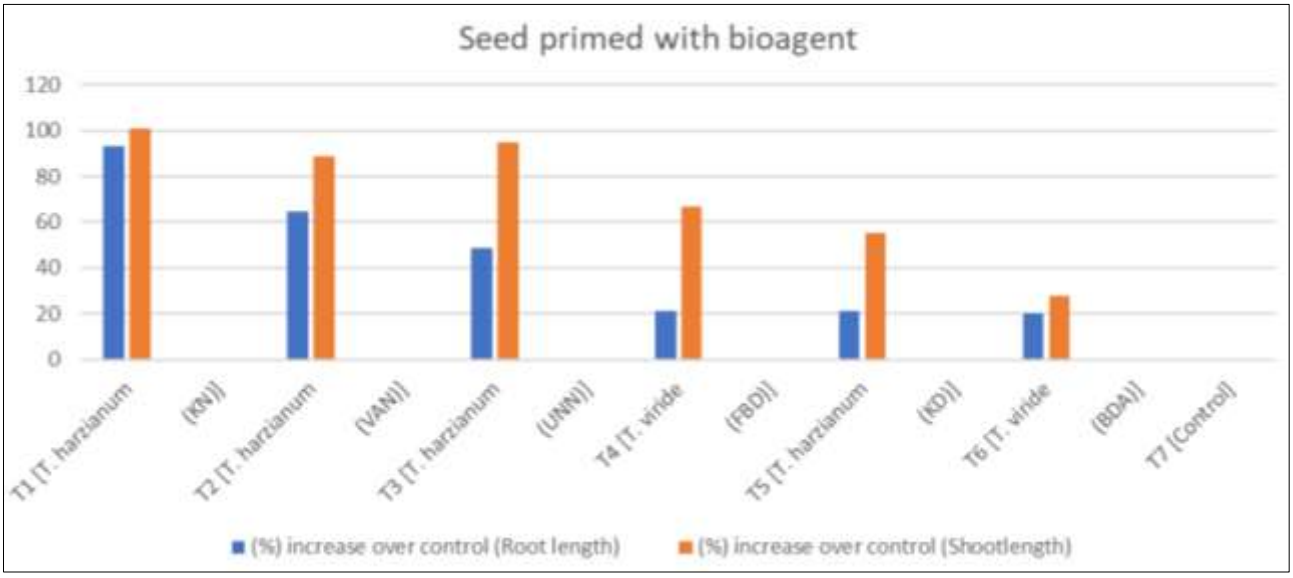

Fig 1: Effect of seed biopriming with different bioagent on morphological attributes of chickpea

Table 2. Effect of seed biopriming 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	42	24.5	36.11
T_2	36	23.51	30.61
T_3	37	22.85	26.94
T_4	35	22.05	22.50
T_5	34	22.50	25.00
T_6	31	20.07	11.50
T_7 (Control)	21	18.00	
C.D.	3.170	0.796969	
SE(d)	1.478	0.371584	
C.V.	5.369	2.076573	

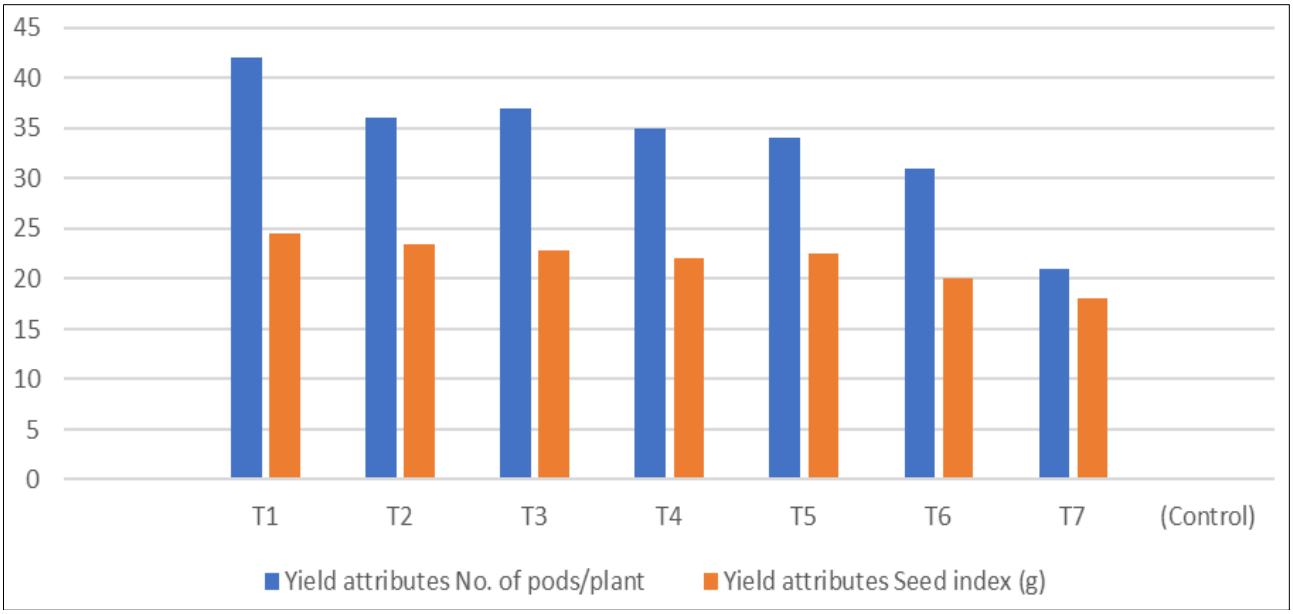

Fig 2: Effect of seed biopriming with different isolates of *Trichoderma spp.* On yield attributes

Table 3: Effect of different isolates of *Trichoderma* on Disease incidence

Treatment no.	Total No. of plants	No. of diseased plants	Disease incidence (%)
T_1	21	4	19.05
T_2	21	5	23.81
T_3	21	6	28.57
T_4	21	3	14.29
T_5	21	7	33.33
T_6	21	9	42.86
T7(Control)	21	12	57.14
C.D.		0.592	
SE(d)		0.276	
C.V.		5.147	

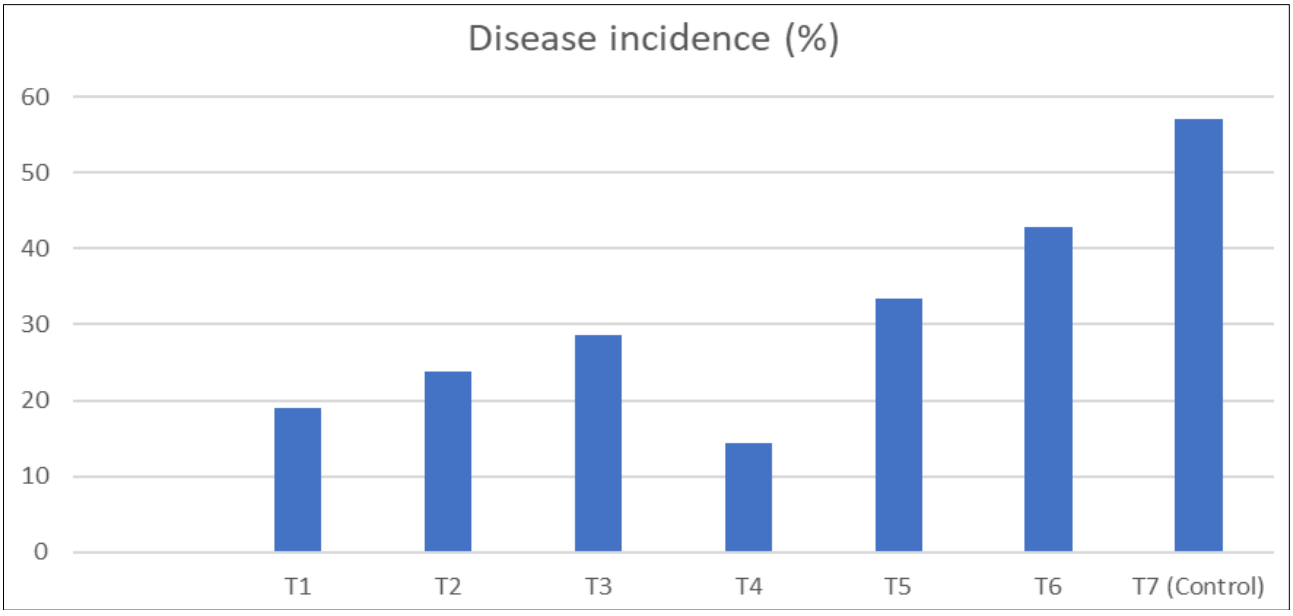

Fig 3: Effect of different isolates of *Trichoderma* on Disease incidence

Conclusion

The different isolates of *Trichoderma* were tested in the form of seed biopriming of chick pea seeds are on the basis of result. It is concluded that *Trichoderma* effective biological agent for management of Fusarium wilt. The most effective among these isolates was Trichoderma KN (Kanpur Nagar) that was significant against other isolates.

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