

# Sustainable Plant Disease Management of Wilt of Chickpea Caused by Fungi-Nematode Interaction

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## Abstract

Fungi and bacteria are being developed into biological pesticides in agriculture. *Pseudomonas fluorescence* and *Trichoderma viride* were tested against *M. incognita* and *Fusarium oxysporum* f. sp. *ciceri* in pots and in infested field. In the pots *Trichoderma viride* were applied in seed, soil and foliar. The results indicated a significantly remarkable reduction of *M. incognita* population in the treatment of *Trichoderma viride* (soil) followed by (seed) and (foliar) application. Further it was also observed the *Trichoderma viride* enhanced plant growth parameters of chickpea and reduced the incidence of Fusarium wilt disease of chickpea up to 70% in soil treatment followed by 66% seed treatment 49% foliar application form control.

In the infested (fusarium wilt and *M. incognita*) field microplot *Trichoderma viride* and *Pseudomonas fluorescence* were applied @ 3kg /ha in soil and @ 3g/kg in seed. Results indicate that both bio-agents treatments significantly reduced the formation of root-knot population as compared to control. Application of both the bio-agents significantly enhanced plant growth parameters and no. of grain pods/ plant as compared with control. Both the bio-agents also reduced significantly incidence of *Fusarium oxysporum* f. sp. *ciceri* as compared to control. In comparing the two bio-agents it was observed that wilt incidence was lowest in the treatments of *Pseudomonas fluorescence* (soil treatment) 15.1% followed by *Pseudomonas fluorescence* (seed treatment) 20.8%, *Trichoderma viride* (soil treatment) 26.2%, and *Trichoderma viride* (seed treatment) 31.6%.

**Keywords:** Bio-agents, *Meloidogyne*, *incognita*, *Fusarium oxysporum* f. sp. *Ciceri*, Chickpea

## 1. Introduction

India is a major pulse producing country of the world which nearly 27 million acres of its agricultural land under pulse crop production. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation by the bacteria prevalent in their root nodules and thus play a vital role in furthering sustainable agriculture. Chickpea (*Cicer arietinum* L.) world's third important pulse crop suffers from root-knot disease caused by *M. incognita* and *M. javanica* reported by Sharma and Mc Donald (1990)

Plant parasitic nematodes cause great economic losses to agricultural crops worldwide. The root-knot nematodes (*Meloidogyne* spp.) are sedentary endoparasites and are among the most damaging agricultural pests, causing a greater loss to different agricultural and horticultural crops through out the world.

On the world basis the estimated losses in chickpea due to plant parasitic nematodes are 10.7% reported by Sassier and Freckmen (1987). In India Upadhy and Dwivedi (1987) reported 4% yield losses in chickpea due to

*M. incognita* and chickpea wilt caused by *Fusarium oxysporum* f. spp. *Ciceri* with *Fusarium oxysporum* f. sp. *ciceri* in chickpea.

Fungi and bacteria are being developed into biological pesticides in agriculture that are both harmless in human and effective in every targeted way. Different fungi are being mass produced and made into highly selective herbicides, insecticides nematocides and fungicides. Uses of organic amendments and bio-agents have been proved as an ecologically sound approach to IDM and an alternate to fungicide use and have potential to promote sustainable agriculture in crop production system (Pandey and Choubey 2003). Devi and Sharma (2002) reported the efficacy of antagonistic fungi and rhizobacteria on *M. incognita* of tomato and Okra. The possible mechanism involved in *T. harzianum* and antagonism had been studied intensively in terms of antibiotic and enzyme products Hyphal interactions (Elad et al., 1982)

Ramanathan *et al.* (2002) reported that the disease management has been largely dependent on chemical control which may not be consistent with sustainable production. There has been an increasing effect to introduce bacterial and fungal bio-control agents for managing plant pathogens. Krishnamurthy and Gnanamickam, (1997) suggested that soil or powder based formulation enhance the ability of antagonistic bacteria to survive for a longer period of time and to control disease development.

Now day's efforts are being made to shift from the conventional use of chemicals in the use of eco-friendly methods. In this direction a preliminary pots and field experiment were conducted on chickpea to find out the effective response of *T. viride* and *P. fluorescence* on *M. incognita* and *Fusarium oxysporum* f. sp. *ciceri*

## 2. Materials and Methods

Two bio-controls isolates viz. *Trichoderma viride* and *Pseudomonas fluorescence* was isolated from AAI-DU research plot and was maintained on TSM and king's B medium slants at 4°C after growing for 2 days at 28°C. The above bio-agents were formulated in CMC (Carboxy methyl Cellulose) with talc powder and uniform formulations @ 4x10<sup>8</sup>cfu/g were maintained.

*Fusarium oxysporum* f. sp. *ciceri* was isolated from infected roots of chickpea and 500ml suspension containing 3x10<sup>5</sup> spores/ml inoculum was also maintained.

The root-knot nematode, *M. incognita* was maintained as pure culture on brinjal plants raised in pots filled with sterilized soil mixture (2:1:1) sand, loam, FYM respectively. Inoculum of *Meloidogyne incognita* were extracted from the roots of brinjal and the suspension of 2<sup>nd</sup> stage juvenile (J<sub>2</sub>) were maintained.

### 2.1 Pot experiment

The earthen pots of 15cm diameter were surface sterilized with 4% formaldehyde solution and filled with steam sterilized soil (1kg/ pot). *Trichoderma viride* were applied @4g/l as seed and soil and 4g/liter of water as foliar spray. Seed of local chickpea variety "Uday" were surface sterilized with 0.1% mercuric chloride for one minute wash three times with sterilized water and air dried the seeds. Five seed were sown to the respective pots. Later, thinned to two healthy seedling /pot inoculated with freshly hatched J<sub>2</sub> of *M. incognita* @ 2 larvae/g of soil, simultaneously *Fusarium oxysporum* of sp. *ciceri*. 4 ml culture filtrate also inoculated into the soil and each treatment were replicated 4 times. The pots were de-potted down-wards and tapped to loose the soil around the roots and observation were recorded at 135 days after inoculation, on plant height (cm), weight (g) , no. of nodules/ root and no. of root-knot system.

### 2.2 Field experiments

Two bio-control isolates viz. *Trichoderma viride* and *Pseudomonas fluorescence* were applied @ 3 kg/ha in soil and treated in seed @ 3g/kg. The treated and untreated chickpea (Uday) seeds were sown to the infested plot of *M. incognita* and *Fusarium oxysporum* f. sp. *ciceri* during Rabi 2007-2008. The trial was laid in R.B.D. with four replications.

Before sowing the seed FYM @5t/ha was applied to the entire plot. The size of the individual plot size was 2x2m. Observation were recorded at 45,90 and 135 days after germination of chickpea on shoot length (cm) weight(g) ; root length (cm) , weight (g), no. of root nodules/root, No. of root knot/ root, weight of 5 pods, No. of pods/ plant, % of fusarium wilt incidence and yield (g) /m<sup>2</sup>

## 3. Results and Discussion

Results of table no. 1 shows that antagonist fungi *Trichoderma viride* suppressed root-knot formation of *M. incognita* in the root of chickpea further it was also observed that the application of *Trichoderma viride* enhanced shoot length, weight, no. of rhizobium nodules as compared with control (nematode and Fusarium

alone). Among the *Trichoderma viride* treated plants significantly reaction the number of root-knot population was found in soil treatment followed by seed treatment and foliar application

At 135 days after foliar application of *T. viride* significantly increased of shoot length and weight was observed. The incidence of *Fusarium oxysporum* f. sp. *ciceri* also recorded at 135 days after inoculation and result shows that the soil treatment of *T. viride* reduced the incidence of disease up to 70% followed by seed treatment (66%) and foliar application (49%) from control Rao and Krishnappa (1994) when inoculated *M. incognita* along with *Fusarium oxysporum* f. sp. *ciceri* to the chickpea plant, the incidence of wilt was recorded up to 18%, where as 6.7% of wilt incidence was recorded in *Fusarium oxysporum* f. sp. *ciceri* alone inoculated plants. Howel (1982) suggested that *T. virens* has capable of destroying pathogen propagules by colonizing the sclerotia of *R. solani*.

Gupta et al., (2006) also reported significantly reduction of *Fusarium oxysporum* f. sp. *ciceri* incidence and higher biomass, dry weight, and grain yield of chickpea after inoculation of *T. viride*.

Gurha (2001) reported the species of *Trichoderma* showed mycoparasitism and strong antibiosis effect against *Fusarium oxysporum* f. sp. *ciceri*

### 3.1 Field experiment

Table no. 2 shows that application of the two bio-agents viz. *T. viride* and *P. fluorescence* in soil and seeds were significantly reduced the formation of root-knot to the roots of chickpea Further it was also noted that both the bio-agents also reduced *F.o.f.* sp. *ciceri* of chickpea. Application of the above bio-agents significantly enhanced shoot length, weight, root length, weight, no of grain pods/ plant as compared with control (without treated plots). In comparison of two bio-agents it was also observed that soil and seed treatment of *P. fluorescence* treated plants significantly increased plant growth and reduced root-knot formation as compared with other treatments. *P. fluorescence* was known to antagonize phyto-pathogens by producing one or more metabolites that included antibiotics also by antibiosis. A considerable suppression of disease by *P. fluorescence* (23% root infection) was also confirmed by Pandey and Chubey (2003), Shanmugam et al., (2003).

Goswami and Singh (1998) reported *Trichoderma* sp. *Aspergillus niger* treated okra plants significantly reduced the parasitic nematode population. Similar increase in plant growth parameters in *P. fluorescence* treated plants was reported by Jaya Kumar et al., (2004) further they reported application of *P. fluorescence* through seed and soil as single and split application either singly and in combination reduced the larvae population of parasitic nematodes in soil and root as compared to control. Effective reduction in nematode population due to split applications in different intervals might be due to maximum building up of bacterial population/root colonization by bacterium inoculation. Eapen et al., (2005) and Devi & Dutta (2002) reported the potential antagonistic of *Trichoderma* sp. on Root-knot nematode eggs and inhibition in egg hatching.

The present study could be concluded that use of the microbial antagonist viz. *T. viride* and *P. fluorescence* may reduce the hazard effect of the chemicals and efficiently useful as bio-control agent and plant growth promoter.

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Table 1. Effect of *Trichoderma viride* against *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *ciceri* on Chickpea (Pot experiment)

Parameters	D.a.s	Control T <sub>0</sub>	<i>F.</i> <i>alone</i> T <sub>1</sub>	<i>M. i.</i> <i>alone</i> T <sub>2</sub>	<i>T. viride</i> (Seed) F+ <i>M.i.</i> T <sub>3</sub>	<i>T. viride</i> (Soil) F+ <i>M.i.</i> T <sub>4</sub>	<i>T. viride</i> (Foliar) F+ <i>M.i.</i> T <sub>5</sub>	S.E	C.D. at 5%
Soot Length (cm)	45	21.1	18.9	15.4	24.7	20.4	22.1	1.49	4.26
	90	25.5	25.1	17.3	24.6	27.3	28.8	2.16	6.1
	135	27.2	28.4	23.2	29.4	30.7	35.1	1.47	4.20
Shoot weight (g)	135	5.08	5.8	3.2	3.9	5.9	7.7	0.75	2.16
Root Length(cm)	135	13.7	9.2	6.7	11.4	14.8	11.9	1.14	3.26
Root weight (g)	135	2.45	1.5	2.2	3.4	4.1	3.4	0.36	1.05
No. of Nodules	135	24	20	6	32	32	28	1.66	4.7
Braches/plant	135	5	4	2	7	7	6	0.67	1.93
No. of Pods/Plant	135	11	10	7	16	15	13	0.82	2.36
Weight of 5pods	135	2.1	0	1.6	3.8	3.6	4.3	1.13	4.73
No. of root-knot/ plants	135	0	0	196	33	17	68	1.9	2.74
% of Fusarium wilt incidence	135	0	89%	0	24.3%	18.9%	41.3%	-	-

*M.i.* *Meloidogyne incognita*

F = *Fusarium oxysporum* f. sp. *ciceri*

Table 2. Effect of bio- control agents on *Meloidogyne incognita* and *Fusarium oxysporum f. sp. ciceri* on Chickpea (Field experiment)

Parameters	d.a.s.	Control T <sub>0</sub>	<i>Trichoderma</i> <i>viride</i> (soil) T <sub>1</sub>	<i>Pseudomonas</i> <i>fluorescence</i> (soil) T <sub>2</sub>	<i>Trichoderma</i> <i>viride</i> (soil) T <sub>3</sub>	<i>Pseudomonas</i> <i>fluorescence</i> (soil) T <sub>4</sub>	SE±	C.D. at .05%
Shoot length (cm)	45	21.1	35.5	39.5	40.0	43.5	1.5	4.6
	90	31.5	44.1	43.6	44.9	46.4	1.1	3.5
	135	37.1	57.0	62.8	45.9	63.1	1.4	4.5
Shoot weight (g)	45	1.4	2.6	2.5	4.4	5.8	0.2	0.6
	90	4.1	14.6	15.3	17.5	18.3	1.5	4.7
	135	5.9	17.7	20.3	20.1	26.8	0.82	2.5
Root length (cm)	45	12.3	14.9	16.9	17.3	18.1	0.8	2.5
	90	14.3	15.9	18.7	19.5	21.1	0.8	2.5
	135	17.3	19.7	19.9	19.1	21.9	0.6	1.7
Root weight (g)	45	1.1	1.8	1.8	1.5	1.8	0.01	0.2
	90	0.8	2.7	2.6	3.5	3.7	0.28	0.87
	135	1.8	4.3	4.6	3.9	4.6	0.31	0.94
No. of nodules/plant	45	12	16	19	20	20	1.8	5.6
	90	18	32	37	32	45	3.7	11.6
No. of root knots/plant	45	33	10	9	11	10	3.1	9.6
	90	174	24	27	31	22	11.9	36.8
	135	289	29	36	25	27	5.8	18.1
No. of pods/plants	135	10	25	35	26	33	1.6	5.1
Weight of 5 pods (g)	135	2.3	3.5	3.6	2.9	44	1.15	0.47
% of wilt incidence	135	67.8	26.2	15.1	31.6	20.8	-	-
Yield /m <sup>2</sup>	135	75.5	116.5	123.3	124.2	125.6	4.07	108

d.a.s. - days after sowing