



## BIO-EFFICACY OF *TRICHODERMA VIRIDE* 2.0% A.S. FORMULATION (STRAIN NO. HBL-TV-72) AGAINST *FUSARIUM OXYSPORUM* F. SP. *SOLANI* CAUSING WILT DISEASE IN CHILLIES

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### Abstract

Chillies (*Capsicum annum* L.), also known as red pepper belongs to solanaceae family. Among various diseases; wilt caused by *Fusarium oxysporum* f. sp. *solani* is one of the most widely spread and prevalent causing considerable loss in India. The incidence of wilt is high in areas where chillies are cultivated intensively. With increasing awareness on toxic effects due to usage of chemical fungicides, the present study was taken up with an aim to evaluate bio- efficacy of *Trichoderma viride* 2.0% A.S. formulation (Strain No.: HBL-Tv-72) as a biological arsenal for the management of fusarium wilt. The results revealed that *T. viride* (Strain No.: HBL-Tv-72) reduced the mycelial growth of the target pathogen (27.12mm) to the minimum in dual culture assay. In field trials also the new strain of the antagonist viz., *T. viride* (Strain No.: HBL-Tv-72) as seed treatment at the dosage level of 20 ml/kg of seeds and as soil application at 2500 ml/ha is found very effective to manage the disease successfully (12.23%&10.96%) and enhance the growth parameters (VigourIndex 2046.4&1946.9) and yield (1.30 &1.28mt/ha)of chillies to the maximum. Further, no phytotoxic symptoms were observed due to seed and soil treatments with *T. viride* 2.0% A.S. formulation and the study showed normal rhizosphere activity of other/beneficial microbes throughout the crop period, at all the dosage levels and delivery systems tested. Hence, it can be concluded that the new bio fungicide *Trichoderma viride* 2.0% A.S. formulation (Strain No.: HBL-Tv-72) may be used to successfully manage fusarium wilt without any deleterious effect on the ecosystem.

**Keywords :** Chillies, *Fusarium oxysporum* f. sp. *Solani* and Bio-Fungicides

### Introduction

Chilli (*Capsicum annum* L.) is one of the most commercialized spice crop. According to Department of Agriculture Cooperation and Farmers Welfare, Government of India, area and production of dried Chilli, for the year 2016-17 is 8,40,000 ha and 20,96,000 MT respectively (<http://agriculture.gov.in>). India contributes 36 percent to total world's production and remained in first position in terms of international trade by exporting nearly 30 percent from it's total production (<http://www.indianspices.com>)

The production of Chilli in India is dominated by Andhra Pradesh (51%), Madhya Pradesh (11%), Karnataka (9%), Orissa (4%), Maharashtra (4%), Rajasthan (5%), Tamil Nadu, Uttar Pradesh and others (Anonymous, 2011) India has produced about 13.2 million tons of Chilli during 2009-10. However, due to unseasonal rains around 40-50% of crop got damaged in major growing regions of Andhra Pradesh. According to trade sources, chilli production in current year is estimated to be lowest in last 3-4 years. Chilli crop is attacked by "''''''''Among those diseases, wilt caused by *Fusarium oxysporum* f. sp. *solani* is one of the most widely spread and prevalent throughout the country causing considerable loss in India. Although chemical control remains highly effective against plant diseases, developing countries with a low gross national product cannot utilize these products without placing an economic strain on national budget. There is also an intensified worldwide concern about environmental pollution due to escalated use of hazardous pesticides. Furthermore, Use of chemicals for disease management calls for pesticides residues in foodstuffs, development of resistance in plant pathogens and appearance of new strains of these pathogens. Hence, a need was felt to develop an eco-friendly economical and alternative method for effective management of wilt disease in chilli. A multitude of microbes has been implicated as

biocontrol agents of plant pathogens sometimes with excellent documentation. Antagonistic effect of rhizosphere on bottle gourd (Gaikwad, 1982) and a few of the studies on suppressive effect of some soils on muskmelon wilt (Alabouvette *et al.*, 1984; Sivan and Chet, 1986; Sharma, 1989) and watermelon wilt (Naik, 1990) demonstrate encouraging trends towards biological control. In recent years, the incidence of *Fusarium* wilt of chilli has increased in irrigated black cotton soil (Devika Rani *et al.*, 2007). Present study is done with an objective to evaluate the bioefficacy of *Trichoderma viride* 2.0% A.S. formulation (Strain No.: HBL-Tv-72) against *Fusarium oxysporum* f.sp. *solani* under *in vitro* conditions. and *in vivo* condition

### Methodology

Bio-efficacy trial were taken up to evaluate the effectiveness of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) in managing *Fusarium oxysporum* f.sp. *solani* causing wilt diseases of chilli crop. The causative pathogens *Fusarium oxysporum* f.sp. *solani* was isolated from diseased chilli plants by direct plating method and maintained as pure culture on potato dextrose agar (PDA) slants.

The population of the antagonist viz., *Trichoderma viride* in the commercial formulation was assessed by serial dilution plating technique and the pure culture was obtained and maintained in *Trichoderma* selective medium slants. To assess the *in vitro* effect of *T. viride* against the target pathogens *F. oxysporum* f.sp. *solani*, dual culture bioassay was conducted at Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar. Observations was made and recorded when the control plates were completely covered by the mycelial growth and percent growth inhibition was calculated by the following formula.

$$\text{Percent growth inhibition} = \frac{(\text{Mycelial growth in control} - \text{Mycelial growth in treatment})}{\text{Mycelial growth in control}} \times 100$$

A field experiment was conducted at papparapatti village, a traditional chilli crop growing village of Dharmapuri district, TamilNadu to find out the effect of *Trichoderma viride* 2.0% A.S. formulation for the management of *Fusarium oxysporum* f.sp. *solani* causing wilt diseases of chilli crop in randomized block design with eight treatments and four replications. The chilli seeds (cv.

K1) treated with *Trichoderma viride* 2.0% A.S. formulation @ 5, 10, 20 ml/kg of seeds were sown in hot spot area (sick soil). The treated seeds were kept for 24 hrs in shade and then sown. Carbendazim (Bavistin 50% WP) @ 2g/kg of seed was used for comparison and a suitable control was also maintained. The crop was maintained with judicial irrigation and all the agronomic practices and fertilizer schedule were followed as per standard procedures.

### Objectives:

a.	To test the efficacy of <i>Trichoderma viride</i> 2.0% A.S. formulation (Strain No.: HBL-Tv-72) against <i>P. aphanidermatum</i> and <i>Fusarium oxysporum</i> f.sp. <i>solani</i> under <i>in vitro</i> conditions.
b.	To test the bio-efficacy of <i>Trichoderma viride</i> 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) for the management of damping off and wilt diseases of Chilli caused by <i>P. aphanidermatum</i> and <i>Fusarium oxysporum</i> f.sp. <i>solani</i> under field conditions.
c.	To test the effect due to application of <i>Trichoderma viride</i> 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) on the biometrics of Chilli crop and phytotoxicity (If any).
d.	To test the effect due to application of <i>Trichoderma viride</i> 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) on non target organisms/beneficial microbes

### Details of the treatments

Tr.No	Treatments
T <sub>1</sub>	Seed treatment with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 5 ml/kg of seed
T <sub>2</sub>	Seed treatment with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 10 ml/kg of seed
T <sub>3</sub>	Seed treatment with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 20 ml/kg of seed
T <sub>4</sub>	Soil application with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 1250 ml/ha (Precolonized in 100 kg FYM)
T <sub>5</sub>	Soil application with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 2500 ml/ha (Precolonized in 100 kg FYM)
T <sub>6</sub>	Soil application with <i>Trichoderma viride</i> 2.0% A.S. Formulation @ 5000 ml/ha (Precolonized in 100 kg FYM)
T <sub>7</sub>	Seed treatment with Carbendazim 50% WP @ 2 g/kg of seed
T <sub>8</sub>	Control

### Method of treatment/application of bio-formulation

#### a) Seed Treatment:

The seeds were spread uniformly on a flat surface and the test product, as per the dosage, was applied to the seeds and mixed twice / thrice to have uniform coating. The treated seeds were kept for 24 hrs in shade and then sown.

#### b) Soil application:

The bioformulation *Trichoderma viride* 2.0% A.S. formulation as per the treatment schedule was mixed well with 100 kg FYM one week prior to application for precolonization and broadcasted to the nursery field and main field.

### Methodology For disease assessment

#### Germination percentage and Vigour index

The germination percentage was assessed at seven days after sowing and calculated by using the following formula

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The observations on shoot length and root length were assessed at the time of transplanting and the seedling vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973)

$$\text{Vigour index} = (\text{Mean root length} + \text{Mean shoot length}) \times \text{Germination (\%)}$$

### Wilt

The Percent wilt incidence was recorded at the time of final harvest using the standard "Phytopathometry" formula given below.

$$\text{Percent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Chilli plants were also observed for phytotoxic symptoms (If any) such as chlorosis, necrosis, scorching, epinasty and hyponasty on 1, 3, 5, 7 and 10 days after treatment/application of *Trichoderma viride* 2.0% A.S. formulation and grading was done as per CIB guidelines adopting 0 - 10 scale. Observations were also made on the effect on other beneficial micro organisms in the rhizosphere due to treatment with *Trichoderma viride* 2.0% A.S. Formulation. This was confirmed by isolating the rhizosphere microorganisms into pure culture and co-inoculating them with *Trichoderma viride* (Strain No.: HBL-Tv-72) using dual culture technique and observed for compatibility among them. The data on disease incidence and other biometrics were analyzed using standard statistical techniques.

### Results

#### a) *In vitro* efficacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) against *Fusarium oxysporum* f.sp. *solani* causing wilt diseases of Chilli crop

The dual culture study conducted to test the efficacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.:

HBL-Tv-72) against *Fusarium oxysporum* f.sp. *solani* causing wilt diseases of Chilli crop revealed the supremacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) in reducing the mycelial growth of the target pathogens. The antagonist recorded 69.86 percent reduction on the mycelial growth *F. oxysporum* f.sp. *solani* respectively (Table 1). Many workers have found similar results. Five species of *Trichoderma* viz., *T. viride*, *T. harzianum*, *T. koningii*, *T. aureoviride* and *T. pseudokoningii* evaluated for their *in vitro* antagonistic potential against *Fusarium oxysporum*, the cause of wilt disease in sweet pepper, *Capsicum annum*. Out of the five species, *T. viride* showed the best performance followed by *T. harzianum*, *T. aureoviride*, *T. koningii* and *T. pseudokoningii*, resulting in 62, 36, 24, 18 and 6% reduction in colony growth (Sahi and Khalid 2007). *T. harzianum* showed maximum growth inhibition (86.44 %) of *Fusarium* wilt of *Solanum melongena* through mycoparasitism. The nonvolatiles produced by the *Trichoderma* sp. exhibited 100 % growth inhibition of the pathogen under *in vitro* condition (Chakraborty and Chatterjee 2008). Cho *et al.* (1989) observed that *T. harzianum* had the highest mycoparasitism and low antibiosis to *F. oxysporum* f.sp. *cucumerinum* causing wilt of cucumber. Pandey and Upadhyay (2000) observed strong antagonism of *T. harzianum*, *T. viride* and *Gliocladium virens* against *Fusarium* species. *Trichoderma* spp. was found antagonistic to *Fusarium solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi*. Naik *et al.* (2009) Found that *T. viride* PDBCTV I0 caused 100.00 per cent inhibition of radial growth of *F. solani* in dual culture

**b) Efficacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) for the management wilt diseases of Chilli caused by *Fusarium oxysporum* f.sp. *solani* under field conditions**

**i) Seed Treatment**

The wilt diseases of chilli crop was significantly reduced due to seed treatment and soil application with *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) (Table 2). In general, the seed treatment delivery system of the antagonist was found better than the soil application of the antagonist. Among the various treatments, Seed treatment with *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) @ 20 ml/kg recorded the least disease incidence of 10.96 percent of wilt incidence. This was followed by the dosage levels with 10 ml/kg and 5 ml/kg of seeds in the decreasing order of merit. Further the dosage levels of 10ml/kg and 20ml/kg of seeds was on par in their effect in reducing the wilt incidences. The untreated control recorded maximum wilt incidences

**ii) Soil application**

Soil application with *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) @ 5000 ml/ha significantly reducing the wilt (11.28%) incidences when compared with other dosage levels of soil application. However, the dosage levels 2500 ml/ha and 5000 ml/ha recorded at par results in reducing the wilt incidences (Tables 2).

**c) Effect of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) as seed and soil treatment on the biometrics of Chilli crop under field conditions**

With regard to the germination and seedling growth parameters, the treatment T3 (Seed treatment with

*Trichoderma viride* 2.0% A.S. Formulation @ 20ml/kg of seeds) (T3) recorded the highest germination percentage (90.23%), maximum shoot length (16.56 cm), root length (6.12 cm), vigour index (2046.4) and dry fruit yield (1.30 mt/ha). The seed treatment dosage levels of 10ml/kg and 20ml/kg of seeds were on par in their effect in enhancing the biometric observations of chilli crop. The least values of biometric parameters were observed in control plots (Table 3).

Among the soil application method of delivery system, the dosage level with Soil application with *Trichoderma viride* 2.0% A.S. Formulation @ 5000 ml/ha (T6) recorded the maximum values of biometric observations viz, germination percentage (89.98 %), maximum shoot length (16.72 cm), root length (5.93 cm), vigour index (2038.0) and dry chilli yield (1.31 mt/ha). This was followed by the treatments T5 and T4 in the decreasing order of merit. However, the soil application dosage levels 2500 ml/ha and 5000 ml/ha recorded statistically at par results in reducing the wilt incidences of chilli crop (Table3). These earlier reports substantiate with the present findings Soil application through FYM as a carrier provides the maximum reduction of test pathogen. This may be due to the maximum growth of bioagent on carrier that favoured its competitive mechanism of bio control. Seed inoculation with the antagonist also recorded good inhibition of test pathogen when it was used alone (Mandhare and Suryawanshi, 2005) by seed soaking and dry seed coating of *T. harzianum* against *Rhizoctonia solani* (Hwang and Benson, 2002), *F. oxysporum* f. sp. *vasinfectum* in cotton, *F. oxysporum* f. sp. *melonis* in melon (Sivan and Chet, 1986). Effect of *Trichoderma* against *Fusarium* and *Sclerotium rolfsii* (Monaco *et al.* 1991), as well as *T. viride* and *Gliocladium virens* against *F. oxysporum* f. sp. *ciceri* (Jha and Singh, 2000) have been documented successfully. Ankita Sinha *et al.* (2018) record that seedling treatment with 1% talc based formulation of *Trichoderma harzianum* and *Trichoderma viride* was found highly significant in reducing wilt incidence in chilli under glass house and field conditions

**Phytotoxicity evaluation**

No phytotoxic symptoms were observed throughout the period of investigation in field trial on the seedlings of Chilli Cv. K 1 treated with *Trichoderma viride* 2.0% A.S. formulation (Strain No.: HBL-Tv-72) at various levels as seed treatment viz., @ 5 ml/kg, 10 ml/kg and 20ml/kg of seeds and as soil application @ 1250 ml/ha, 2500 ml/ha and 5000 ml/ha (Table 4).

**Effect on non target organisms**

The results showed that the activity of the non target organisms viz., *Pseudomonas* spp., *Trichoderma* spp., *Bacillus* spp. etc have not been affected due to seed treatment with *Trichoderma viride* 2.0% A.S. formulation (Strain No.: HBL-Tv-72) and no adverse effect was observed on these beneficial microbes in the rhizosphere of chilli crop at all the dosage levels tested. All these microorganisms showed compatibility with *Trichoderma viride* (Strain No.: HBL-Tv-72).

**Conclusion**

The results of the present study have clearly revealed that *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) could be successfully used to manage wilt

diseases of chilli crop. As seed treatment the dosage level of 10 ml/kg of seeds and as soil application 2500 ml/ha is found very effective to manage the disease successfully and enhance the growth parameters and yield of chilli crop. The same dosage levels produced better results than the Carbendazim seed treatment in reducing the disease incidence and improving the plant growth parameters and

yield of chilli crop. Further, no phytotoxic symptoms were observed due to seed treatment with *Trichoderma viride* 2.0% A.S. Formulation and the study showed normal rhizosphere activity of other/beneficial microbes throughout the crop period and at all the dosage levels of seed treatment and soil application method of delivery systems tested.

**Table 1 :** *In vitro* efficacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) against *Fusarium oxysporum* f.sp. *solani* causing wilt disease on Chilli crop

Tr. No	Treatment details	Radial growth of <i>F. oxysporum</i> f.sp. <i>solani</i> (mm)*	Percent growth reduction
T1	<i>Trichoderma viride</i> 2.0% A.S. (Strain No.: HBL-Tv-72)	27.12	69.86
T2	Carbendazim 50% WP @ 0.1 % conc.	7.28	91.91
T3	Control	90.00	-
	SE	0.15	-
	CD (p=0.05)	0.33	-

\* Mean of seven replications

**Table 2 :** Bio-efficacy of *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) for the management of *Fusarium oxysporum* f.sp. *solani* causing wilt disease of Chilli under field conditions

Tr. No	Treatment details	Wilt incidence (%)*	Disease control (%)
T1	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 5 ml/kg of seed	18.12 (26.01)	40.05
T2	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 10 ml/kg of seed	13.23 (20.75)	56.23
T3	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 20 ml/kg of seed	10.96 (18.66)	63.74
T4	Soil application with <i>T. viride</i> 2.0% A.S., @ 1250 ml/ha	19.42 (29.57)	35.70
T5	Soil application with <i>T. viride</i> 2.0% A.S., @ 2500 ml/ha	12.23 (22.25)	59.51
T6	Soil application with <i>T. viride</i> 2.0% A.S., @ 5000 ml/ha	11.28 (20.49)	62.6
T7	Seed treatment of Carbendazim 50% WP @ 2 g/kg of seed	09.12 (16.66)	69.83
T8	Untreated control	30.23 (36.21)	-
	SE	0.81	-
	CD (p=0.05)	1.71	-

\* Mean of four replications

The data in parentheses are arc sin transformed values

**Table 3 :** Effect of seed and soil treatment with *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) on the biometrics of Chilli crop under field conditions

Tr. No	Treatment details	Germination (%)*	Shoot length (cm)*	Root length (cm)*	Vigour Index	Yield (Dry fruits) mt/ha*
T1	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 5 ml/kg of seed	79.23 (63.64)	13.21	5.25	1462.5	1.08
T2	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 10 ml/kg of seed	89.72 (71.98)	16.23	6.02	1996.2	1.29
T3	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 20 ml/kg of seed	90.23 (72.12)	16.56	6.12	2046.4	1.30
T4	Soil application with <i>T. viride</i> 2.0% A.S., @ 1250 ml/ha	80.31 (63.94)	14.23	5.12	1553.8	1.01
T5	Soil application with <i>T. viride</i> 2.0% A.S., @ 2500 ml/ha	89.72 (71.95)	15.89	5.81	1946.9	1.28
T6	Soil application with <i>T. viride</i> 2.0% A.S., @ 5000 ml/ha	89.98 (71.54)	16.72	5.93	2038.0	1.31
T7	Seed treatment of Carbendazim 50% WP @ 2 g/kg of seed	89.72 (71.87)	14.13	4.58	1678.6	1.29
T8	Untreated control	62.74 (50.33)	12.49	3.23	986.2	0.75
	SE	0.38	0.18	0.03	-	0.03
	CD (p=0.05)	0.71	0.35	0.08	-	0.05

\* Mean of four replications

The data in parentheses are arc sin transformed values

**Table 4 :** Phytotoxicity study of seed and soil treatment with *Trichoderma viride* 2.0% A.S. Formulation (Strain No.: HBL-Tv-72) against damping off and wilt diseases of Chilli crop

Sl. No	Treatments	Phytotoxic symptoms*																								
		Yellowing/DAT					Necrosis/DAT					Scorching/DAT					Epinasty/DAT					Hyponasty/DAT				
		1	3	5	7	10	1	3	5	7	10	1	3	5	7	10	1	3	5	7	10	1	3	5	7	10
1	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 5 ml/kg of seed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 10 ml/kg of seed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	Seed treatment with <i>T. viride</i> 2.0% A.S., @ 20 ml/kg of seed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	Soil application with <i>T. viride</i> 2.0% A.S., @ 1250 ml/ha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	Soil application with <i>T. viride</i> 2.0% A.S., @ 2500 ml/ha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	Soil application with <i>T. viride</i> 2.0% A.S., @ 5000 ml/ha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	Control (Water treated)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

\* Mean of four replications

DAT- Days after treatment

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