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Exploiting the bio-control activity of Trichoderma viride isolates against Wilt complex disease of Chilli

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ABSTRACT: Investigation was undertaken to screen the potential native isolate of *Trichoderma viride* for bio suppression of corn rot pathogen complex, as *Trichoderma viride* are the most successful and widely used biocontrol agents. Taking the advantage and constraints of *Trichoderma viride* into consideration, efforts were made to encourage the nauve isolate against corn rot pathogens. Nine isolates of *Trichoderma viride* namely Tv_1, Tv_3, Tv_4, Tv_6, Tv_8 Tv_9, Tv_{10}, Tv_{11}, and Tv_{15}, were isolated from soils and which modified *Trichoderma* Specific Medium (TSM). The isolates were studied for their cultural, Micrometric i.e. characters and antagonistic potential against six newly recoided majoi fungal pathogens of chilli viz sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Phytophthora* sp., *F. oxysporum* and *F. solani* individually on Potato Dextrose Agar, the culture morphology of all the isolates was found to be similar The isolate Tv_1, Tv_3, Tv_4, Tv_8, Tv_9, Tv_{11}, and Tv_{15}, were found fully overgrown on Pathogens of chilli wilt complex, where as the isolates Tv_{13} failed to inhibit the *Phytophthora* sp Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods.

Key words: Bio-control, Wilt complex. *Rhizoctonia solani*, *Phytophthora* sp., *Fusarium* sp.,

INTRODUCTION

Chilli (*Capsicum annum L.*) belongs to the family Solanaceae and is popularly known as red pepper and is considered as one of the most important vegetable as well as spice crop. Chilli (*Capsicum annuum* L.) has a unique importance amongst the Solanaceous crops grown in India, because of its high nutritive value and manifold uses. In India Chilli occupies an area of 840 thousand hectares with an annual production of 2096 M. tonnes (2016-17). The incidence of wilt varied from 0.0 to 75.0 percent in different states of India. Andhra Pradesh (A.P.), Karnataka, Tamil Nadu, Maharashtra are major chilli growing states in India which together contributes about 75% of total cultivated area. Though India is the leading producer,

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the average yield of chilli is very low (1.11 t/ha dry chilli) as compared to developed countries like USA, China etc, where the average yield ranges from 3-4 t/ha (Source: Spice Board, India).

Besides the evaluation in improved varieties and use of recommended package of practices, the yield of chilli in the country is still very disheartening compared to its productivity in the developed countries. Chilli production and productivity is constrained due to many biotic and abiotic stresses. Several constraints for low productivity of Chilli but diseases are important.

The vascular wilt complex disease of chilli, incited by a number of pathogens, is the devastating soil-borne disease and hence difficult to manage. The disease has been observed to be caused by *Fusarium* f sp. *capsici*, *Fusarium oxysporum* and *Fusarium solani Fusarium spp.*, *Phytophthora capsici*, *Rhizoctonia solani*, (Shali, 2000; Najar, (2001)). Other pathogens from different genera including *Phytophthora*, *Pythium* and *Rhizoctonia* have been found associated with infections on roots and stems (Vatchev, 2007). Among of them *Fusarium oxysporum* causing vascular wilt is the most predominant and causes 10-50 per cent crop losses around the world and 10-80 per cent in India.

Transmission of disease occurs by means of its chlamydospores which remains active for several years. In sweet pepper browning of the vascular tissue is a strong evidence of *Fusarium* wilt. To have a control over vascular wilts a variety of fungicides remained as a primary means with a negative impact on growth of useful soil microorganisms, thereby causing an imbalance in ecological niche with a harmful effect on environment. Simultaneously as a counter effect even pathogens are developing innate resistance towards many fungicides hence control of *Fusarium spp*. remains to be a challenging job in the provisions of disease management (Srinon, *et al.*, 2006).

Chemical fungicides are extensively used in contemporary agriculture. However these products may cause problems such as environmental pollution and have adverse effects on human health. Microorganisms as bio control agents have high potential to control plant pathogens and have no negative effect on the environment (or) other non target organisms. *Tricoderma* spp are used as effective biocontrol agents against several soil borne fungal plant pathogens (Howell, 2003).

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Successful reductions of *Fusarium* wilt in many crops with application of different species of *Trichoderma* have been found (John, *et al.*, 2010; Ramezani, 2009). In addition all isolates of *Trichoderma* spp are not equally effective (Ramezani, 2008). But controlling of *Fusarium oxysporum* f.sp. *capsici* by fungal species through biological approach is necessary. Considering all these points, present study is designed *in vitro* to evaluate the antifungal efficacy of *Trichoderma harzianum* and *Trichoderma Viridae* in preventing *Fusarium* wilt of chilli.

Biological control means antagonistic activity of micro-organism for another micro-organism. Antagonism is a term for micro-organism association which is harmful to one another and is often used for cases in which toxic metabolites are involved. It has been studied principally in relation to the rhizosphere, and competition between micro-organism in the soil.

During last fifteen years, chilli crop has been affected by severe rotting caused by sterile Basidiomycetes fungus. *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium solani* and reduction in yield has been reported. In 1980 the yield per hectare was 5.66 kg ha^{-1} and now its present productivity is 1.53 kg ha^{-1} (Anonymous, 2009) which is the lowest in the world.

In recent years, attempts were also made to use a consortium of biocontrol agents to gel persistent control, of plant pathogens (Chaube and Sharma, 2002). Biological control, therefore, holds promise as a strategy for disease management and it is environment friendly too. Antagonistic fungi especially *Trichoderma* spp has been widely used against a number of phytopathogens (Rini and Sulochana, 2006) and parasitized hyphae of other fungi *in vitro* and brought about several morphological changes during destruction (Anitha and Murugesan, (2001)) Screening of potential *Trichoderma* strains was done by Bandopadhyay *et al* (2003) against major root pathogens and it was found that more or less all the strains checked the growth of the pathogen and stimulate plant defensive mechanisms (Hanson and Howell, 2004. Harman *et al*, 2004, Yadav *et al*, 2011)

Trichoderma harzianum is one efficient biocontrol that is commercially produced to prevent development of several soil pathogenic fungi (Jegathambigai *et al*, 2009). Biocontrol is an important approach for plant disease management under changing food habits and commercialization of agriculture (Manczinger *et al.* 2002)

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Therefore, keeping in view medicinal importance and to remove the pesticidal residue of such valuable medicinal crop, the present study was undertaken for screening of several local antagonistic isolates of T. *viride*, obtained from different field of chilli , under *in vitro* conditions against few pathogens sterile Basidiomycetes fungus, *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani*, *Phytophthora* sp. causing chilli wilt.

MATERIALS AND METHODS

Collection of pathogen: Four pathogenic isolates namely sterile Basidiomycetes fungus. *Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium solani* and *Phytophthora* sp were isolated, from infected chilli plant in growing area The pathogens were maintained on PDA medium at 4^0C.

Isolation of *Trichoderma* spp. (Tv_1, Tv_3, Tv_4, Tv_6, Tv_8, Tv_9, Tv_{10}, Tv_{11}, and Tv_{15}) was done from randomly collected soils from different vegetable fields by dilution plate technique using *Trichoderma* specific medium TSM (Elad *et al.*, 1981) modified by Sana and Pan (1997)

Antagonistic potential of *Trichoderma viride* isolates on chilli wilt complex pathogens: The antagonistic properties of fifteen isolates of *Trichoderma viride* were tested on PDA by dual culture plate technique Paired cultures were observed for a total of 12 days before being discarded. All the ratings were done after contact between pathogen and the antagonist using a Bells scale (Bell *et al*, 1982) which is slightly modified (Class I -7) as follows.

- S_1 = The pathogen and the antagonism locked at the point of contact.
- S_2 = The antagonism starts overgrowth on pathogen.
- S_3 = The pathogens starts overgrowth on mycoparasite.
- S_4 = The antagonist overgrew at least 15% of pathogen.
- S_5 = The antagonist overgrew of least 30% of patliogen.
- S_6 = The antagonist overgrew at least 60% of pathogen.
- S_7 = The antagonist completely overgrew the pathogen (100% overgrowth)

RESULTS AND DISCUSSION

Identity of Isolates of *Trichoderma* spp.: In general, colony morphology of all the isolates was more or less similar showing sparse to thin colony mycelial mass with whitish border in some cases Speculation started after 48h of incubation at 28±l°C for all the isolates (Table 2).

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Micrometric measurements of *Trichoderma* viride (Table 1) showed that phialospore length ranged between 2.98-5.52 µm and, breadth ranged from 2.71-4.6 µm and phiahdes length 9.22-12.56 and breadth 1.3-2.5. These characteristics, particularly the trifid phialophore with short phiahdes clearly resembled the identical characters of *Trichoderma viride* (Rifai, 1969).

Antagonistic potential of *Trichoderma viride* isolates against chilli wilt complex pathogen.

***Phytophthora* sp.:** The results showed that isolate Tv_1, Tv_8, Tv_4, and Tv_{11}, were antagonistic to *Phytopthora* by totally overgrowing the pathogen within seven, nine and eleven day respectively Isolate Tv_{10}, Tv_{15}, and Tv_3, Tv_6, and Tv_9 were antagonistic to Phytopthora overgrowing 75, 45 and 60%, respectively.

Fusarium solani: The results showed that isolate Tv_1, Tv_4, Tv_6, Tv_8, Tv_9, and Tv_{11}, were antagonistic to *Fusarium solani* by totally overgrowing the pathogen within 8 to 12 days. Isolates Tv_3 and Tv_{10}, were overgrowing the pathogen 90 and 45%, respectively.

Sterile Basidiomycetes fungus: The results against Basidiomycetes fungus showed that five isolates Tv_1, Tv_8, Tv_{15} and Tv_9, and Tv_6, totally overgrowing within nine, eight and 12 day respectively. The remaining isolate Tv_{11}, Tv_4, Tv_3, and Tv_{10}, overgrew 90, 75, 45 and 15%, respectively.

Table1 : Micrometric measurement of phialospores. phialides and dilamydospores of isolate

Isolate	Conidia (urn)		Phialidc (um)		Chlamydosporc *(pm)*	
	L	B	L	B	L	B
Tv_1	3.50-4.17	2 71-3 22	10 2-11 9	1.3-20	11.04-11.05	11.4-11 0
Tv_3	4.21-4.87	3.20-3.62	9 41-9.51	10-24	8.3-11.4	8 3-11.4
Tv_4	4 92-5.52	3.62-4.60	9.25-10.12	1.3-1.9	6.1-7.2	6.1-7.2
Tv_6	3 79-4 22	3 10-4 52	9 82-1045	1.8-2.1	8.5-96	8 5-9 6
Tv_8	3 41-4 04	2.56-3 21	922-10.33	1.4-1 9	7.4-89	74-8.9
Tv_9	3 62-3 97	11-3 96	9 99-11 22	1 <-l 8	6.2-7 5	6 2-7 J
Tv10	298-3.60	2.89-2.79	10.56-12.22	2.1-2.5	8.7-9.2	8.7-9.2
Tv11	4.27-162	3.56-3 88	9.66-9 22	12-14	10.2-11 5	10.2-11.5
Tv15	3.91-4.53	.< 8<M <6	11 22-12 56	1.8-15	9.5-10.6	9.5-10.6

Table 2 : Colony characters of *Trkltoderma viride* isolates

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Isolate name	After 3 days dia (cm)	36 h	After 60 h	After 90 h
Tv_1	3.8	White growth appears inoculum, sparse very dim mycelium hardly - ccti	Sparse 4 cm mycelium growth, media become yellow around inoculum, after light green sporulation	Light green away from inoculum, inner circle sparse and outer circle with dense growth, encircled dense white fluffy mycelium
Tv_3	3.5	White mycelial growth on tiic inoculum, very thin mycelium surround the inoculum	Compact white mycelium on inoculum, light green sporulation around the inoculum 2.5 cm after raised cottony growth 8 6 cm	Inoculum covered with snow white mycelium surrounded sparse growth, later thick doty green slightly fluffy raised 1.5 cm. then dark green
Tv_4	6.3	Sparse whitish thick growth	Contact fluffy light great sporulation on older regions	Around inoculum 2 cm dia Sparse whitish green after 15 cm dia. dark green fluffy raised
Tv_6	6	White growth on the inoculum. surround sparse mycelium Yellow growth on inoculum, surround spares white growth	On inoculum while growth. Inisc lightly sparse fluffy green sporulation 4 3 cm Yellow growth on inoculum. around 2 cm sparse light green sporulation	On inoculum snow white growth, surround dull green sparse 4 cm media, encircled whitish green raised growth Inoculum covered with yellow growth, surrounded dirty green growth 2 cm. encircled with slightly raised growth
Tv_8	3.9	Hun sparse giowth 3.9 cm around the inoculum	Yellow growth appeals on inoculum sparse light green sporulatioii appears while dense growth at periphery	Inoculum covered with green growth, surrounded by dark green band encircled by off white mycelium
Tv_9	4	Very thin mycelial growth around the inoculum	M i cm thin mycelial growth around inoculum encrcled with compact dark green sporulation	Around inoculum 5-5.3 cm dia White mycelial growth. Surrounded by sparse whitish green mycelium 15 cm dia. encrcled by dark green 0 5 cm dia
Tv10	7	Thick raised white mycelium	Around the noculum very light green flu fly mycelium encircled b 1 cm dense green sproulation	Around inoculum 5-5 3 cm dia White y mycelial growth Surrounded by sparse whitish green mycelium 15 cm dia. cue axled by dark green 0.5 cm dia.
Tv11	6.3	Around inoculum 3 cm while mycelium	Fluffy mycelium lake balls, sunouiided dark gjeen spoiulalioi	Fluffy mycelial balls with lightly i green sporulatioii

Table 3: Hyperparasitic potential of *T. viride* wild isolates on fungal pathogens of chilli

Isolates	Basidiomycetes fungus		*Rhizoctonia solani*		*Phytophthora* sp		F. oxysporum		F. solani	
	D	R*	D	R	D	R	D	R	D	R
Tv_1	3	$9S_7$**	3	$6S_7$	3	$7S_7$	3	87	3	$10S_7$
Tv_3	3	$5S_4+S_5$	3	$8S_7$	3	$7S_4+S_5$	3	$9S_7$	3	$11S_7$
Tv_4	3	$11S_6+S_4$	3	$7S_7$	3	$9S_7$	3	$7S_7$	3	$9S_7$
Tv_6	4	$12S_1$	4	$8S_7$	4	$11S_4+S_5$	4	$5S_1$	4	$5S_1$
Tv_8	3	$9S_7$	3	$8S_7$	3	$7S_7$	3	$6S_7$	3	$6S_7$
Tv_9	3	$8S_7$	3	$6S_7$	4	$10S_6$	3	$8S_5$	3	$7S_5$

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Tv10	3	$4S_7$	3	$6S_7$	4	$10S_6+S_4$	3	$9S_5$	3	$7S_4$
Tv11	3	$4S_6+S_5$	4	$9S_7$	3	$10S_7$	3	$9S_5$	3	$7S_5$
Tv15	3	$9S_7$	4	$10S_6+S_4$	4	$10S_6+S_4$	3	$9S_7$	3	$8S_7$

D: Days before contact, R: Rating. **An average of five individual observation *The numerical value represents the days required for attaining S_1 to S_7 stage of modified Bell's scale

Rhizoctonia solani: The results showed that all isolates were antagonistic to *Rhizoctonia solum* by totally overgrowing the pathogen with six to nine day except isolate Tv_1. It overgrew only 75% even after day.

Fusarium oxysporum: The results showed that isolate Tv_1, Tv_3, Tv_4, Tv_8, and Tv_{15}, were antagonistic to *F. oxysporum* by totally overgrowing the pathogen within 6 to 9 days Isolates Tv_9. Tv_{10}, and Tv_{13}, did not progress beyond 30% ever after day The remaining isolate Tv_6 totally fails to overgrow the host pathogen even upto 12 days of inoculaUon inspite of attaining the point of contact of the third day.

Fusarium solani: The result shows that five isolates Tv_1, Tv_3, Tv_4, Tv_8, and Tv15, were highly antagonistic to ***Fusarmm solani***, totally overgrowing the pathogen within 6 to 11 days Isolates Tv_9 and Tv_{11}, were overgrew the pathogen 30% whereas Tv_{I0} 15% and Tv_6 failed to overgrew the host pathogen even after 12 days of inoculation, in spite of attaining the point of contact on the 4th day of inoculation.

The overview of the results (Table 3) showed that the isolates Tv_1, Tv_3, Tv_4, Tv_8, Tv_9, Tv_{11}, and Tv_{15}, were found fully overgrown on all corm rot Pathogens of chilli, where as the isolates Tv_{13}, failed to inhibit the *Phytophthora* sp. To identify then, isolates of *Trichoderma* spp. have been listed m the tables that reached class-I (S_7) stage within 6-11 days of inoculation However, based on this information the antagonistic *Trichoderma viride* did not allow an early selection of isolates, as the variability in the antagonistic characteristic within the isolate and isolate-pathogen interaction was very high.

The above observations established the fact that Tnchodenna isolates existing m their natural conditions in natural eco system do differ with reaped to then growth and antagonistic potential Similarly Li *el al* (2001) studied eighteen isolates of *Trichoderma* spp of these isolates. TR13 showed greatest antagonists effects against *Rhizoctonia solani* Several research papers that have appealed in the literature do reveal reveal the fact that vanous species and isolates of fungal antagonist Trichoderma suppress mycelial growth reduce root

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rots, increase plant growth and induce resistance in various crops with which *Sclerolium rolfsu* (Tian *et al*, 2001. Das and Dutta. (2002). Palomar *el al*, (2002), *Rhizoctonia solani (Li et al.* 2001, Burgess and Hepworth, 1996, Zapata *et al*, 2001, Znedan and Mahmoud, 2002, Gaikwad and Nimbalkar, 2003. Yossen *et al.*, 2003, Fravel and Lewis. 2004; Hajlaoui *et al*, 2001; Singh *et al.*, 2003; Huang and Erickson 2004. Salhpour *et al*. 2005) are associated. It is clear that the success of bioagents introduces in soil does not guaiantee the control the target pathogens because plant, physicochemical and biological factors of soil affect establishment. proliferation and antagonistic activities of the introduced bioagents It is necessary that, identified antagonist efficiency against foot, root rot and damping off should be investigated and examined *in vivo* conditions also, the results of such survey would be reported by die authors in near future (Shaigan *et al*, 2008)

It is in this context that to ensure success of introduced bioagents, they should be isolated for the local areas where they exit. Since, they have already faced various processes of evaluation, their application would be feasible and result oriented. We reviewed the literature to find out that have others worked on these aspects. Literature analysis revealed that comparative studies have been done with various species (Kucuk and Kivanen, 2003, Chang *et al*, 2006) studied *Trichoderma* isolates from different soil sampled and grouped them according to then antagonistic potential and chitin utilization

CONCLUSION

The overgrowth by the antagonist under *in vitro* conditions may be good criteria of selecting an isolate shows good performance under *in vitro* conditions. The trend of the results also indicated that there was not only variability amongst the isolates of *Trichoderma viride* With differential degree of antagonism towards a single pathogen but also towards different pathogens.

The results of the study are the pointer to the fact that the antagonists should be isolated from different systems and locations to create a huge genetic pool and tested for their antagonistic potential against variety of die targeted plant pathogens and recommended specifically for different locations and systems. The present study clearly indicates the high potential of biocontrol agent. *Trichoderma viride* isolates for different plant pathogens. Efforts are onto evaluate the performance of promising isolate in field by soil and seed application methods

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