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Evaluation of Consortium of Bioagents against *Fusarium oxysporum* f. sp. *pisi*

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ABSTRACT: In India, particularly in Madhya Pradesh (Jabalpur), the pea is a significant pulse crop. Pea crop suffers from many diseases caused by fungi from which wilt is reported as one of the emerging disease in last few year. Fungal and bacterial bioagents have been identified as one of the greatest alternatives that doesn't harm the environment for minimizing the use of chemicals. The majority of biocontrol agents used to combat a variety of root, shoot, and postharvest infections are Trichoderma spp. The idea of creating microbial consortia for bio-control is based on the observation that in their natural habitats, bioagents exist in communities that provide some advantages to plants. In this study, the ability of consortium of Trichoderma in dual culture as well as its volatile and non-volatile compounds were evaluated. In all tested consortium in dual culture *T. virens* + *T. harzianum* recorded maximum percent growth inhibition (JC-1, 69.83%). In volatile compounds Percent growth inhibition 86.80 % was recorded highest in *T. viride* + *T. harzianum* (JC-2, 76.51%) and Percent growth inhibition in non-volatile compound will increase as concentration of bioagents increases in highest concentration 100% growth inhibition was recorded in five consortiums of bioagents that was *T. virens* + *T. harzianum* (JC-1), *T. viride* + *T. harzianum* (JC-2), *T. viride* + *T. virens* (JC-3), *T. asperellum* + *T. longibrachiatum* (JC-6) and *T. longibrachiatum* + *T. Virens* (JC-7). The in-vitro test was performed October-November 2021.

Keywords: Consortium, Fusarium, Trichoderma, wilt, pathogen, bioagent.

INTRODUCTION

Pea is one of the most important legumes harvested worldwide, after soybeans, chickpeas and peanuts (Foyer *et al*., 2016). In India, it is mainly cultivated in Uttar Pradesh, Madhya Pradesh, Himachal Pradesh, Maharashtra. After Uttar Pradesh, Madhya Pradesh is the second largest pea producer state (Anonymous, 2017; Tiwari *et al*., 2019). The soilborne plant pathogen *Fusarium oxysporum* has formae speciales that have been designed to infect particular plant hosts and is the cause of disease in many commercially significant crops. More than 150 host-specific formae speciales of the fungus *F. oxysporum* have been identified, with examples including the well-researched and commercially significant *F. oxysporum* f. sp. *cubense*, *lycopersici*, *cepae* and *pisi* serving as notable examples. *F. oxysporum* f. sp. *lycopersici*, *cubense*, and *pisi* are additional examples of *f. sp.* that contain numerous economically harmful races and are specialized to infect specific cultivars of a host species d (Edel-Hermann and Lecomte, 2019). For instance, f. sp. *cubense* tropical race 4 is wreaking havoc on the well-known Cavendish cultivar of bananas in the tropics. The thick-walled chlamydospores of this soilborne pathogen can survive and persist in the soil for more than ten years (Kraft, 1994; Khan *et al*., 2017; Cha *et al*., 2016). The root cause of pea wilt disease is *F. oxysporum* f. sp. *pisi* (Jenkins *et al*. 2021). Wilt in pea is not a serious problem but it is emerging disease in every pea growing country (Merzoug *et al*. (2014). There are four distinct races of Fop (races 1, 2, 5, and 6) has been identified (Grajal-Martin *et al*., 1987; Haglund and Kraft 1993). Races 1 and 2 are found nearly everywhere, however Races 5 and 6 are, as of right now, exclusively significant in western Washington State, USA (Infantino *et al*., 2006). The numerous advantages of sustainable agriculture are facilitated by biocontrol agents. Utilizing fungus, bacteria and actinomycetes to control plant diseases is known as biological control (Tripathi *et al*., 2020).

Numerous crop plants have been reported to gain advantages from using *Trichoderma* spp. as possible biocontrol and growth-promoting agents (Bai *et al*., 2008; Savazzini *et al*., 2009; Chaudhary *et al*., 2016; Magar *et al*., 2020). The three most significant processes in biocontrol activity are parasitism, competition with pathogens and the synthesis of antifungal chemicals (Verma *et al*., 2007; Savazzini *et al*., 2009). Improved microbial efficacy, dependability and consistency under various soil and environmental situations is made possible by a consortium of effective microbial strains for biological control. In a consortial formulation, different genera occupy various root zone niches, which limits competition between them. In the agriculture, bioagents are frequently used as plant protectors that have a significant impact on the plant community by promoting plant growth and host-specific biotic and abiotic tolerance. The majority of biocontrol agents used to combat a variety of root, shoot, and postharvest infections are *Trichoderma* spp. (Siemering, 2016). The idea of creating microbial consortia for bio-control is based on the observation that in their natural habitats, bioagents exist in communities that provide some advantages to plants. The use of a consortium of bioagents may increase their effectiveness, dependability, and consistency even in a variety of soil and environmental situations (Amirthalingam *et al.,* 2020). In this study effectiveness of Eight consortiums from Trichoderma species (*T. virens + T. harzianum, T. viride + T. harzianum, T. viride + T. virens, T. asperellum + T. ressei, T. longibrachiatum + T. ressei, T. asperellum+ T. longibrachiatum, T. Longibrachiatum + T. virens*) were tested against the fusarium pathogen for the purpose of environmentally friendly and long-term disease control (Raut *et al*., 2014; Patel *et al*., 2021). In Trichoderma-treated hosts, particularly after application of the consortium, defense-related enzymes and antioxidants also shown significantly greater activity (Bisen *et al*., 2019).

MATERIAL AND METHOD

A. Isolation and purification of the pathogen

Small piece of infected tissues 1-2 mm in size from the propelling edge of the wilted root, with healthy segments were cut with sharp blade, washed well in distilled water to take out dust stuck to the tainted pieces. Pieces were pre-treated 1% NaOCl (Sodium hypochlorite) for 1 minute and consequently washed well in three changes of disinfected refined water. The pieces were then moved to PDA test tube with the help of inoculating needle under aseptic condition and incubated at 28 ± 1°C. After 72 hrs, fragments of hyphal growth from the growing tips were moved to new PDA test tube. Pure culture was made, following rehashed hyphal tip transfer method.

The following Trichoderma consortiums were which were listed below were evaluated to test the antagonism against *Fusarium oxysporum* f. sp. *pisi* in the Department of Plant Pathology Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (M.P.) during 2020-21.

T1	*T. virens* + *T. harzianum*	(JC-1)
T2	*T. viride* + *T. harzianum*	(JC-2)
T3	*T. viride* + *T. virens*	(JC-3)
T4	*T. asperellum* + *T. ressei*	(JC-4)
T5	*T. longibrachiatum* + *T. ressei*	(JC-5)
T6	*T. asperellum* + *T. longibrachiatum*	(JC-6)
T7	*T. Longibrachiatum* + *T. virens*	(JC-7)

B. Effect of consortium on growth and sporulation of Fusarium oxysporum

A dual culture technique developed by Morton and Straube (1955); Patole (2017) was adopted to study the effect of Trichoderma consortia against *F. udum.* Twenty ml sterilized melted PDA media was poured into sterilized petriplates @ 20 ml/plate aseptically, allowed to solidify. For the Trichoderma consortium, 8 mm disc of Trichoderma was placed at equiditance on potato dextrose agar media in a petriplate. Immediately after inoculation, the plates were sealed with plastic film and incubated at 27 ± 1ºC for 1 week period. Observations were recorded after 96 hrs of inoculation on the growth of individual Trichoderma in the presence of its co-inoculant. Pairs of consortia were considered compatible if they grow without any inhibition zone in the culture plate. Then, 8 mm disc of test pathogen and the consortia cut with the help of sterilized cork borer were placed on PDA approximately 4 cm apart from each other and incubated in BOD incubator at 27± 1ºC for 144 hours. Three replications were maintained for each treatment. Observations on colony diameter of individual antagonist and the pathogen were recorded after 144 hours of incubation. Inhibition of radial growth of *Fusarium oxysporum* over control was calculated by the formula given by Vincent (1947).

$$\text{Inhibition of growth of target pathogen} = \frac{\text{Radial growth of target pathogen in check} - \text{Radial growth of target pathogen in dual culture}}{\text{Radial growth of target pathogen in check}} \times 100$$

In order to study the viability of target pathogen, isolation was done by transferring 5 mm mycelial disc cut by cork borer from the zone where the test fungus was already overgrown by the antagonist on PDA medium.

C. Effect of volatile compounds from Trichoderma consortium agents on radial growth and sporulation of F. oxysporum

The effects of volatile compounds of consortium on radial growth of *Fusarium oxysporum* were studied as per the method given by Dennis and Webster (1971). The two bottom portion of Petriplates containing PDA were inoculated with a 5 mm disc of pathogen and other and sealed with cellophane adhesive tape and incubated in BOD incubator at 28 ±1°C. The petriplate containing PDA without antagonist serves as control. The observations on the radial growth of the test fungus were recorded after 120 hours of incubation at 28 ± 1°C. The radial growth of the test fungus in the

treatment in comparison with that of check gave percent growth inhibition.

D. Efficacy of non-volatile compounds from Trichoderma consortium agents radial growth and sporulation of F. oxysporum

The biocontrol agents were grown in Potato dextrose broth at 27°C with irregular shaking at 150 rpm. The metabolites were collected after 15 days and filtered. The sterilized filtrate was amended in PDA to make 5, 10 and 15% concentration in petriplates. The solidified agar plates in sets of three were inoculated at the middle with 5 mm diameter mycelial disc of target pathogen and incubated at 28 ± 1°C for 96 hours. The Plates without filtrate used as control. The colony diameter was recorded and percent inhibition of radial growth was determined utilizing the formula given by Vincent, 1947. The Statistical method used to conduct the experiments was in completely randomized design method, during 2020-2021.

$$\text{Inhibition of growth of target pathogen} = \frac{\text{Radial growth of target pathogen in check} - \text{Radial growth of target pathogen in treatment}}{\text{Radial growth of target pathogen in check}} \times 100$$

RESULT AND DISCUSSION

A. Evaluation of antagonistic efficacy of consortium

Consortium is an association of two different bioagent. So to know the effect of two bioagents together the trial was conducted and data presented in Table 01. Percent growth inhibition was highest in *T. virens* + *T. harzianum* (JC-1, 69.83%) followed by *T. viride* + *T. harzianum* (JC-2, 55.08%) and *T. asperellum* + *T. longibrachiatum* (JC-6, 50.61%). And lowest 32.18% growth inhibition was recorded in *T. asperellum*+ *T. ressei* (JC-4) followed by *T. longibrachiatum* + *T. ressei* (JC-5, 33.61%), *T. viride* + *T. virens* (JC-3, 44.31%). Excellent sporulation was recorded in untreated plate followed by *T. asperellum* + *T. ressei* (JC-4) and *T. viride* + *T. Virens* (JC-3), *T. longibrachiatum* + *T. Virens* (JC-7), result fair sporulation and remaining four consortium show good sporulation.

B. Evaluation of antagonistic efficacy of volatile and non-volatile compounds of consortium

Volatile compounds of all seven consortiums were also evaluated and results cent percent growth inhibition and presented in Table 1. Effect of volatile consortium compound was recorded maximal in *T. viride* + *T. harzianum* (JC-2, 76.51%) followed by *T. virens* + *T. harzianum* (JC-1, 68.64%) and *T. asperellum* + *T. longibrachiatum* (JC-6, 66.64%). Minimal growth inhibition was recorded in plated treated with (T7-58.94%) followed by *T. viride* + *T. virens* (JC-3, 60.03%) and *T. longibrachiatum* + *T. ressei* (JC-5, 61.93%)

Non- volatile compounds of consortium of bioagents (5, 10 and 15%) was evaluated and extracted from the liquid culture were assessed for their antifungal activity on radial growth, percent growth inhibition and sporulation of test pathogen. And data presented in Table 02. Maximum percent growth inhibition was recorded in *T. viride* + *T. harzianum* (JC-2, 70.30%) followed by *T. virens* + *T. harzianum* (JC-1, 42.90%), *T. asperellum* + *T. longibrachiatum* (JC-6, 39.50) and *T. longibrachiatum* + *T. Virens* (JC-7, 38.94%). Minimum inhibition was recorded in *T. longibrachiatum* + *T. ressei* (JC-5, 0.99%) followed by *T. asperellum* + *T. ressei* (JC-4, 10.89%) at 5% concentration. Eexcellent sporulation was recorded in control and (JC-5) plate. Good sporulation was recorded in *(*JC-1, JC-3 and JC-6*)* treated plate. At 10% concentration 100% percent growth inhibition was recorded in *T. viride* + *T. harzianum* (JC-2) followed by *T. virens* + *T. harzianum* (JC-1), and *T. asperellum* + *T. longibrachiatum* (JC-6) that is 94.89% and 86.87% respectively. And sporulation was recorded excellent in control only. Good sporulation was recorded in JC-5, JC-1, JC-4, JC-6 and JC-7 show fair sporulation. 15% concentration of consortium all bioagents result excellent and 100% growth inhibition was recorded in five consortiums of bioagents that was *T. virens* + *T. harzianum* (JC-1), *T. viride* + *T. harzianum* (JC-2), *T. viride* + *T. virens* (JC-3), *T. asperellum* + *T. longibrachiatum* (JC-6) and *T. longibrachiatum* + *T. Virens* (JC-7). Percent growth inhibition was recorded lowest in *T. longibrachiatum* + *T. ressei* (JC-5, 91.36.5%), followed by *T. asperellum* + *T. ressei* (JC-4, 91.36%). No sporulation was recorded in any treatment except control.

Trichoderma has gained popularity as a reliable and practical biological control agent for treating soil-borne illnesses. Pythium, Fusarium, Rhizoctonia, Sclerotium, and Macrophomina are just a few of the serious soil-borne diseases that Trichoderma species have demonstrated capacity to combat (Choudhary and Mohanka 2012; Kumar *et al.*, 2012; Ommati and Zaker 2012; Raut *et al.*, 2014; Magar *et al.*, 2020). Trichoderma then gets ready to combat pathogens by producing secondary metabolite compounds or volatile compounds. *Trichoderma asperellum* GDSF1009 (CGMCC NO. 9512), *Trichoderma asperelloides* Z4-1 (CGMCC NO. 40245), *Trichoderma harzianum* 10569 (CGMCC NO. 40246), and *T. asperellum* 10264 (CGMCC NO. 22404) were combined to determine the best consortium for co-culture, the factors underlying the levels of *Fusarium oxysporum* antagonistic and growth-promoting activity in plants, as well as the enhancement of seed germination in monocultures of a single strain (Hao *et al.*, 2022). Percent growth inhibition was highest in *T. virens* + *T. harzianum* (69.83%) followed by *T. viride* + *T. harzianum.* And lowest 32.18% growth inhibition was recorded in *T. asperellum* + *T. ressei* followed by *T. longibrachiatum* + *T. ressei.* In contrast to studies carried out by many researchers, including Duffy *et al*. (1996); Mishra *et al.* (2011), fusarium wilt occurs. By combining various biocontrol agent isolates, they have shown improved biocontrol activity. There are several findings that reveal specific microbial consortia were unable to exhibit at least comparable impacts on plants in relation to their unique applications (Kumar and Jagadeesh

2016). The incompatibility of the microorganisms in the mixture with one another is one of the main reasons why microbial mixes can produce such contradictory results. Additionally, according to Wong *et al.* (2020), using bioformulations including *Pseudomonas aeruginosa* DRB1 and *Trichoderma harzianum* CBF2 successfully reduced the severity of Fusarium Wilt and induced host defence in banana against *Fusarium oxysporum* f. sp. *cubense* disease. The combination of *T. hamatum* + *T. viride* (JC-4) was discovered to be the most effective against *Fusarium udum*, according to Patel and Kumar's study (2021). The consortiums of *T. hamatum* + *T. viride* (JC-4) and *T. hamatum* + *T. harzianum* (JC-5) in volatile compounds completely inhibited *Fusarium udum* growth. The impact of consortium of DRB1 and CBF2 on the development and biochemical alterations of bananas infected with Foc-TR4 is studied by Wong *et al.* (2021). Consequently results that, a promising approach to managing Foc-TR4 sustainably in both in-vitro and greenhouse conditions are the use of bioformulations including BCA consortia. Izquierdo-Garca and coworkers (2020) studied *Trichoderma virens* Gl006 and *Bacillus velezensis* Bs006 as a consortium and came to the conclusion that it had a good ability to suppress Fusarium wilt of cape gooseberry induced by *Fusarium oxysporum* f. sp. *physali*. It was also recorded by Wong *et al.* (2020) that applying bioformulations containing microbial consortium of biocontrol agents (BCAs), *Pseudomonas aeruginosa* DRB1 and *Trichoderma harzianum* CBF2 was promising in growth promotion as well as successfully reduces disease severity (Fusarium Wilt) and induce host defence in banana against *Fusarium oxysporum* f. sp. *cubense.*

Table 1: Antagonistic efficacy of bioagents consortium under dual culture technique and their volatile compounds against *Fusarium oxysporum* f. sp. *pisi* after 124 hrs of incubation.

Treatment		Dual culture			Volatile compounds		
		Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation
T 1	*T. virens* + *T. harzianum*	21.17	69.83	++	24.45	68.64	++
T 2	*T. viride* + *T. harzianum*	31.51	55.08	++	18.31	76.51	++
T 3	*T. viride* + *T. virens*	39.07	44.31	+++	31.16	60.03	+++
T 4	*T. asperellum*+ *T. ressei*	47.58	32.18	+++	27.98	64.11	++
T 5	*T. longibrachiatum* + *T. ressei*	46.57	33.61	++	29.68	61.93	+++
T 6	*T. asperellum*+ *T. longibrachiatum*	34.65	50.61	++	26.01	66.64	++
T 7	*T. Longibrachiatum* + *T. virens*	38.52	45.09	+	32.01	58.94	+++
T 8	Control	70.15	0.00	++++	77.96	0.00	++++
	SE (m)	**0.88**			**0.61**		
	CD (0.5)	**2.66**			1.88		

Table 2: Effect of non-volatile compounds (5, 10 and 15%) of bioagents consortium against *F . oxysporum* f.sp. *pisi* under *in vitro* of condition after 124 hrs of incubation.

Treatment		5%			10%			15%		
		Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation	Radial growth of pathogen	% growth inhibition	Sporulation
T 1	*T. virens* + *T. harzianum*	17.3	42.90	+++	2.11	94.89	+	0	100.00	-
T 2	*T. viride* + *T. harzianum*	9	70.30	++	0	100.00	-	0	100.00	-
T 3	*T. viride* + *T. virens*	21.17	30.13	+++	10.12	75.48	++	0	100.00	-
T 4	*T. asperellum* + *T. ressei*	27	10.89	+	12.6	69.48	+++	5.14	93.45	-
T 5	*T. longibrachiatum* + *T. ressei*	30	0.99	++++	13.78	66.62	+++	6.78	91.36	-
T 6	*T. asperellum*+ *T. longibrachiatum*	18.33	39.50	+++	5.42	86.87	+	0	100.00	-
T 7	*T. Longibrachiatum* + *T. virens*	18.5	38.94	++++	7.8	81.10	+	0	100.00	-
T 8	Control	30.3	0.00	++++	41.27	0.00	++++	78.46	0.00	++++
	SE (m)	**0.49**			**0.21**			**0.89**		
	CD (0.5)	**1.29**			**0.65**			**0.29**		

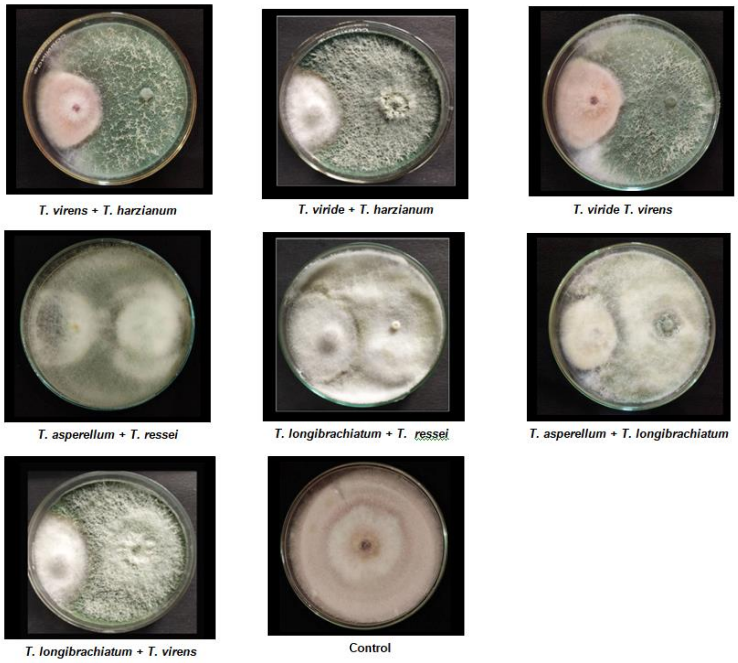

Plate 1. Antagonistic efficacy of bioagents consortium against *Fusarium oxysporum* by using dual culture technique.

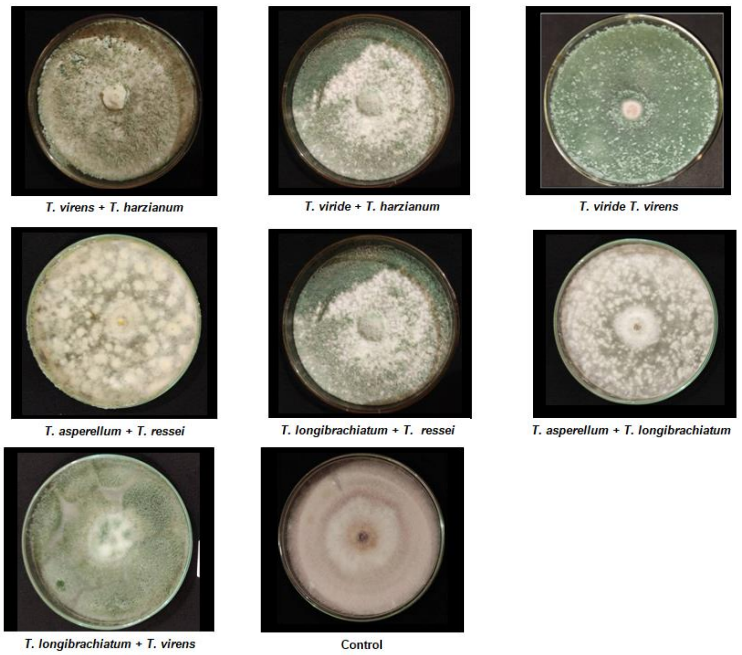

Plate 2. Effect of non-volatile compounds (15%) of consortium of bioagents against *Fusarium oxysporum.*

CONCLUSIONS

Trichoderma consortium in dual culture give best result in *T. virens* + *T. harzianum* (JC-1, 69.83%) in volatile compound *T. viride* + *T. harzianum* (JC-2, 76.51%) and in non-volatile compound all were found satisfactory and in highest concentration 100% growth inhibition was recorded in five consortiums of bioagents that was *T. virens* + *T. harzianum* (JC-1), *T. viride* + *T. harzianum* (JC-2), *T. viride* + *T. virens* (JC-3), *T. asperellum* + *T. longibrachiatum* (JC-6) and *T. longibrachiatum* + *T. Virens* (JC-7).

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Conflict of Interest. None.

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