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Antagonistic activity of *Trichoderma* sp. against pathogenic fungi

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Abstract
Antagonistic activity of *Trichoderma* sp. against five pathogenic fungi *viz*., *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* was assessed by dual culture plate method (Fig.1). On an average the radius of pathogenic fungi in control plates (i.e. when those were grown alone) was 38.2 mm. It was highest (70 mm) for *Colletotrichum* sp. while least for the pathogen *Alternaria citri* (23 mm) however, the radius of almost all fungi under investigation reduced within the range of 12 to 17 mm with an average value of 14 mm in dual culture. Among the fungi maximum percent inhibition was observed against *Colletotrichum* sp. (80%) followed by *Colletotrichum musae* (61.29%), *Fusarium incarnatum* (56.67%), *Gibberella avenacea* (54.05%) and *Alternaria citri* (39.13%) with decreasing orders. This indicated strong antagonistic activity exhibited by *Trichoderma* sp. against the pathogenic fungi. There was significant difference between the radius of colonies by fungi when grown alone and those with *Trichoderma* sp. (Table 1, Fig.1).

Keywords: antagonistic activity, *Trichoderma* sp., *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea.*

1. Introduction
Using microorganisms to suppress plant disease is the best alternative to the use of synthetic chemicals. Biological control is an innovative, cost effective and eco-friendly approach to chemical control. The use of naturally occurring microbial antagonists was the most promising alternatives, either alone or as part of an integrated control strategy to reduce synthetic fungicide inputs (Fan and Tian, 2001) [10]. Biological control is also likely to be stronger than disease control that is based on synthetic fungicides (Emmert and Handelsman, 1999) [9].
Application of the fungicides is not economical in the long time because they pollute the environment leave harmful residues and can lead to the development of resistant strains of the pathogen with repeated use studied by Vinale (2008) [43]. Replacement of fungicides with bio-control agents is an alternative mean to manage the plant pathogens produce safety food and reduce the environment pollution observed by Barakat and Al-Masri (2005) [3]. One of the most important biocontrol agents is *Trichoderma* sp. which is the most frequently isolated soil fungi and present in plant root ecosystems. *Trichoderma* sp. was also commercially marketed as biopesticides, biofertilizers and soil amendments. The use of *Trichoderma* fungi in agriculture can provide numerous advantages; 1) Colonization of the root and rhizosphere of plant, 2) Control of plant pathogens by different mechanisms such as parasitism, antibiosis production and induce systemic resistance, 3) Improvement of the plant health by promote plant growth and 4) Stimulation of root growth studied by Harman (2004) [16].
Some of the common antagonists include *Bacillus subtilis*, *Gliocladium virens* and *Trichoderma* spp., *Bacillus subtilis* and *Gliocladium virens* were used antibiosis as the main mechanism of antagonism. Whereas *Trichoderma* spp., use mycoparasitism as the chief mechanism of antagonism (Baker and Paulitz, 1996) [2]. *Trichoderma* spp. was well documented as effective biological control agents of plant diseases caused by soil borne fungi (Sivan and Chet, 1994; Basim, 1999) [38]. The different species of *Trichoderma* were well known for their biological control capabilities against a wide range of commercially important plant pathogens (Whipps and Lumsden, 2001; McLean, 2004) [44, 42].
Microbial antagonist has been used to reduce the hazardous effect of pesticides for the control of root infecting *Fusarium* spp. (Ehteshamul Haque, 1990; Perveen and Ghaffar, 1991) [8, 28]. Qureshi and Ghaffar (1966) [30] found that most of the microorganisms used by them were unable to inhibit the growth of *Fusarium solani*. In contrast to them *Trichoderma* spp. gave maximum mycelial inhibition of *Fusarium solani* (Tomar, 2004) [41]. Coating of seed with *Trichoderma harzianum, Trichoderma viride* at 1% improved germination, reduce root rot and increase cotton yield (Monga and Dad Raj, 2000) [23]. In presence of *Trichoderma harzianum, Trichoderma viride* and *Gliocladium virens*, the infection by *Fusarium solani* was greatly reduced (Perveen, 1994) [29]. In solarised soil, *Trichoderma harzianum* and *Trichoderma viride* alone and in combination gave maximum reduction of *Fusarium* wilt of *Carnation* and also enhanced the plant growth parameters (Kumar, 2005) [21]. In view of this antagonistic activity of *Trichoderma* sp. was seen against *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* pathogenic fungi.
Singh (1998) [36] confirmed that *T. harzianum* showed strong mycoparasitism and covered 100 % colony growth of *S. sclerotiorum*. Kapil (2002) [17] reported maximum inhibition (73.3%) of *S. sclerotiorum* with *T. viride* in dual culture. *T. harzianum* and *T. viride* were found to decrease the root rot caused by *R. solani* in bell pepper plants upto 70.9 % studied by Gaikwad and Nimbalkar (2003) [11]. Singh (2004) [37]

conducted an experiment on four antagonistic against *F. oxysporum* f. sp. *lycopersici* under glass house conditions. They found that out of four antagonist *viz*. *T. viride, T. harzianum, Gliocladium virens* and *Aspergillus nidulans*, 86% disease controlled by *T. viride* followed by 81% by *T. Harzianum.* Application of *Trichoderma* sp. effectively controls a large number of foliar and soil-borne fungi such as *Fusarium* sp, *Rhizoctonia solani, Pythium* sp. and *Sclerotium rolfsii* studied by Ngo (2006) [25].

Use of *Trichoderma* sp. against number of plants pathogenic fungi have been reported by Brisa (2007) [5]. High inhibitory effect of volatile toxic substances emitted by *Trichoderma* sp. on the radial growth of *Fusarium* sp has also been reported by Dubey (2007) [7]. Kumar (2007) [20] tested three species of *Trichoderma* i.e. *T. virens, T. viride* and *T. harzianum* against *F. moniliforum* var *subglutinas* and found them effective. Kapoor (2008) [18] studied an in vitro efficacy of *Trichoderma* sp. against soil-borne pathogens. Gupta and Mishra (2009) [15] studied the inhibition was high with the direct use of *Trichoderma* sp. in dual culture against *Fusarium oxysporum* f. sp. p*sidii* (61-69%) and *Fusarium solani* (58-68%).

Rajkonda (2011) [32] performed dual culture experiment and observed antagonistic efficacy of *Trichoderma* spp. against pathogenic fungi such as *Alternaria alternata, Rhizoctonia solani, Aspergillus niger, Geotrichum candidum, Fusasrium oxysporum* f. sp. *spinaciae* and *Macrophomina phaseolina* under in vitro conditions. Choudhary (2012) [6] studied antagonistic potential against phytopathogen *Fusarium oxysporum* causing wilt of lentil, a disease common in Bihar. In this work nineteen isolates of *Trichoderma* were isolated and these were attributed to three species *viz*. *Trichoderma harzianum, Trichoderma viride* and *Trichoderma koningii*. Efficacy of these bio-antagonists was tested in vitro by employing double culture method and liquor culture filtrate analysis. Keeping this in view, antagonistic activity of *Trichoderma* sp. against five pathogenic fungi *viz*., *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* was assessed by dual culture plate method.

2. Materials and Methods

2.1 Isolation of the pathogens

Pathogens were isolated from infected leaves samples of *Ixora coccinea* L., *Clitoria ternatea* L., *Chrysanthemum morifolium* Ramat. and *Dianthus caryophyllus* L. plants. The pathogens i.e. *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* were identified on the basis of their cultural, morphological and reproductive characteristics with the help of available literatures (Gilman, 1957; Mukadam, 2006). Some mycoflora were identified by Agharkar Research Institute, Pune. For microscopic observations, different fungal isolates were stained with lactophenol cotton blue and observed under the microscope at different magnifications.

2.2 Antagonistic activity *Trichoderma* sp. by dual culture method

Dual culture method was used for the antagonistic activity of *Trichoderma* sp. against five pathogenic fungi i.e. *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* were studied in vitro (Rahman, 2009; Nguyen, 2014) [31, 26]. In this method, the 90 mm petriplates contain PDA medium is used. On the medium of dual control plate *Tricoderma* sp. was placed at 2 cm. away from the edge of the petri plate. Targeted fungus was similarly placed 2 cm. away from the edge of the petri plate and on the opposite side with *Tricoderma* sp. Plates without antagonistic fungi served as control. The plates in triplicate were inoculated in laboratory at temperature 26 ± 3^0C. Antagonistic activity was tested after seven days of incubation by measuring the radius (R_2) of the targeted fungus colony in the direction of the antagonistic colony and the radius (R_1) of the targeted fungus colony in the control plate.

$$\text{Percent inhibition of growth} = [(R_1 - R_2) / R_1] \times 100.$$

Where

R_1 = The radius of targeted fungus mycelium in the control plate.

R_2 = The radius of targeted fungus mycelium in the dual culture plate.

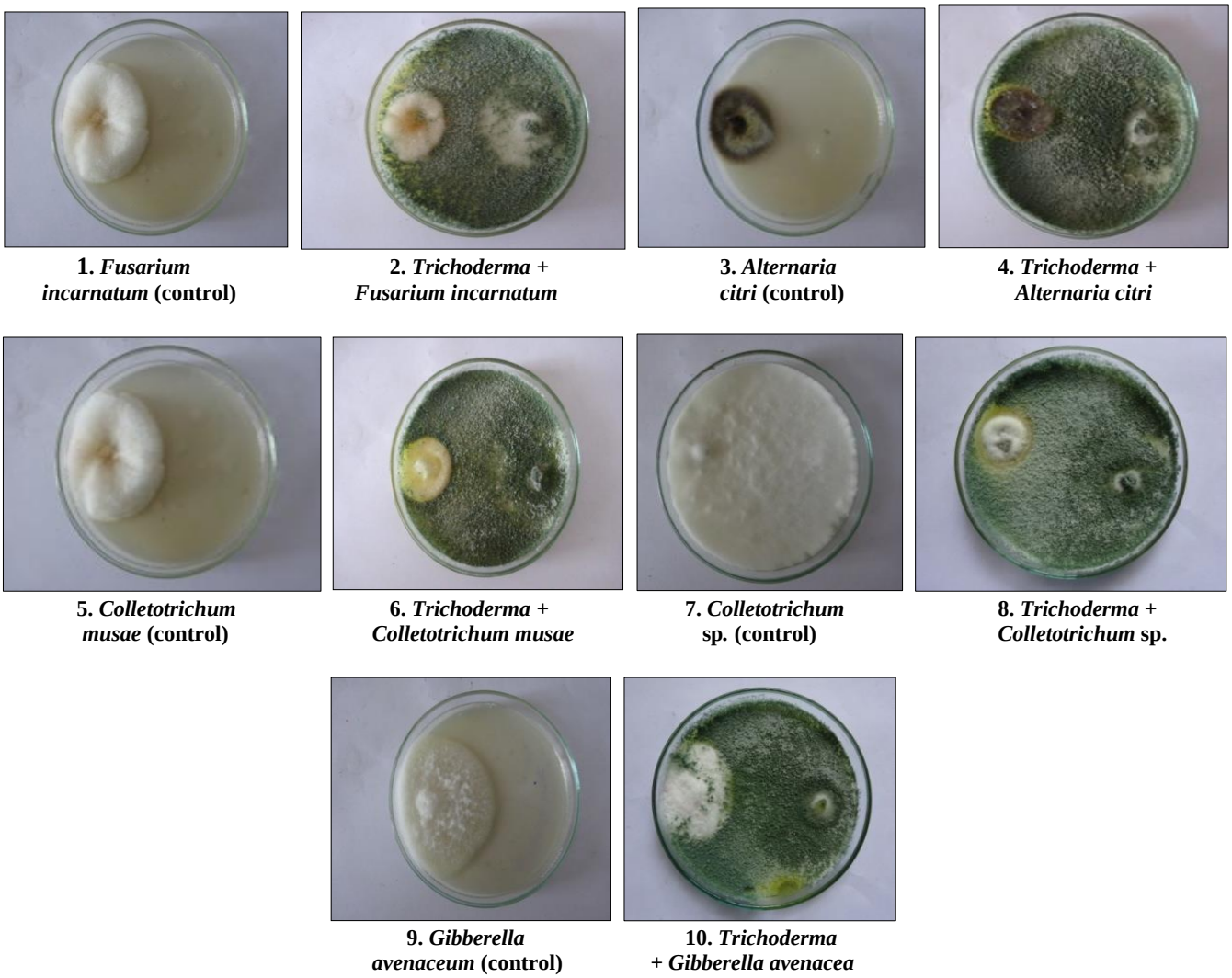
3. Results and Discussions

Antagonistic activity of *Trichoderma* sp. was studied under in vitro condition against *Fusarium incarnatum, Alternaria citri, Colletotrichum musae, Colletotrichum* sp. and *Gibberella avenacea* by dual culture plate method. *Trichoderma* sp. shows antagonistic activity against all pathogenic fungi of ornamental plants. Among the fungi maximum percent inhibition was observed against *Colletotrichum* sp. (80 %) followed by *Colletotrichum musae* (61.29 %), *Fusarium incarnatum* (56.67 %), *Gibberella avenacea* (54.05 %) and *Alternaria citri* (39.13 %) with decrease in order. It indicates strong antagonistic activity exhibited by *Trichoderma* sp. against the pathogenic fungi. There was significant difference between the radius of colonies by fungi when grown alone and those with *Trichoderma* sp. (Table 1) (Fig. 1). These results are supported by the work of Gautam and Gupta (2014) [13]. They studied that *Trichoderma* comprises a number of fungal strains that act as biological agent. Genus *Trichoderma* is efficient as bio-control agent against fungal and bacterial pathogen. *Trichoderma viride* and *Trichoderma harzianum* grow quickly in culture and simply isolated. *T. viride* control 75 % mycelial growth of *Fusarium moniliforme* and 45 % of *Fusarium sacchari*. *T. harzianum* control 70% growth of *Fusarium moniliforme* and 55% of *Fusarium sacchari.*

Ramaraju (2017) [33] evaluated the antagonistic activity of seven *Trichoderma* species against brinjal vascular wilt causing pathogen, *Fusarium oxysporum* f. sp. *melongenae* (Fom) under in vitro conditions. Out of the seven fungal antagonists studied for their efficacy, *T. harzianum* shown maximum extent of inhibition 81.11 %, followed by *T. koningii* 80 %, *T. pseudokoningii* and *T. viride* 78.88 % each, *T. virens, T. atroviride* and *T. reesei* 77.77 % each by nonvolatile compounds. *T. koningii* shown least antagonistic efficacy of 28.88 % by the production of volatile compounds. Gajera (2012) studied in vitro potentialities of seven species of *Trichoderma* against phytopathogen *Macrophomina phaseolina* by double culture method. Isolation and Characterization of *Trichoderma* sp. for antagonistic activity against root rot and foliar pathogens were studied by Krishna (2012) [12]. Patil (2012) [27] observed in vitro screening tests of three *Trichoderma species* for antagonism against *Pythium species* isolated from *Lycopersicon esculentum*. Seema (2012) [34] evaluated four fungal and one bacterial bio-agent in vitro against *Rhizoctonia solani*. Sumana (2012) [39] used

bio-agents, *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and fungicides, Carbendazim, Copper hydroxide, Propiconazole, Difenoconazole, Thiophanate methyl, Mancozeb, Tridemorph, Metalaxyland Triadimefon to manage the *Fusarium* wilt and root rot diseases of tomato both in vitro and in vivo condition in Karnataka.

Usha (2012) [42] observed antagonistic activity of *Trichoderma* sp. and *Aspergillus* sp. against *Fusarium oxysporum* causing *Fusarium* wilt disease in vitro. They observed both the bioagents effectively suppressed the pathogen. Sundaramoorthy and Balabaskar (2013) [40] studied the efficacy of *Trichoderma species* to manage the *Fusarium* wilt disease under in vitro and in vivo condition. Sharma (2014) [35] reviewed that status *Trichoderma* research in India. *Trichoderma* is known as world wild for bio-controlling of other fungal microbial community and widely exploited in industry as a source of enzyme. In India researcher is working on various aspects of *Trichoderma Viride.* Adolf (2016) [1] studied root rot of geranium transplants and its biological control and indicated that *Trichoderma harizinum* was highly antagonistic against *F. anthophilum*, *F. proliferatum* and *F. semitectum*, while it gave slight antagonistic effect against *P. ultimum*.



1. *Fusarium incarnatum* (control) | **2. *Trichoderma* + *Fusarium incarnatum*** | **3. *Alternaria citri* (control)** | **4. *Trichoderma* + *Alternaria citri***

5. *Colletotrichum musae* (control) | **6. *Trichoderma* + *Colletotrichum musae*** | **7. *Colletotrichum* sp. (control)** | **8. *Trichoderma* + *Colletotrichum* sp.**

9. *Gibberella avenaceum* (control) | **10. *Trichoderma* + *Gibberella avenacea***

Fig 1: Antagonistic activity of *Trichoderma* sp. against pathogenic fungi

Table 1: Antagonistic activity of *Trichoderma* sp. against pathogenic fungi

S. No.	Name of pathogenic fungi	R_1	R_2	Percent inhibition
1	*Fusarium incarnatum*	30	13	56.67
2	*Alternaria citri*	23	14	39.13
3	*Colletotrichum musae*	31	12	61.29
4	*Colletotrichum* sp.	70	14	80
5	*Gibberella avenacea*	37	17	54.05
Mean		38.2	14	-
S.D.		18.46	1.87	-
Value of t		2.92 (p = 0.01)		

R_1 = Radius of pathogenic fungi in control plate.

R_2 = Radius of pathogenic fungi in dual culture plate.

4. Conclusion

Antagonistic activity of *Trichoderma* sp. against five pathogenic fungi *viz*., *Fusarium incarnatum*, *Alternaria citri*, *Colletotrichum musae*, *Colletotrichum* sp. and *Gibberella avenacea* was assessed by dual culture plate method (Fig.1). On an average the radius of pathogenic fungi in control plates (i.e. when those were grown alone) was 38.2 mm. It was highest (70 mm) for *Colletotrichum* sp. while least for the pathogen *Alternaria citri* (23 mm) however, the radius of almost all fungi under investigation reduced within the range of 12 to 17 mm with an average value of 14 mm in dual culture. Among the fungi maximum percent inhibition was observed against *Colletotrichum* sp. (80%) followed by *Colletotrichum musae* (61.29%), *Fusarium incarnatum*

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