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Research Article

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In vitro antifungal activity of *Trichoderma* strains on pathogenic fungi inciting hot pepper *(Capsicum annuum L.)*

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ABSTRACT

Biological control agents with ecofriendly approach now a day's remained as a requisite in crop protection strategy against indiscriminate usage of chemicals. Contemporarily integration of technology made fungal agents potent with broad spectrum control. Hence present work aims at determining the antimycotic ability of Trichodermae species as biocontrol agent in controlled conditions against phyto-pathogens like fusarium and Colletotrichum species. Results revealed that Trichodermae viridae showed maximum inhibition on radial growth of fusarium oxysporiumf.s.p.capsici (76.74±0.4) in dual culture while its volatile metabolites on growth of C.gleosporides (53.3±0.3) and nonvolatile metabolites on C.capsici (70.94±0.6). While Trichodermae harzianum exhibited its maximum potentiality in arresting radial growth of C.capsici at 87.6±0.9 percent in dual culture while 58.6±0.2 reduction by volatile metabolites and over growth at 5% filtrate application by nonvolatile metabolites. From the results it has been illustrated that both Trichodermae species may serve as biological agents and in combination work effectively to control plant diseases with respect to their mode of infection and host pathosystem promoting harmful chemical free agricultural practices.

Key words: Trichodermae species, Antagonism, phytopathogens, antifungal activity.

INTRODUCTION

Chilli (*Capsicum annuum* L) an economically fourth essential vegetable crop grown unanimously all over the world for its outstanding nutritional and therapeutic values. India is the world's largest producer, consumer and exporter of chilli accounting to 36 percent of global chilli production. Although production is high in India, the average productivity is less (1ton/ha), when compared to other important producers of chilli viz, China, Mexico, Taiwan where the productivity is 3 tons/ha, as intermittently its production was being challenged by many biotic stresses. Among them fungal diseases are reported to be most devastative especially Colletotrichum and fusarial species were more common causing Anthracnose and fusarium wilt resulting in yield loss more than 50 percent [1] at both qualitative and quantitative aspects. *Colletotrichum capsici* (Syd.) Butler and Bisby is an efficiently significant cosmopolitan fungi reported to infect 45 genera of plant kingdom and most commonly found to occur in India [2]. Mostly transmits by air or moist and hemibitrophic in nature of infection. *Colletotrichum gloeosporioides* Penz. a facultative parasite infecting wide range of hosts at both mature and immature stages of fruit majorly spread by rain splashes and air causing anthracnose in chili. *Fusarium oxysporium f s.p.capsici* is a soilborne fungus, infects by means of its chlamydospores which remains active in soil for several years. All these fungi are highly pathogenic with complex nature exhibiting variability with respect to their environmental conditions. In such scenario farmers are relying on the usage of pesticides and fungicides which sequentially impart negative impact onto environment. As an alternative biological control with an eco-friendly approach is considered to be best. Especially research area

on fungal biological control was rapidly developing [3] with successful stories. Few fungi like *Verticillium lecanii* against Whitefly and thrips , *Lagenidium giganteum* against Mosquito larvae, *Trichoderma harzianum* against Wide range of fungal diseases, *fusarium oxysporium* against *F. moniliforme* and *Gliocladium virens* against several plant diseases like damping off & root pathogens etc., Hence this study was aimed to identify potentiality of *Trichodermae* species as biocontrol agent on phyotpathogen inciting chilli.

Trichoderma are, highly opportunistic, soilborne green spored filamentous-ascomycete found all over the world exhibiting adaptability to various ecological conditions [4, 5]. Mycoparasitism, antimicrobial metabolites, induction of plant defense system are key factors contributing to bio control measures of these fungi. On the other hand, *Trichoderma* spp. had also been employed in a wide range of commercial enzyme productions, i.e., cellulases, hemicellulases, proteases, and ß-1, 3-glucanase. All these qualities have made *Trichoderma* spp. acknowledged in industry as sources of enzymes, and in agriculture as biofungicides/growth promoters. *Trichoderma* spp. are living entities, the activity and survival of which are dependent on biotic and abiotic environmental factors which limited it to be a potent chemical fungicide. Alternative is to understand the exact molecular mechanism of biocontrol and search for strains of *Trichoderma* with improved biocontrol potential, as well as ability to survive in adverse environmental conditions may pave a way to improve sustainable crop production without causing any harm to surroundings. Therefore the present work aims at evaluating antimycotic ability of *Trichoderma* species against *Colletotrichum capsici* and *Fusarium oxysporum* phyto pathogens using dual, volatile and non volatile assay.

EXPERIMENTAL SECTION

Isolation of *Trichoderma strains*

Trichoderma strains viz., T.viridae and *T.harzianum* were collected from Varsha Bioscience Pvt. Ltd., Hyderabad. Strains were confirmed based on morphological characters *viz.,* colony growth, color, conidia shape, conidiophores branching, phialide length etc. Pure cultures were maintained on PDA agar slants at 4^0C and used for inhibition studies.

Isolation of phytopathogens *strains*

Fruit rot and vascular wilt infected fruit and stem of chilli cultivars were collected from farmer's field. Isolation was carried out using tissue transplanting technique [6]. Infected stem bits (5mm) were surface sterilized with 4% sodium hypochlorite solution for 5-10 minutes followed by subsequent washings with sterile distilled water. Then bits are transferred onto Potato dextrose agar (PDA) medium plates and pure cultures were obtained following single spore technique [7] when incubated at 25 ± 2^0C for a week. Pathogens were confirmed based on morphological characters described by [8] *viz.,* colony growth, color, conidia size and shape. Monospore culture was maintained on PDA agar slants, stored at 4^0C and used for inhibition studies.

Dual culture assay

Antagonistic activities of *Trichoderma* spp. were determined using dual culture technique proposed by [9]. A 5 mm plug of 7 day old pure culture of antagonistic fungi & pathogen were carefully excised and inoculated on opposite ends of a PDA plate with 3 cm away from each other, whereas control plates were inoculated with sterile agar disc in place of antagonist and incubated at 25 ± 2^0C for 7 days. In case of slowly growing pathogen antagonist is placed two days after inoculation. Radial growth of pathogen was measured every day after inoculation. A set of three replications were maintained and radial growth of pathogen and its inhibition by *Trichoderma species* were assessed by using the formulae: Percent of inhibition (PI) = C-T/C x 100 Where, C = Growth of test pathogen with absence of antagonist (cm), T = Growth of test pathogen in presence of antagonist (cm).

Antimycotic efficiency of Volatile Metabolites

The antimycotic effect of *Trichodermae* volatile metabolites was evaluated according to the method described by [10]. Petri plates containing PDA medium were centrally inoculated with a 5 mm diameter disc of antagonistic strains and pathogen individually. The plates were incubated for 3 days at 28^o C. The upper lid of each Petri plate was removed aseptically and lower plate containing pathogen was placed over a plate containing antagonist strain. The plates were enclosed by three layers of para film or cellophane adhesive tape to prevent the loss of volatile substances from sides of a petriplate and incubated for 5 days at 28^o C. The Petri plate containing PDA without antagonist serves as control. Each assay was performed in triplicate. The percent inhibition was obtained using the following formula: Inhibition (%) = (**D1 – D2)/D1 x 100**; where D1 represents the diameter of radial growth of pathogen in control and D2 represents the diameter of radial growth of pathogen with antagonist [11].

Antimycotic efficiency of Non Volatile Metabolites

Poisoned food technique was used to determine the antagonistic activities of potent antifungal metabolites present in *Trichoderma spp*. culture filtrate obtained after 10 days of incubation in potato dextrose broth at 28^0C. Ensuing 10 ml of culture was centrifuged at 10,000 rpm for 20 minutes. The supernatant was filtered by Whattman No.1 filter paper and conceded through 0.34 µm Millipore filter. Different volumes of filtrates were added to the molten PDA medium to obtain final concentrations of 5, 10, 15, (v/v). The medium was poured into Petri plates and inoculated with 1 cm mycelial plug of 4-day-old actively growing pathogen culture. The Petri plates were incubated at 28 ± 2^0 C for 4 days. Control plates were maintained without culture filtrate. Radial mycelia growth of the pathogen (colony diameter) was measured and inhibition percentage was calculated and recorded by using formulae proposed by [11].

RESULTS AND DISCUSSION

Isolation of *Trichoderma strains*

Collected sample powder was serially diluted and observed for growth and abundant growth of monospore culture was observed at 10^{-4} dilution plate on PDA medium. Culture was examined under microscope for structural conformation and morphological characteristic features. Color from upper surface varied from watery white to bluish green color and lower surface from yellow to amber. Surface mycelium was whitish and floccose in nature, Growth rate for 4 days ranged from 8-9.0 cms. Microscopic features conidia are round in shape with diameter 1.5 µm and tuft green in color. From the above observation strain was conformed to be *Trichodermae viridae* (Fig.1) in accordance with key given by [12].

Fig 1: *Trichodermae viridae* at macro and microscopic examination

Color from upper surface varied from white green to dark green color and lower surface from colourless to drab color. Surface mycelium was whitish and compact in nature with concentric circles, Growth rate for 4 days ranged from 9.5 cms. Microscopic features conidia are smooth and subglobose round in shape and yellow to pale green color. From the above observation strain was conformed to be *Trichodermae harzianum* (Fig.2) in accordance with key given by [12].

Fig 2 : *Trichodermae harzianum* at macro and microscopic examination

Isolation of plant pathogens:

Colletotrichum gleosporides is confirmed based on tophographic features proposed by [13]. Upper surface varied from white to dark orange or pink color with delicate and thin mycelium. Growth rate is 0.9 cm/ day. Size of conidia is 13.5 ±4.0µm. cylindrical conidia while Colletotrichum capsici is confirmed based observations and characteristic features described by [14]. Upper surface varied from white or grey to black color with cottony mycelium. Growth rate 0.8 cm /day Presence of setae, Size of conidia is 21±3.0µm , Aseptate conidia with Falcate or half moon shape and *fusarium oxysporium f.s.p.capsici* was conformed based on [15]. Colonies initially appear in white and then turn to orange or purple at maturity. Growth rate is 0.7 5 cm / day. Conidia are fusiform in shape slightly curved and pointed at tip mostly 3-5 septate, basal cells pedicellate.

Dual culture assay

Results from present investigation by dual culture assay indicated that both isolates of *Trichoderma harzianum and viridae* exhibited varied antimycotic activity over radial growth of serious plant pathogens in *invitro* condition. *Colletotrichum capsici* radial growth was inhibited by Trichodermae viridae (66.1%) and *Trichodermae harzianum* (87.6%) after 5 days of inoculation. Among them *T.harzianum* had revealed highest inhibition when compared to that of [16]. While inhibition by *T. viridae* was in accordance with the results of Naglot (2015) [17]. Potent *Colletotrichum gleosporides* mycelial growth was restricted by *Trichodermae viridae* (64%) with maximum inhibition than that of [17]. While *Trichodermae harzianum* (50%) inhibition rate was in harmony with results of [18]. *F. oxysporum f. sp Capsici* growth was reduced utmost by *Trichodermae viridae* (76.7%) when compared to that of [19] showing 68.4 percent of inhibition while *Trichodermae harzianum* with (68%) in dual culture assay performed shown in (Table 1 and Fig 3)

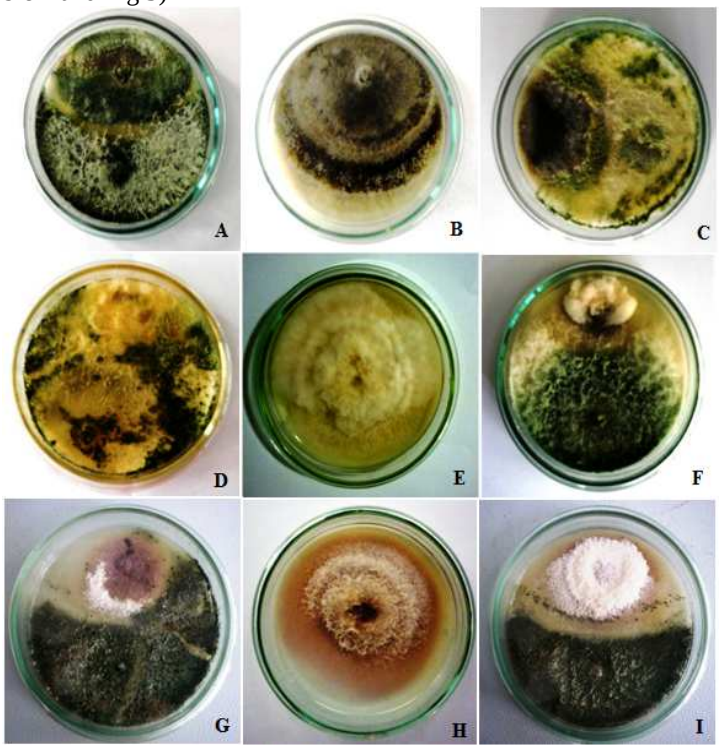

Fig 3: A *Colletotrichum capsici* with *Trichodermae harzianum* , *B : C.capsici* , *C: C.capsici* with *Trichodermae viridae. D: Fusarium oxysporium F.sp.capsici* with *T.viridae. E: F.sp.capsici F : F.sp.capsici* with *T.harzianum. G: Colletotrichum gleosporides* with *T.harzianum H: C.gleosporides I: C.gleosporides* with *T.viridae*

Table 1 : Assessment of antimycotic effect of *Trichodermae* species on Plant pathogens

Percentage of mycelia growth inhibition by antagonist in Dual culture		
Plant pathogen	T. viridae	T. harzianum
Colletotrichum capsici	66.19 ± 1.0	87.6 ± 0.9
Fusarium.oxysporium f.sp.capsici	76.74 ± 0.4	68.66 ± 0.3
Colletotrichum gleosporides	64.24 ± 0.1	50.65 ± 1.0

**Values are mean of three replicates ±Standard error*

Volatile assay:

Volatile metabolites present in the filterate of *Trichodermae viridae* revealed its highest potentiality by inhibiting *Colletotrichum gleosporides* at 53% and *Fusarium oxysporium f.s.p.capsici* growth by 46% superior than that of [20] and least inhibition on *Colletotrichum capsici* at 25% after 7 days of inoculation on PDA medium. While *Trichodermae harzianum* restricted *C. capsici* growth by 58% followed by 37 % of *f.s.p.capsici* and 34% on *C.gleosporides* (Table 2) respectively. Biocontrol capability of *Trichodermae* varies with respect to pathogen and cultural conditions.

Table 2 : Effect of *Trichodermae* species volatile metabolites on radial growth of pathogens

Plant pathogen	Percent growth inhibition of antagonist	
	Trichodermae viridae	*Trichodermae harzianum*
Fusarium oxysporium f.s.p.capsici	46.6±0.8	37.3±0.7
Colletotrichum Gleosporides	53.3±0.3	34.2±1.6
Colletotrichum capsici	25.5 ±0.2	58.6±0.2

**Values are mean of three replicates ±Standard error*

Poisoned food technique

This technique is mostly used to estimate the efficacy of non volatile metabolites present in antagonist culture filtrate bioassay over phytopathogen. Both strains of *Trichodermae* showed significant restriction over their growth when employed at 5, 10, 15% v/v concentration (Table 3).

Among different proportions (5, 10, 15) 5 % of *T.harzianum* filterate had completely arrested *C.capsici* growth and T.*viridae* 15% could be able of inhibiting its growth upto 70 percent signifying its capability to be an proficient biocontrol agent with minimum aliqout in agreement with that of work done by [16]. Then *C.gelosporides* strain growth was more reduced from 35, 52 to 65 percent in presence of *T.viridae* filtrate than *T. harzianum* with 31, 42 and 48 percent of inhibition. Consequently *Fusarium oxysporium f.s.p.capsici* motility was restricted at 51, 60 and 66 percent of inhibiton by *T.viridae* filtrate when compared to that of 24, 42, 63 percent by *T.harzianum* respectively whose Inhibition rate was superior when reviewed with work done by [21].

Table 3: Effect of *Trichodermae* species nonvolatile metabolites on radial growth of pathogens

Plant pathogen	Antagonist	5% Culture filterate	10% Culture filterate	15% Culture filterate
Fusarium oxysporium f.s.p.capsici	T.V	51.53±0.5	60.66±0.6	66.43±0.3
	T.H	24.71±0.4	42.82±0.3	63.48±0.5
Colletotrichum Gleosporides	T.V	35.64 ±0.3	52.52 ±0.1	65.32 ±0.1
	T.H	31.82±0.5	42.81±0.5	48.92±0.4
Colletotrichum capsici	T.V	50.79±0.4	66.45±0.4	70.94±0.6
	T.H	Completely inhibited	Over growth	Over growth

**Values are mean of three replicates ±Standard error*

CONCLUSION

Among two strains of *Trichoderma* screened against three phytopathogens for mycoparasitism and antibiosis, *T. viridae* was considered to be more potent by inhibiting *C.gleosporides* and *fusarium oxysporium f.s.p. capsici* radial growth via dual culture assay, volatile and non-volatile assay while *T. harzianum* showed its maximum inhibitory ability over *C.capsici* in three methods when compared with other two fungi. On a whole both organisms had showed their maximum and minimum percent of inhibition to be as biocontrolagents. Biological control is now being a key strategy in crop protection program meant for controlling pests worldwide. From this case study both species can be considered as biocontrol agents and remain more efficient when used in combinations only after large scale of field level application. These preliminary studies serve as a primary step in developing sustainable agriculture especially in controlling devastative diseases leading to severe yield loss in chilli crop.

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