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Modes of Parasitism Between the Necrotrophic Fungus *Botrytis cinerea* and *Trichoderma* spp

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Abstract

This study aims to understand the differential antagonistic activity of the *Trichoderma* spp. against *Botrytis cinerea* (grey mould) on tomato plants. The antagonistic efficiency between *Botrytis cinerea* and *Trichoderma* spp. viz., *Trichoderma reesei*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma longibractum* were studied *in vitro* using dual plate technique. The results revealed that all of the *Trichoderma* isolates had the ability to inhibit the mycelial growth of grey mould. The percentage reduction in the growth of *B. cinerea* after seven days of incubation at 23 ± 2 ℃ varied between 35-84%. The *Trichoderma* spp. such as *T. reesei* (A1) and *T.harzianum* (E1) showed the highest antagonistic activity (*T. reesei* (A1) – 84%; *T. harzianum* (E1) – 72.8%). SEM studies at cellular level have shown the collapse of hyphal wall of *B. cinerea* at an early stage. Clear evidence on direct parasitism was recorded on most of the *Trichoderma* spp. tested in this experiment. In bioassay experiments, *B. cinerea* applied alone was found throughout the leaf tissues in high densities after an incubation period of five days at 18 ℃ in a moist chamber rather than when pathogen and antagonists were applied together. Based on previous records of *Trichoderma* spp., biocontrol potential and observations of its colonizing properties, it appears that *T. reesei* can compete and reduce the growth of *B. cinerea* in tomato plants at an early stage and enhance the growth of the plants.

Keywords: Biological control, antagonistic potential, cell damage, grey mould, *Trichoderma* spp.

1. Introduction

Grey mould caused by the fungus *Botrytis cinerea* Pers.:Fr., is one of the most common crop diseases that is responsible for serious crop losses in more than 200 plant species worldwide (Williamson *et al*., 2007). This fungus can negatively affect all of the above ground organs of plants, especially the buds, flowers and fruits (Elad *et al*., 2007). It normally enters through a wound or infect plants that are under stress, although it can also infect healthy plants, especially under humid conditions. Biological control of necrotrophic pathogens such as *Botrytis cinerea* can be achieved by the suppression of spore production by the pathogen on necrotic plant tissues, resulting in a lower density of initial or secondary inoculum of the pathogen within the crop (Fokkema, 1993; kohl, 1995). The use of fungicides immediately before or after harvest to prevent rots is being increasingly limited because of environmental, toxicological and technical risks (Jamalizadeh *et al*., 2008). Moreover, the onset of resistance towards the few authorized fungicides is a frequent phenomenon in the population of fungal pathogens (Spotts and Cervantes 1986; Guizzardi *et al*.,1995; Stehmann and DeWard, 1996). Recently, a worldwide tendency has been increased towards use of eco-friendly methods in plant protection (Hajieghrari *et al*., 2008).

The application of biocontrol agents against postharvest rots of biotic origin is more suitable since the environmental factors are more stable and can be controlled. Considerable research effort has been devoted to select organisms that effectively control postharvest diseases of fruit, vegetables, and grains (Wilson *et al*., 1996). A number of studies have been demonstrated on the potential to control grey mould rot of bacterial and fungal inoculants such as *Trichoderma harzianum* (Harman *et al*., 1996;Zimand *et al*., 1996; Fravel *et al*., 1998), *Gliocladium roseum* (Yu *et al*., 1997; Köhl *et al*., 1995; Sutton *et al*., 1993), *Bacillus pumilus*, *B. amyloliquefaciens* (Nari *et al*., 1996), *B. subtilis* and *Pseudomonas syringae* (Fravel *et al*., 1998). The antagonism of *Trichoderma* spp. has been observed *in vitro* (Mishra *et al*., 2011) greenhouse conditions and field trials (Kexiang *et al*., 2002). Some strains of *Trichoderma* also promote plant growth and yielding through enhanced production of plant hormones and vitamins, improved nutrient uptake and acquisition, etc. (Joshi *et al*., 2010). Consequently, the antagonistic potential of *Trichoderma* spp. against pathogens is considered to be successfully used in biological control instead of the application of chemical plant protection products against phytopathogens. Population densities of the antagonists in the necrotic substrates necessary to achieve sufficient control levels and the timing of antagonist applications may depend on the underlying mechanisms.

Furthermore, necrotic tissues from different crops may have a differential effect on the expression of such mechanisms.

The experiments on biocontrol activity by *Trichoderma* spp. against grey mould were carried out with the following objectives. (i) To evaluate the differential antagonistic activity of *Trichoderma* spp. against *Botrytis cinerea* under *in vitro* conditions and to identify the species of highest competence for grey mould inhibition. (ii)To illustrate the mode of antagonism between *Botrytis cinerea* and *Trichoderma* spp. (iii) Bioassay on tomato leaves to test the antagonistic effect under *in vitro*. (iv) Microscopic and cellular level studies to analyze and compare the behavior of the four antagonists against *B.cinerea*.

2. Materials and Methods

2.1 *Trichoderma* strains

The biocontrol agents such *T. reesei* (A1, A2, A3, B1), *T. viride* (C1, C2, C3), *T. harzianum* (E1), *T. hamatum* (F1), *T. longibractum* (D1) were obtained from National Collection of Industrial Microorganism in National Chemical Laboratory, Pune. They were sub cultured and maintained in PDA medium at 25^0c throughout the course of work.

2.2 *Botrytis cinerea*

Two strains of *Botrytis cinerea* obtained from NRC Grapes and MACS Agharkar Research Institute, Pune were tested. Among them MACS Agharkar strains seems to virulent, hence it is used throughout the research. They were sub cultured and maintained in PDA medium at 18^0c throughout the course of work.

2.3 Estimation of antagonistic efficiency of *Trichoderma* spp. against *Botrytis cinerea*.

Interactions between antagonistic and pathogenic fungi were determined by dual plate technique (Dennis and Webster, 1971). The mycelial discs of 5mm diameter was removed from the margin of one week-old culture of *Trichoderma* spp. *(T. reesei, T. viride, T. hamatum, T. harzianum* and *T. longibractum)* and *B.cinerea* were placed on the opposite of the plate at equal distance from the periphery. The completely randomized block design with four petri dishes for each antagonist was used for the experiment. In control plates (without *Trichoderma*), a sterile agar disc was placed at opposite side of *B. cinerea* inoculated disc. The plates were incubated at 23 ± 2 ℃ and observed after 3, 5and 7 days for growth of antagonist and test fungus. Index of antagonism as percent growth inhibition of *B. cinerea* was determined by following the method of (El-Naggar *et al*., 2008). A portion of mycelia samples were removed from the interaction region (–pathogen hyphae) in dual-culture tests (5days after inoculation), were fixed on slide and observed under an inverted binocular light microscope (Axioplane 2, 40x) for the presence of spores attachment and coiling structures for wall disintegration. Samples were taken on 5 days after application of the antagonists, since this time period to be optimal. Agar block (2 × 2 mm) were cut from the interaction region of *Trichoderma* spp. and *Botrytis cinerea.* Each treatment has been replicated five times. Samples from same treatments were bulked.

Growth reduction of *Botrytis cinerea* by dual plate technique were determined as follow: R = (A-B)/A × 100, where: R = Percentage reduction in the growth of pathogen, A = Radius (cm) of pathogen colony in control culture, B= Radius (cm) of pathogen colony in test dish.

2.4 Scanning electron microscope (SEM) studies

For SEM observations, samples were taken at 5 days after application of the antagonists. During 3^{rd} day after inoculation the rate of interaction is minimum, while 7 days after inoculation, there is complete overgrowth of the antagonists. Hence 5 day after inoculation seems to be optimal to evaluate the mode of interaction between *B. cinerea* and *Trichoderma* spp. The samples were mounted on aluminium stubs with the help of double sided conducting carbon tape. Analysis was done by keeping the samples in a variable pressure mode (low vacuum mode) and irradiated with an accelerating voltage of 15 KV. Images were recorded by using FEI make Quanta 200 3D Dual beam ESEM. The experiment was repeated twice on two replicate plates for each interaction. For each replicate, 5 agar blocks for each sampling time were examined using scanning electron microscopy (SEM).

2.5 Bioassay on detached tomato leaves

Detached tomato leaves were washed thoroughly with tap water to remove soluble nutrients and subsequently blotted dry with sterile filter paper (No. 21). A pair of leaves was placed in each moist chamber consisting of a sterile plastic petri dish (90 mm in diameter) lined with sterile cotton followed by two sterile filter papers (80 mm in diameter) moistened with 1 ml of sterile water. The treatments were each of the above mentioned antagonists applied with *B. cinerea,* while the control was *B. cinerea* alone. Pair of tomato leaves were placed in a moist chamber, conidial suspension of *Trichoderma* spp.(5×10^6 cells ml^{-1}) followed by *Botrytis cinerea* (5×10^6 cells ml^{-1}) were applied. Thereafter, leaves were further incubated at 18 ℃ for 24h in the dark. Visual observations were taken 3, 5, 7 days after application of the antagonists. Small section of leaves about 2mm X 2mm were cut from the central part of randomly selected leaf segments of each replication of each treatment

were bulked, fixed and viewed through light microscope (Data not shown). The above mentioned six treatments along with control were experimented under completely randomized block design with three replicates.

2.6 Statistics

The data were statistically analyzed through completely randomized block design by online software WEB AGRISTAT PACKAGE provide by ICAR, Goa

3. Results

3.1 Mycelial interactions between *Trichoderma* strains and *Botrytis cinerea*

In Petri dish dual cultures, the first apparent contact between hyphae of *B. cinerea* and those of *Trichoderma* spp. occurred within 2 to 3 days after inoculation (DAI) followed by growth inhibition. The rate of inhibition of *B.cinerea* during *in vitro* interaction with bio-control agents of *Trichoderma* spp. at 3, 5 and 7 DAI are depicted

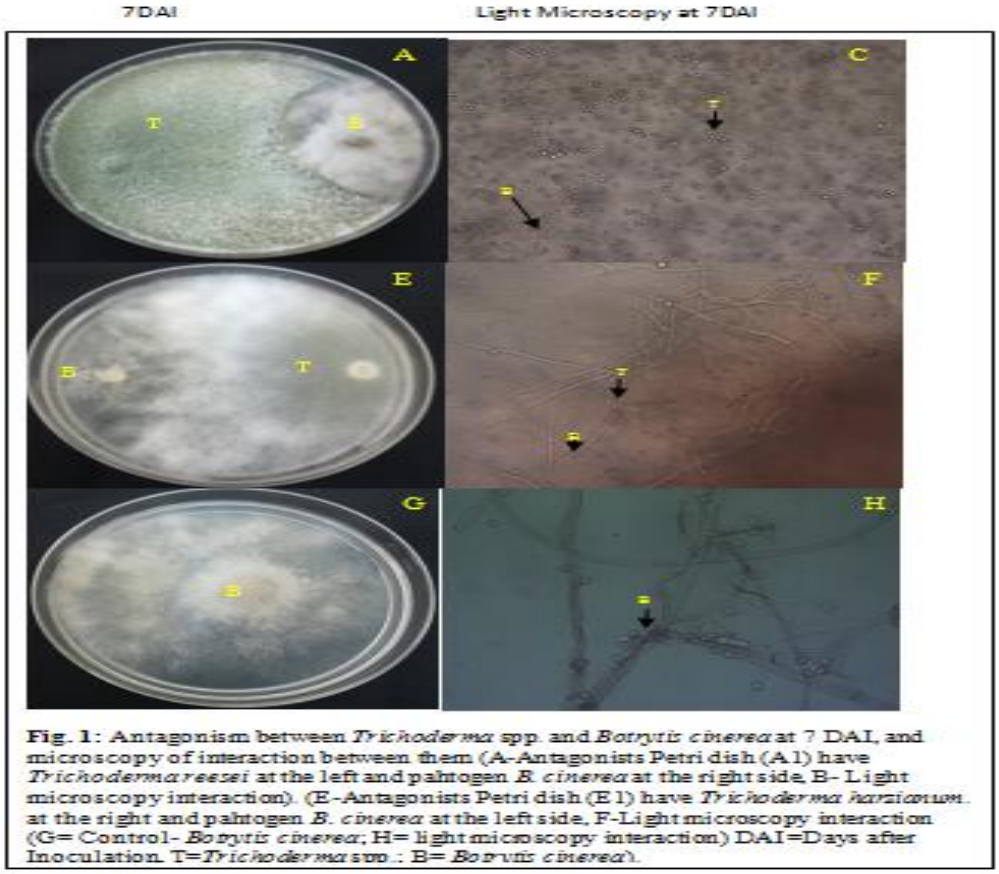

Fig. 1: Antagonism between *Trichoderma* spp. and *Botrytis cinerea* at 7 DAI, and microscopy of interaction between them (A-Antagonists Petri dish (A1) have *Trichoderma reesei* at the left and pahtogen *B. cinerea* at the right side, B- Light microscopy interaction). (E-Antagonists Petri dish (E1) have *Trichoderma harzianum*. at the right and pahtogen *B. cinerea* at the left side, F-Light microscopy interaction (G= Control- *Botrytis cinerea*; H= light microscopy interaction) DAI=Days after Inoculation. T=*Trichoderma* spp. ; B= *Botrytis cinerea*).

Table. 1. Growth of antagonists during *in-vitro* antagonism with *B.cinerea* at 3, 5 and 7 DAI

	Growth of antagonists (mm)		
Antagonists	3rd day	5th day	7 th day
T. reesei(A1)	6.47[a]	7.39[a]	8.00[a]
T. reesei (A2)	4.18f[c]	5.10[e]	5.80[fg]
T. reesei(A3)	4.38d[c]	5.29[de]	6.00[de]
T. reesei(B1)	4.27[c]	5.19[de]	5.90[ef]
T. viride (C1)	3.79[d]	4.71[f]	5.43[gh]
T. viride(C2)	5.15[b]	6.07[c]	6.73[c]
T. viride(C3)	4.15[c]	5.07[e]	5.76[g]
T. longibractum(D1)	4.60[c]	5.52[d]	6.20[d]
T. harzianum(E1)	5.70[b]	6.62[b]	7.26[b]
T. hamatum(F1)	3.40[d]	4.32[f]	5.06[h]
Control	0.53[e]	1.78[g]	2.60[i]
CV	3.77	1.85	1.70
CD(0.01)	1.01	0.55	0.54
CD(0.05)	0.74	0.40	0.40

*****Values represent mean of three experiments, each performed in duplicate.

[z]Treatments in each column followed by a common letter are not significantly different from each other according to ANOVA-CRBD

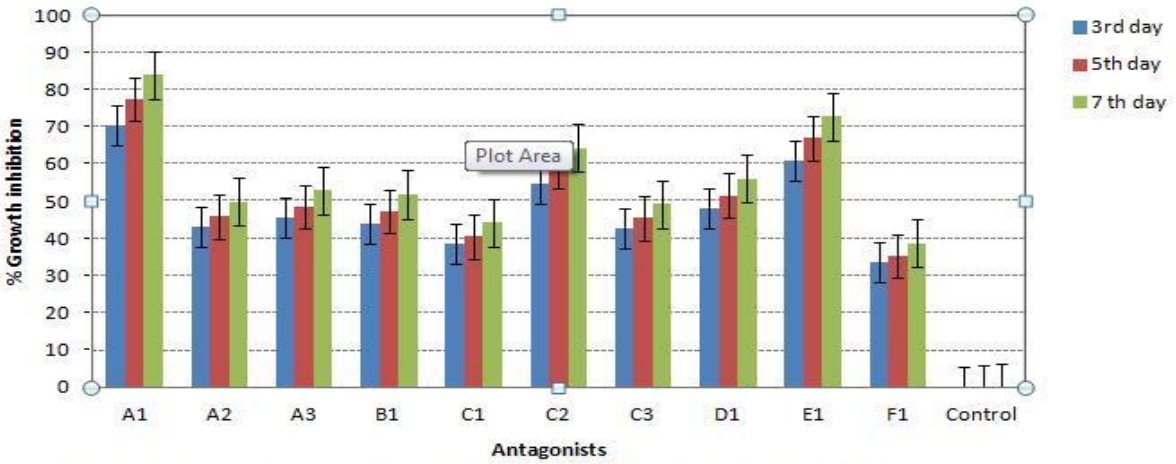

Figure 2: Percent growth inhibition of *Botrytis cinerea* during *in-vitro* antagonism with *Trichoderma spp.* at 3,5 and 7 DAI

T1=*T. reesei* (A1)×*B.cinerea* ; T2= *T. reesei* (A2)×*B.cinerea*; T3 = *T. reesei* (A3)×*B.cinerea*; T4= *T. reesei* (B1)×*B.cinerea*; T5=*T. viride*(C1)×*B.cinerea*; T6 = *T. viride*(C2)×*B.cinerea*; T7= *T. viride*(C2)×*B.cinerea*; T8=*T. viride*(C3)×*B.cinerea* T9=*T. longibrctum*(D1)×*B.cinerea* T10=*T. harzianum*(E1)×*B.cinerea* T11=*T. hamatum*(F1)×*B.cinerea* T12=*B.cinerea*–Control, DAI=Days After Inoculation.

Percent growth inhibition of pathogen (*B.cinerea*) was higher in *T.reesei* (A1) (84.37%) followed by *T.harzianum* (72.91%), *T. viride* (C2) (64.58%), *T. longibractum* (56.25%) and *T.hamatum* (53.85%) at 7 DAI. Mycoparasitism of antagonists were observed upto 10DAI. The antagonist *T. reesei* (A1) and *T. harzianum* were recorded above 60% and *T. hamatum* was recorded below 40% at all time intervals. The

mode of parasitism at cellular level was studied using light and scanning electron microscopy (40X).

3.2 Evaluation of parasitism through light microscope

Trichoderma spp. tested in this work had a marked significant variation in inhibition of pathogen growth under *in vitro*. These results were tested under light microscope on 5 DAI. Maximum growth inhibition of *B.cinerea* was recorded with *T. reesei* (A1). By 3 DAI the rate of sporulation was minimum, while at 5 DAI the sporulation was optimum and there was clear zone of inhibition (Fig. 1). The antagonistic spore attachment on *B. cinerea* was visible. At 10 DAI, *T. reesei* completely destroyed the host and sporulated. This process occurred at different time period and intensities depending on the *Trichoderma* spp. Formation of apressoria-like structures enabled the hyphae of *Trichoderma* spp. to attach firmly to the surface of its host mycelium. Microscopic studies showed that *T. reesei* (A1) and *T.harzianum* showed attachment of spores, overgrowing and degrading *B. cinerea* mycelia (Fig. 1), whereas *T. viride* and *T. hamatum* used different mechanism against *B. cinerea* by touching the hyphae without coiling.

3.3 Evaluation of parasitism through scanning electron microscope

3.4 *B.cinerea* and *T. reesei* interaction

SEM investigation of mycelial samples collected at the zone of interaction of *B.cinerea -T.reesei* dual plate showed the typical parasitism on *B. cinerea* colony at 3 days after inoculation (DAI). The antagonist established close contact with the host by means of attachment of spores around the hyphae. At the later stage of the interaction, the pathogen appeared to be disturbed by a marked loss of turgor and by obvious morphological alterations (Fig. 3). By 5 days after inoculation, the *B. cinerea* hyphae were markedly collapsed and the growth of antagonist (*T.reesei*) was predominant on the plate.

3.5 *Botrytis cinerea* and *T. harzianum* interaction

SEM observations of mycelial samples from the interface region revealed that as soon as 3 days after inoculation the antagonist multiplied abundantly and attached around the *B. cinerea* hyphae (Fig. 3). The antagonist hyphae were very dense and tightly surrounded the host hyphae, leading to strong hyphal compression, as illustrated by the wrinkled appearance of the cell surface when compared with hyphae grown in pure cultures. By 5 DAI, the penetration of *T.harzianum* into *B. cinerea* hyphae increased and signs of cell collapse were easily noticeable (Fig. 3). Tight attachment, cell wall penetration together with hyphal collapse was typical features of reactions observed in the interaction between *B. cinerea* and *T. harzianum*.

3.6 *Botrytis cinerea* and *T. viride* interaction

At the initial stage, T.*viride* established close contact with *B.cinerea* by attachment of the spores with the host hyphae (4 days after inoculation). The antagonist multiplied abundantly and attached around the *B.cinerea* hyphae. At the later stage of the interaction (7 DAI), the antagonist had multiplied and enhanced proliferation. These events were followed by host cell wall collapse as revealed by wrinkled appearance and loss of turgor of *B.cinerea*. The pathogen (*B. cinerea*) appeared to be disturbed by a marked loss of turgor and by obvious morphological alterations.

3.7 *B.cinerea* and *T.longibractum* interaction

At an early state of parasitism (4 days after inoculation), *T.longibractum* hyphae established close contact with the host by means of frequent coiling around the pathogen hyphae (Fig. 3). At this stage of the colonization process, no external damage was visible on the intertwined hyphae of *B.cinerea*, although some shrinkage could be seen at the hyphal surface. At the later stage of the interaction, the pathogen appeared to be distressed by a marked loss of turgor and by obvious morphological alterations. By 7 DAI, the pathogen hyphae were markedly collapsed, and the antagonists profusely occupied the whole plate.

3.8 *Botrytis cinerea* and *T. hamatum*

SEM observations of mycelia samples collected in the zone of interaction between *B.cinerea* and *T.hamatum* of 4-day-old dual cultures exhibited the hyphae of *T. hamatum* by their smaller diameter (Fig. 2). They grew along the host hyphal walls and established tight binding. As the early symptom of parasitism, *T. hamatum*

hyphae were turgescent and the integrity of the cell surface was similar to that observed in single cultures. By 7 days after inoculation, active growth of the antagonist was associated with pronounced host hyphal collapse and loss of turgor and wrinkled appearance.

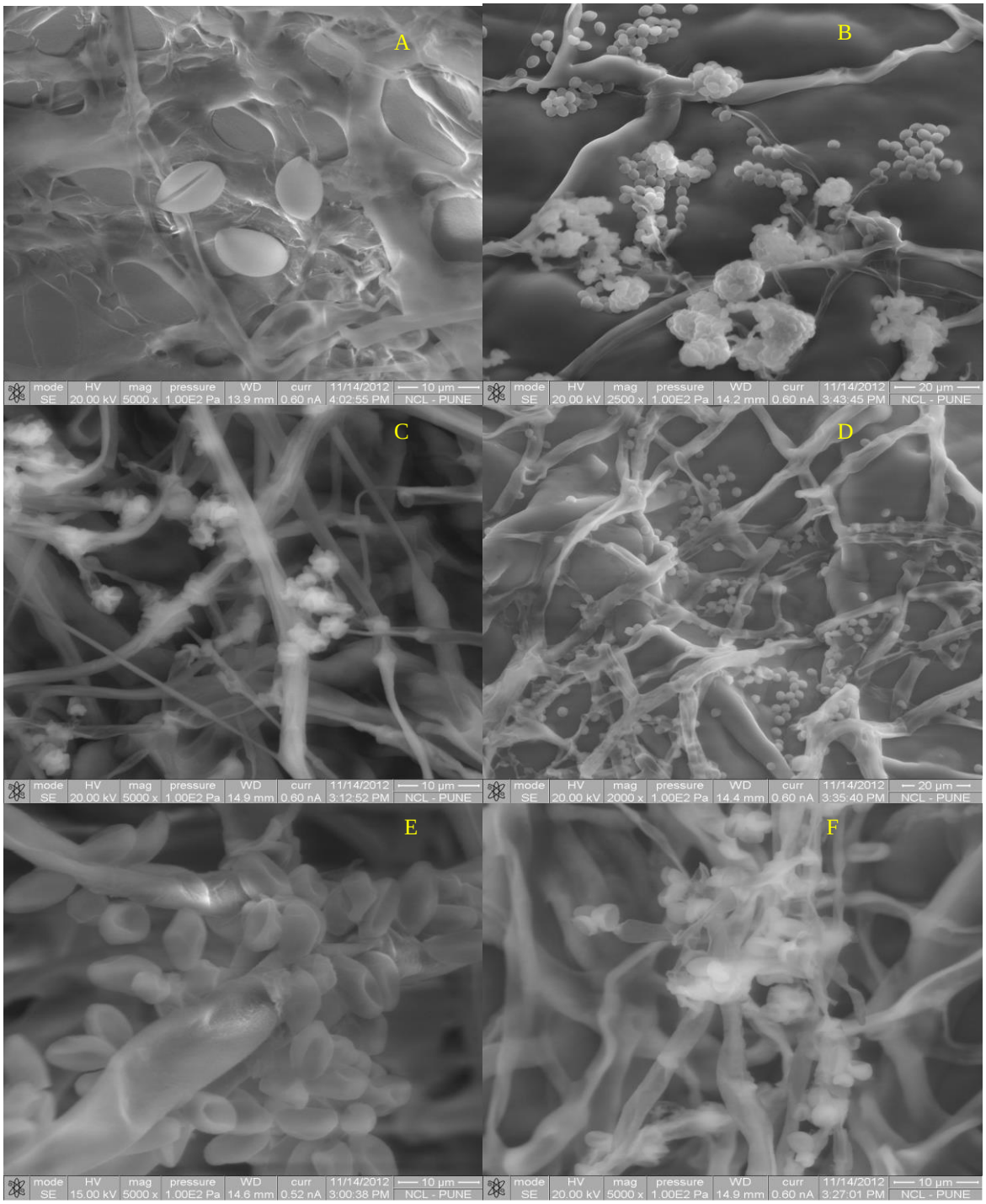

Fig. 3. Scanning electron micrograph of dual plate interaction of *Trichoderma* spp 7 DAI. A. Condia of B.cinerea. from the control plate B. Hyphal coiling of *Trichoderma hamtum* on B.cinerea hyphae. C. Spore attachment and hyphal penetration of *Trichoderma reesei* on B.cinerea hyphae. D. Spore attachment and hyphal coiling of *Trichoderma longibractum* on *B.cinerea* hyphae. E. Spore attachment, shrunken and collapsed hyphae of *B.cinerea* by *T. harzianum* F. Spore attachment and shrunken hyphae by *Trichoderma viride* on *B.cinerea* hyphae. . Bars: A, C, E, F=10 mm, B, D=20 mm.

3.9 Bioassay

Under the constant humid conditions, four antagonists viz *T.reesei, T.harzianum, T.viride, T.longibractum* efficiently suppressed the sporulation of *B. cinerea*. Symptoms developed on control leaves at 3 DAI while

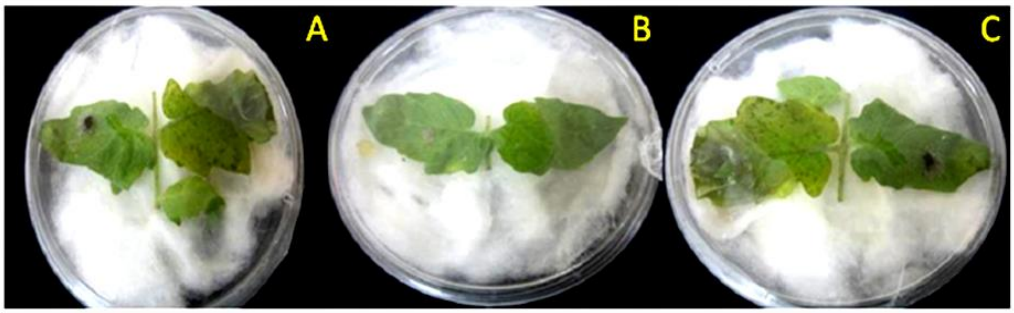

Fig. 4. Bioassay on detached tomato leaves 7 DAI. A-Control (*B. cinerea* alone), B. *T. reesei* challenge inoculated with *B.cinerea*, C. *T. harzianum* challenge inoculated with *B. cinerea*

on the treated leaves at DAI. The lesion length increased over time period. Isolations from these lesions confirmed the presence of *B.cinerea* (Fig. 4c). *T.reesei* and *T.harzianum* showed minimum infection, while *T.hamatum* exhibits maximum infection at 7DAI (Data not shown).

4. Discussion

Trichoderma spp. are ubiquitous fungi as saprophytes in the soil, highly competitive to plant pathogens. They have been shown to be successful in controlling soilborne diseases in the greenhouse and under field conditions. The most evaluated species are *T. reesei* (El-Naggar *et al*., 2008), *T. harzianum* (Lone et al., 2012; Aleksandra Bogumił *et al*., 2013), *T. hamatum* (Mathys J *et al*., 2012) and *T. viride* (Mishra *et al*., 2011). Several reports available on *Trichoderma* spp. are against *Rhizoctonia solani, Macrophomina phaseolina, Sclerotium rolfsii* (Baek *et al*., 1999; Ramezani, 2008), few reports document antagonism of *T. reesei* against *B. cinerea* (El-naggar, 2008).

Current study discusses the differential antagonistic activity of *Trichoderma* spp. against grey mould. The work on dual culture with different *Trichoderma* spp. tested, revealed that *T. reesei* (A1) showed maximum (84.37%) growth inhibition of test pathogen followed by *T. harzianum* (E1) (72.91%) while *T. hamatum* (F1) showed minimum inhibition (33%) at 7 DAI as compared to control (*B. cinerea* only) (Fig. 1, 2,Table 1). Pattern of growth inhibition of *B.cinerea* was continued with maximum 14.5% increase in A1 (85.2%) followed by 7.5% elevation in E1 (65.6%) antagonists during 3 to 7 DAI. Most of the *Trichoderma* isolates grew rapidly and intensively covered the entire surface of the Petri dishes after 7 days. A clear zone of inhibition between *T. reesei* and *B. cinerea* was observed. Based on the time interval evaluation between 1-7 DAI, it was observed that *T.reesei* starts the multiplication at 24 to 48hr after inoculation which activates earlier suppression of *B.cinerea* compared to other *Trichoderma* spp. (Fig. 1, 2, Table 1). Earlier report by El naggar (2008) demonstrated 30% growth inhibition of grey mould by *T. reesei*. Fiume and Fiume (2006) observed the antagonistic activity of *T. harzianum* against grey mould at a range from 4.7% to 75.76% between three to seven days of incubation.

SEM studies have shown slight variation in the mode of interactions of *Trichoderma* spp. against *B. cinerea* in their antagonism. Mycoparasitism involves morphological changes, such as coiling and formation of appressorium- like structures, which serve to penetrate the host. Differential antagonistic activity has been observed for various *Trichoderma* spp. (Blakeman and Fraser, 1971; Schirmbock *et al*, 1994, Tronsmo and Dennis. 1977). Our results on light and scanning electron microscopic study revealed that *T. reesei* (A1) and *T.harzianum* (E1) showed effective coiling on pathogen *B. cinerea* at 3 DAI, and may start the parasitism earlier during antagonism compared to other *Trichoderma* spp. Similar results have been reported for *T. reesei* against *B. cinerea* (El -Naggar, 2008), however the rate of inhibition was 30% at 5DAI as against 77.45% in the present study.

SEM investigations on interaction regions in dual plate technique demonstrated that growth inhibition and structural alterations of *B.cinerea* started to appear soon after contact with hyphae of *T. reesei* mostly at 3 DAI compared to other *Trichoderma* spp. viz., *T.viride, T.harzianum, T.hamatum, T.longibractum*. Coiling, penetration and stunted hyphae were observed as signs of hyphal parasitism of *B. cinerea* (Fig. 3). These observations provided support to the assumption that the outcome of the interactions likely was determined by early recognition events that triggered firm antagonist binding to the host cell surface, leading to subsequent responses such as attachment of spores, hyphal wall shrinkage and host penetration. Positive correlations between surface-associated components and recognition events in microbial interactions often have been (Elad, 1996; Benhamou and Chet, 1987) considered as key determinants in the outcome of a given interaction.

Based on the colonization pattern between *T.reesei* and *B.cinerea,* it is known that nutrient competition within the substrate would play a major role in antagonism (Howell, 2003), since the substrate would only be depleted

locally at the site where colonies are formed. Generally necrotrophic pathogens, such as *B. cinerea* depend on exogenous nutrients for growth and survival. Reduction of nutrients generally leads to reduction in conidia and germ tube germination (Blakemen 1971). Based on the cell wall deformities, such as shrunken, wrinkled and loss of turgor of *B. cinerea* hyphal wall strongly suggests the release of antibiotics by *T.reesei* (Ghisalberti and Sivasithamparam, 1991). Certain cell wall degrading enzymes, i.e. cellulase and chitinase, play important role in penetration process (Chet 1987, Schirmböck *et al* 1996). An isolate of *T.reesei* studied by (Martinez *et al*., 2008) showed hemicellulases and other plant cell wall degrading enzymes in comparison to other filamentous fungi. These extracellular enzymes are connected with mycoparasitism that is initiated against phytopathogenic fungi. Chitinases are able to lyse the hard chitin cell wall of mature hyphae, conidia, chlamydospores and sclerotia (Harighi *et al*., 2007). Multiple modes of action have been demonstrated by *T.harzianum*, such as competition (Belanger *et al*., 1995), induced resistance (Brožová, 2004; Harman 2000), solubilization of inorganic plant nutrients (Altomare *et al*., 1999), inactivation of the pathogen's enzymes involved in the infection process (Claydon *et al*., 1987; De Meyer *et al*., 1998) and mycoparasitism (Cruz, et al., 1995; Barnett and Binder 1973). To confirm the efficacy of *Trichoderma reesei*, bioassay has been performed with detached tomato leaves using B.*cinerea* alone and in combination with *Trichoderma* spp. Minimum infection was recorded in *T. reesei* as compared to other antagonists tested (Fig. 4). Although the mechanisms by which *Trichoderma* spp. operates differ according to the host species, the current study reveals that competition and antibiosis are the common features of the antagonism. *T. reesei* has showed higher antagonistic activity against *B. cinerea* which starts earlier as compared to other findings. Green house studies are in progress to confirm the biocontrol activity of this species and its potential involvement in the antagonistic process. The microscopic observations have provided insight into the mode of action of *Trichoderma* spp. paving the way for selection of biocontrol agents for management of diseases. This information would play a key role in establishing the respective behaviour of the antagonists and its influence on the eventual success of biocontrol of *Botrytis* spp.

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