

Original Research Article

BIOLOGICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* ISOLATED FROM IVORIAN COTTON BY *TRICHODERMA* SPP.

ABSTRACT

Evaluation of the antagonistic activity of some *Trichoderma* species by direct confrontation method was used in this study. The mycoparasitism inhibitory effects of *Trichoderma* species on the growth of the causal agent of cotton Fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) were investigated by dual culture under *in vitro* condition. Seventeen isolates of *Trichoderma* and one strain of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) were used. These different isolates of *Trichoderma* were *in vitro* confronted to FOV. The invasion ability of antagonist colonies was measured during 14 days. Thus, at the end of these control trials, isolates of *Trichoderma* have controlled the growth of FOV. Indeed, FOV is confined to a small area and is unable to extend its growth after the third day of incubation in the culture room at 30 °C. Beyond this period and after then days, they never sporulated, revealing the inhibitory action of *Trichoderma* against FOV. Consequently, the *Trichoderma* isolates were found able to biologically control of FOV growth.

Keywords: Cotton, Biological control, Fusarium wilt, Mycoparasitism, *Trichoderma* spp.

1. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is not only the most important fiber crop in the world, but also the second best potential source for plant proteins after soybean and the fifth best oil-producing plant after soybean, palm-tree, colza and sunflower [1]. Cotton is infected by a number of pathogens. But *Fusarium oxysporum* and *Rhizoctonia solani* are recognized as the main pathogens in cotton seedling disease. Moreover, *F. oxysporum* f. sp. *vasinfectum* (FOV) cause Fusarium wilt of cotton disease. It occurs in most major cotton production regions of the world [2]. Wilt of cotton is a vascular disease caused by the soil borne pathogen FOV. The disease is widespread and causes substantial crop losses. Control of FOV may be achieved by applying tremendous volume of fungicides during the cotton plant culture. However, the continued use of these products has created serious problems for the environment and human health. Biological control of phytopathogens by microorganisms has been considered to be more natural and environmentally acceptable alternative to the existing chemical treatment methods [3]. In addition, biological control using microbial pesticides or microorganisms is accepted as an efficient mean of reducing environmental pollution and poisoning of consumers [4- 7].

The genus *Trichoderma* includes the most common saprophytic fungi in the rhizosphere and is found in almost any soil. The harmful ability of *Trichoderma* species against pathogens for induce resistance at plants allows the development of different bio-control strategies [8, 9]. Several *Trichoderma* spp. reduce the incidence of soil borne plant pathogenic fungi under natural conditions [10]. Recently there have been numerous attempts to use *Trichoderma* spp. against soil borne pathogens such as *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia* species [11]. *Trichoderma* is often used to prevent development of several pathogenic fungi in biological controls. Moreover, John *et al.* [12] reported that *Trichoderma* can be considered as a biological control agent against *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes* and as a growth promoter of soybean.

Different isolates of *Trichoderma* have various strategies for fungus antagonism as well as several indirect effects on plant health. Possible mechanisms of antagonism applied by *Trichoderma* spp. includes nutrient and niche competition, antibiosis and myco-parasitism [13] that act as inhibitors against many soil-borne fungi, and last parasitism [14]. *Trichoderma* strains have a better competence to mobilize and take up soil nutrients compared to other organisms. It was noticed by Tjamos *et al.* [15] that *T. harzianum* controls

Fusarium oxysporum by competing for both rhizosphere colonization and nutrients. They observed that biocontrol became more effective as the nutrient concentration decreased. *Trichoderma* can be used in direct biocontrol. They parasitize a variety of fungi and they are able to detect other fungi, and growing towards them for prevents from developing. The distant sensing is partly due to the sequential expression of cell wall degrading enzymes, mostly chitinases, glucanases and proteases [16]. Most *Trichoderma* strains produce volatile and nonvolatile toxic metabolites that obstruct colonization by antagonized micro organisms. Some of these metabolites have been studied and the production of harzianic acid, alamethicins, tricholin, peptaibols, 6-penthy- α -pyrone, massoilactone, viridin, gliovirin, glisoprenins, heptelidic acid and others have been described [17]. The role of *Trichoderma* is also indicated in plant growth promotion. Chang *et al.* [18] (1986), observed enhanced germination, rapid flowering and increase in height and fresh weigh of plants treated with *Trichoderma*. Many reports indicate the involvement of *Trichoderma* in induction of defense mechanisms in plants. It was found that the addition of *Trichoderma* metabolites results in the synthesis of phytoalexins, PR proteins and other compounds in plants. And, thereafter, may increase resistance in plants against several plant pathogens, including fungi and bacteria [10, 19]. *Trichoderma* produce three types of propagules: hyphae, chlamydospores and conidia [20]. Conidia have been commonly employed for use in biological control. In spite of the above listed advantages, expected market value has not yet been achieved by *Trichoderma*. Substantially rapid effect of chemical pesticide on pathogen is responsible for its high market value. Therefore, it is necessary to find more aggressive strains of *Trichoderma* which can compete with chemical pesticide in terms of quick action on pathogen. The aim of the present investigation is to evaluate the *in vitro* biological potential of the *Trichoderma* species against *Fusarium oxysporum* f. sp. *vasinfectum*, causal agent of Fusarium wilt of cotton.

2. MATERIALS AND METHODS

2.1. Fungal isolate

Seventeen strains of *Trichoderma* and one strain of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) were used in the present study. The seventeen *Trichoderma* isolates (T1 to T17) were obtained from the Laboratory of Plant Physiology fungus collection of Félix Houphouët Boigny University (Abidjan, Côte d'Ivoire). Antagonism of these fungi was assessed against the pathogen, FOV which was provided by the Phytopathology Laboratory of the Agronomy school of Félix Houphouët Boigny, National Polytechnic Institute (Yamoussoukro, Côte d'Ivoire). FOV were isolated on cotton in zone of Béoumi (center of Côte d'Ivoire, West Africa). Stock cultures of test fungi were maintained on PDA (Potatoes Dextrose Agar) slants and stored at 4 °C in refrigerator.

Pure culture of isolates of *Trichoderma* and FOV were identified using Barnett and Hunter [21] method. The pure culture of the isolates was inoculated on Potatoes Dextrose Agar (PDA). It was fortified with penicillin and streptomycin antibiotics at a concentration of 0.1 ml stock solution (0.5 g of streptomycin and 1.0 UI of penicillin in 20 ml of distilled and sterile water) according method described by Tuite [22], modified and adapted to our biological material.

2.2. Pathogenicity test

Pathogenicity test was carried out when isolates of antagonists (*Trichoderma*) and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) were together inoculated on PDA in Petri dishes. Three replicates samples of each inoculation test were obtained. To achieve this one 5 mm (in diameter) culture disc was obtained from the edge of a seven days old pure

isolates each of antagonists are placed 5 cm from each other and incubated at 30°C. FOV and *Trichoderma* were inoculated simultaneously.

2.3. Dual culture technique

The antagonistic activity of *Trichoderma* spp against FOV was evaluated by dual culture technique as described by Morton and Strouble [23]. Mycelia disc (5 mm diameter) from the 7-day-old margin of the *Trichoderma* spp culture was placed on a plate. Each pathogen was then placed on the opposite of the plate at equal distance from the periphery. The experimental design used was a completely randomized with three Petri dishes for each isolate. In the control plates (without *Trichoderma* spp.), a sterile agar disc was placed at opposite side of the pathogens inoculated plates. Inoculated plates were incubated at $25 \pm 1^\circ\text{C}$ for 7 days. Twenty days after the incubation period, radial growth of mycelia was measured. PDA plates were used in triplicates and inhibition percentage of average radial growth was calculated in relation to the controls growth as follows:

$I = [(C - T)/C] \times 100$, where I is inhibition of radial mycelia growth; C is radial growth measurement of the pathogen in control; T is radial growth of FOV in the presence of *Trichoderma* isolates [24].

2.4. Statistical analysis

Experiments were performed using a completely randomized design. Data were subjected to analysis of variance (ANOVA) using STATISTICA software (release 7.0) and differences between means were compared using Newman-Keuls test. Differences at $P \leq 0.05$ were considered as significant.

3. RESULTS

3.1. Difference in growth of *Fusarium* under direct intense or less influence of *Trichoderma* on dual culture plate

Due to rapid spread of *Trichoderma* on dual culture plate, *Fusarium* was almost encircled by *Trichoderma*. The distal side of the disc colonized by *Fusarium* was curved by *Trichoderma* metabolites. Later, *Trichoderma* was trapped by *Fusarium* on all sides (Fig. 1).

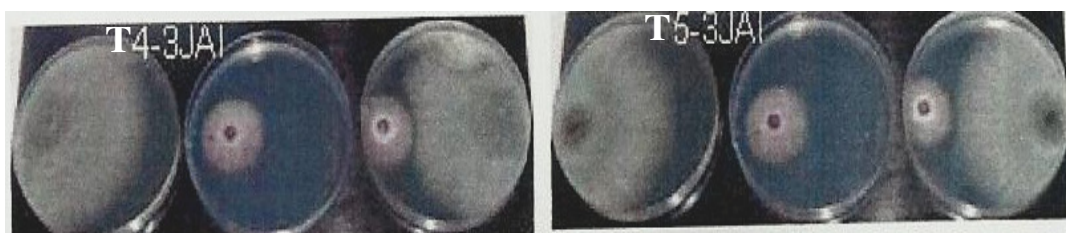


Fig 1. *Trichoderma* isolates 4 and 5 with *Fusarium oxysporum* f. sp. *vasinfectum* on a dual culture plate

Strong antibiosis between *Trichoderma* and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). FOV is confined to a small area and is unable to extend its growth due to the inhibitory action exerted by *Trichoderma*; JAI, day after infection; T, *Trichoderma*.

3.2. Effect of *Trichoderma* on *Fusarium* growth

Difference in growth of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) on dual culture plate and on control plate (FOV alone) was plotted against zone of inhibition. This was done to assess actual magnitude of *Trichoderma* influence on FOV. Figure 2 showed an irregular pattern of relationship between the inhibition zone and FOV growing. This irregular pattern of

relation implies that each of the seventeen *Trichoderma-Fusarium* interactions was unique in its expression. Every isolate of *Trichoderma* exerted a different degree of stress on *Fusarium*.

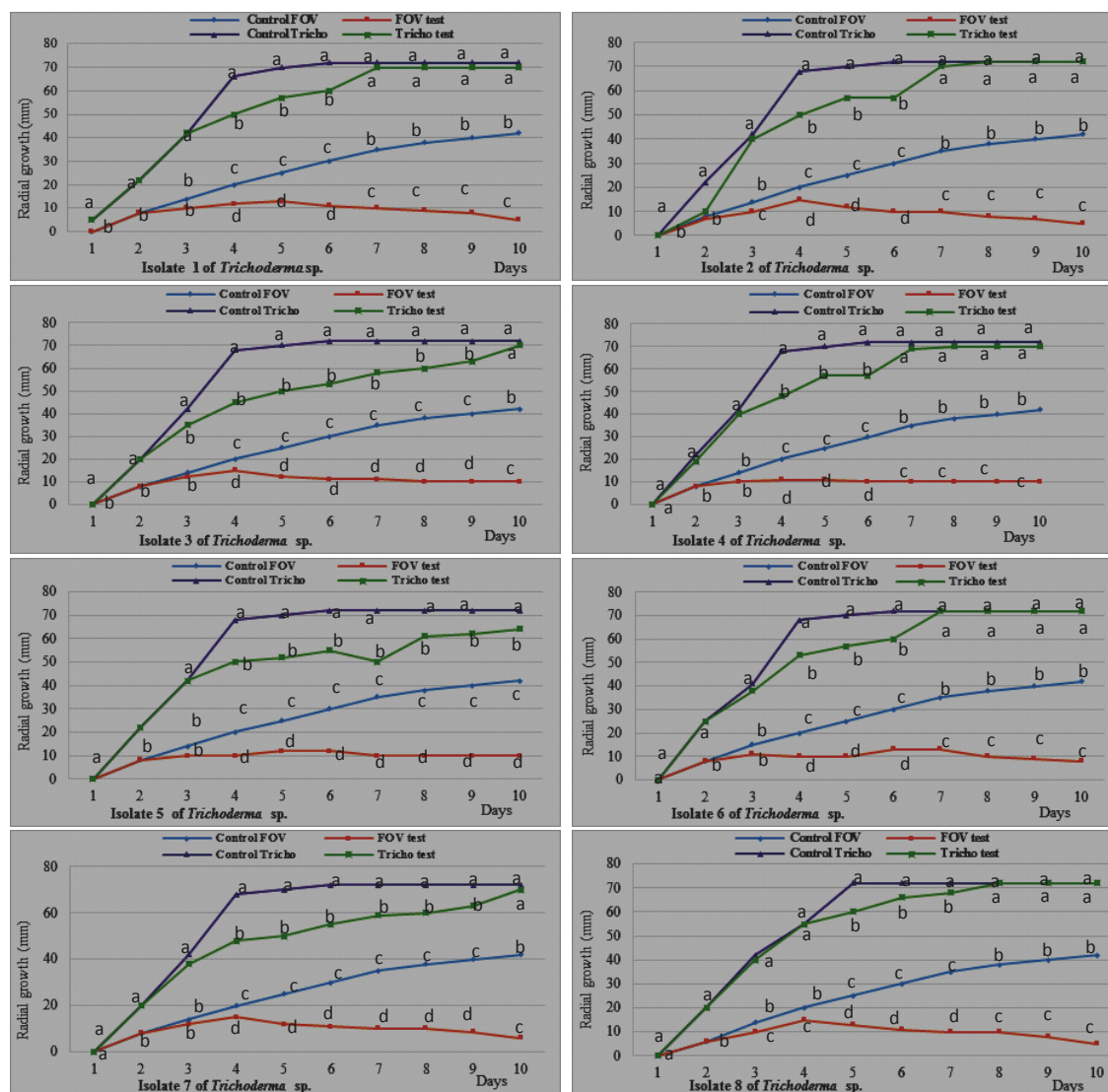


Fig. 2. Zone of inhibition analyzed in relation to growth of *Fusarium oxysporum* f. sp. *vasinfectum* influence *Trichoderma* isolates 1 to 8

Data are expressed as mean of three replicates; for a given day, values followed by a different letter are significantly different according Newman-Keuls test at 5%.

Isolate 12 of *Trichoderma* (T12) showed highest degree of antibiosis on *Fusarium* as depicted by Figure 3. Some differences were observed in this study, but, their inferences remain similar. These differences may result from the fact that the metabolites of *Trichoderma* act distance. T12-*Fusarium* interaction is the best example of long distance influence of metabolites released by both the organisms on each other.

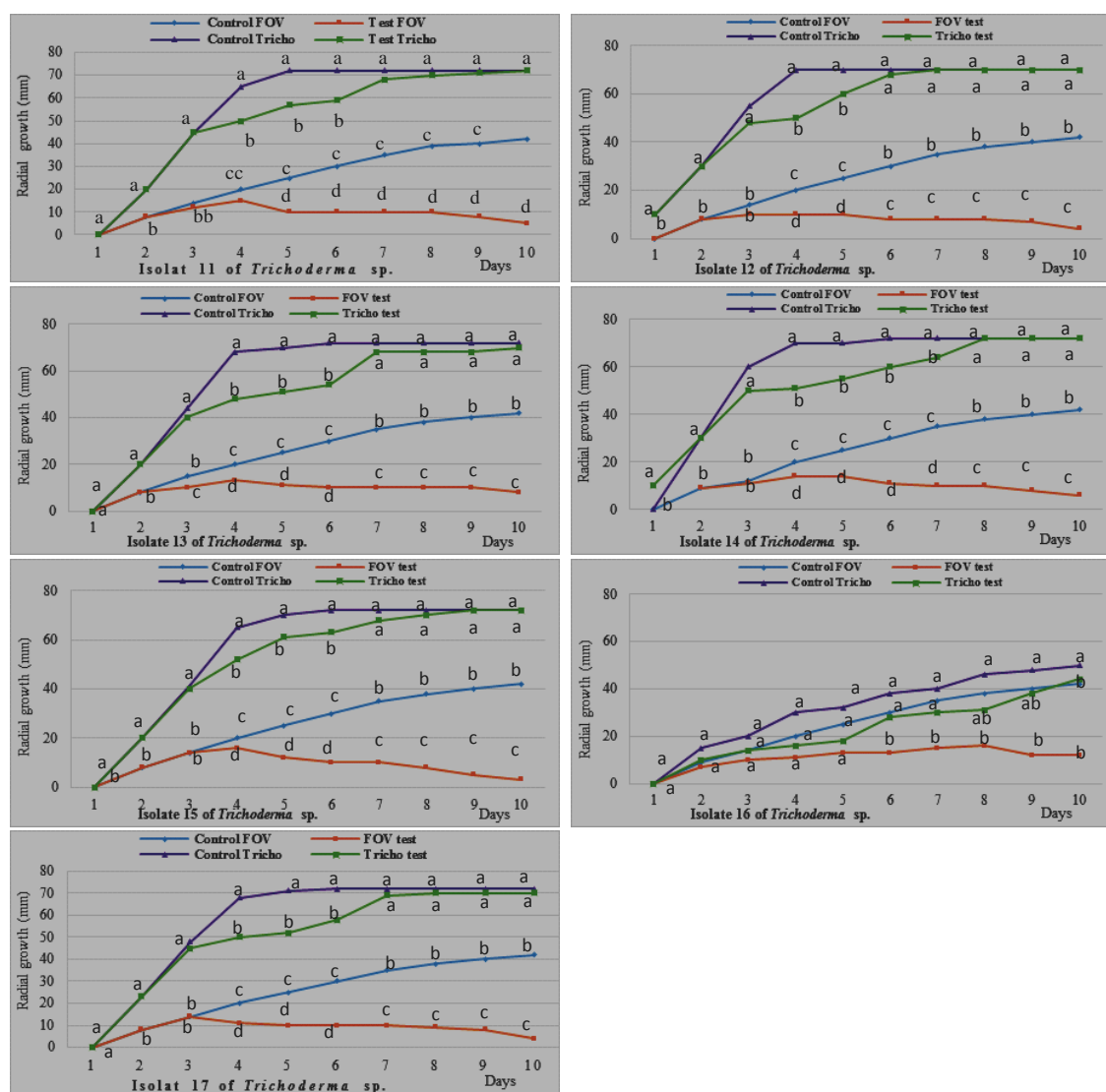


Fig. 3. Zone of inhibition analyzed in relation to *Fusarium oxysporum* f. sp. *vasinfectum* growth under the influence of *Trichoderma* isolates 11 to 17

Data are expressed as mean of three replicates; for a given day, values followed by a different letter are significantly different according Newman-Keuls test at 5%.

3.3. Zone of inhibition analyzed in relation to growth of *Trichoderma* under influence of *Fusarium oxysporum* f. sp. *vasinfectum*

The pattern of plot (Fig. 4) suggests that the growth difference of *Trichoderma* (C0) largely corresponds to zone of inhibition (C1) which implicates that *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) definitely exerted an inhibitory effect on the growth of *Trichoderma*.

No correspondence wasn't found between the growth *Trichoderma* under influence of *Fusarium* and conversely.

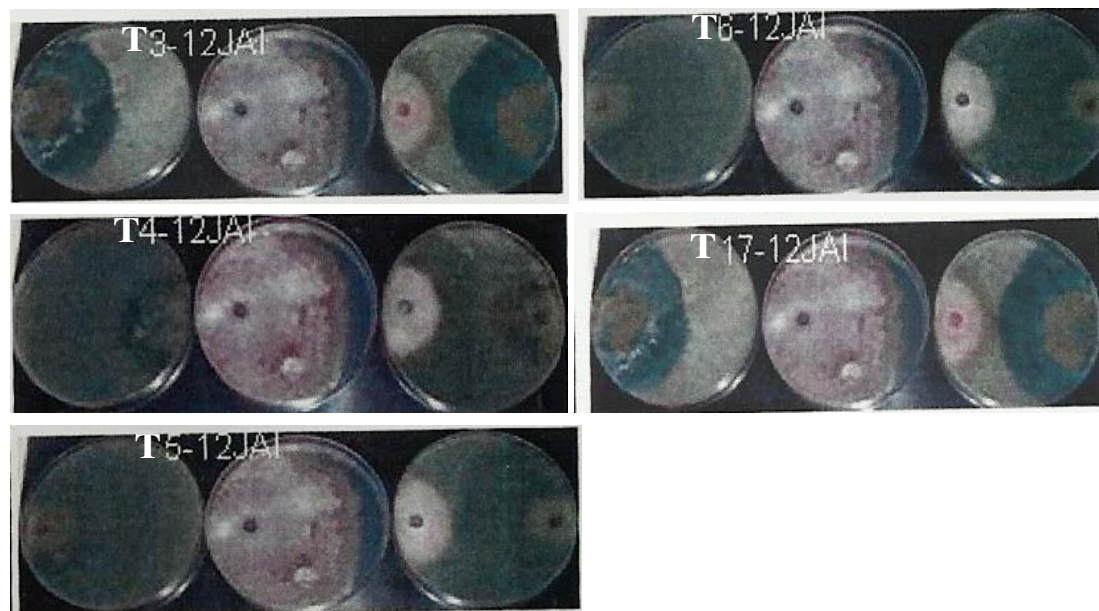


Figure 4. Isolates of *Trichoderma* greatly affected by *Fusarium oxysporum* after 12 days of infection

JAI : day after infection; T3, T4, T5, T6 and T17 : isolates 3-6 and 17 of *Trichoderma*

3.4. Analysis of counter inhibition shown by *Trichoderma* and *Fusarium oxysporum* f. sp. *vasinfectum*

The results show that most of the isolates of *Trichoderma* have inhibitory effect on *Fusarium*. Isolate of *Trichoderma* such as T3, T5, T7, T10 and T13 suffered strong opposition from *Fusarium* (Fig. 4). Exceptionally intense and almost equal opposite inhibitory counter action was observed between T8 and *Fusarium*. It is similar for isolates T13 and T17 but the inhibition counter was weaker. Moreover, isolate T2 showed similar feature but with medium intensity. All the four plots suggest that the degree of antibiosis imposed by each *Trichoderma* isolate on *Fusarium* and the level of competitiveness and opposition shown by *Fusarium* towards each *Trichoderma* isolate was uniquely different. In other words, the consequence of counter stress shown by both the organisms on each other was specifically a character of individual *Trichoderma-Fusarium* interaction for each of these the isolates of *Trichoderma*.

3.5. Interaction *Trichoderma-Fusarium oxysporum* f. sp. *vasinfectum*

After the phase of antibiosis, the next phase is to cross the zone of inhibition and initiate the mechanism of parasitism. The measure of efficiency of a biocontrol agent such as *Trichoderma* is determined by its ability to cross the zone of inhibition posed by the pathogen and to overwhelm the pathogen. The best biocontrol agent is the one which takes least time to perform this process. The total time taken by biocontrol agent to cross zone of inhibition and overwhelm pathogen was the prime criterion for evaluation of biocontrol potential of *Trichoderma* isolates. Besides *Trichoderma* isolate T1 other isolates such as T2, T7, T8, T9, T10, T11, T12, T13, T14 and T15 also showed considerable activity against *Fusarium*. While

T16 was the slowest of all, T1, T3, T4, T5, T6, and T17 were unable to cross the inhibition zone imposed by *Fusarium* (data not show).

4. DISCUSSION

Antibiosis and mycoparasitism are the well known mechanisms involved in biocontrol of pathogens by *Trichoderma*; competition for nutrition, space and dominance being equally important and mutually inclusive phenomenon. The complete course of interaction between *Trichoderma* and *Fusarium* as observed on the dual culture plates can be divided into three phases. The initial phase was marked by interaction without mycelia contact. In this phase, metabolites from both organisms determine the interaction importance. The intermediate phase, in which *Trichoderma* may be inhibited or not by *Fusarium* In this intermediate phase, some chemo-attractive mechanisms may be activated. The final phase is characterized by the inhibition of *Fusarium* by *Trichoderma*. Seventeen *Trichoderma* isolates which showed biocontrol potential were analyzed in this research. The initial interaction between *Fusarium* and *Trichoderma* seem involve defensive and offensive mechanisms from both the organisms. The pattern of zone of inhibition between the two organisms clearly indicated that *Fusarium* initially induced the inhibition of *Trichoderma* with its metabolites secreted. However, it suffered of stress caused by metabolites released by *Trichoderma*.

Physiological changes in *Fusarium* were materialized by the morphological changes like waxy moist appearance, scanty or no mycelia and excessive pigmentation. According to Lorito *et al.* [25] this modification noticed in most of the test plates as a prominent feature seems to be under the effect of *Trichoderma*. On the other hand, *Trichoderma* sporulation seemed to be influenced by the presence of *Fusarium* metabolites. In certain cases, *Trichoderma* sporulation was hindered initially by *Fusarium* but in later stages, a heavy sporulation by *Trichoderma* was seen at the verge of inhibition zone marked by influence of *Fusarium*. Exceedingly fast growth rate and also heavy and quick sporulation activity of *Trichoderma* is an added advantage to its ability to overcome inhibition posed by *Fusarium*. Further in the course of interaction, *Trichoderma* reinforced itself after an initial halt and extended its mycelia towards *Fusarium*. This advancement was possible only when *Trichoderma* succeeded in effectively overcoming the inhibitory upshot of *Fusarium*. These characteristics were observed in most of the strains of *Trichoderma* tested against *Fusarium*. After a period of cross signaling by pre-contact chemical interactions between the two fungi, competent strains of *Trichoderma* were able to reach and overwhelm *Fusarium* in later stage of interaction. In all, except T16, the plates *Trichoderma* was able to encroach into the inhibition zone of *Fusarium* and extend its mycelia towards *Fusarium* followed by heavy sporulation immediately on the colony of *Fusarium* which depicts a step in the mechanism of parasitic activity of *Trichoderma* [26].

Therefore, after an initial phase of intense counter inhibition, *Trichoderma* was not only able to overcome inhibitory effect of *Fusarium*, but could also attract *Fusarium* towards itself as observed in this study. This is a striking feature of *Trichoderma* in which specific signal transduction cascade is believed to play a role. Omann and Zeilinger [27] mentioned the importance of G-protein signaling in mycoparasitic activity of *Trichoderma*. In T3, T4, T5, T6 and T17 heavy pigmentation by *Fusarium* and heavy sporulation by *Trichoderma* around *Fusarium* colony was observed. At the same time, *Trichoderma* was seen growing, precisely, on the colony of *Fusarium* and showed a great sporulation. In isolates T7, T8, T9, T10, T11, T12, T13, 14 and T15 the colony of *Fusarium* was completely overwhelmed and covered by *Trichoderma*. Precisely, *Trichoderma* covered the area on which *Fusarium* was growing, leaving the peripheral area of the colony. Later, this peripheral zone is colonized. This behavior of *Trichoderma* suggests that *Fusarium* was the target towards which *Trichoderma* was specifically attracted. Similar results were shown by all the other isolates except T3, T4, T5, T6, T16 and T17 which did not cross the zone of inhibition to parasitize *Fusarium*. *Trichoderma* isolate T3 was the quickest of all isolates in crossing the zone of inhibition and parasitizing *Fusarium*. On agricultural field, dominance of biocontrol agent through its high growth rate and offensive mechanisms against pathogen are decisive in

manifestation of disease control. Parasitism by *Trichoderma* is its powerful weapon in destruction of pathogen. But, *Trichoderma* must overwhelm pathogen before the pathogen proliferates and infects germinating plant seeds. Therefore, a measure of speed with which biological control on pathogen is achieved is important when searching an aggressive strain of *Trichoderma*. The analysis of *Trichoderma-Fusarium* interaction has been discussed in four sections mentioned below.

Two broad phenomena were observed in the results obtained in dual culture experiment. First, antibiosis; in terms of counter inhibition which clearly states that not only *Trichoderma* inhibits growth of *Fusarium* but *Fusarium* also exerts inhibitory activity on *Trichoderma*. Each isolate of *Trichoderma* exhibits uniqueness in its response to the presence of pathogen as shown by the graphs. Second, parasitism is shown by *Trichoderma* on *Fusarium* only in case *Trichoderma* is able to overcome inhibition posed by *Fusarium*. Also, the time taken by each *Trichoderma* isolate to overcome inhibition posed by pathogen and thereafter enter parasitic phase is important in determining its efficiency as a potential biocontrol agent. Other researchers have also scrutinized these mechanisms of biocontrol shown by *Trichoderma*. Vinale *et al.* [28] studied pre-contact events of the mycoparasitic interaction and divided the interaction into two phases. In the first phase, the mycoparasite (*Trichoderma*) produces specific high molecular weight compounds that reach the host (pathogen). In the second phase, low molecular weight degradation products are released from the host cell walls reach the mycoparasite and activate the mycoparasitic gene expression cascade. A number of secondary metabolites produced by *Trichoderma* spp. are involved in pre-contact events. These belong to three categories. First, volatile antibiotics such as 6-pentyl- α -pyrone (6pp) and isocyanide derivatives, second, water soluble compounds such as heptelidic acid or koningic acid, and third, peptaibols which are linear peptides known to inhibit radial growth of many fungi [29]. It has also been stated that low molecular weight, non polar, volatile compounds such as 6pp have a quite long distance range of influence on the host. Production of these secondary metabolites is stated to be strain dependent [27]. In the present research, this could be one reason why each *Trichoderma* isolate showed distinct response in presence of *Fusarium*. A number of antibiotics have been isolated from *Trichoderma* spp by other workers. Harzianolide from *T. harzianum* was found to inhibit germination of *Fusarium oxysporum* conidia and chlamydospores. Viridin produced by *T. viride* also inhibits spore germination of a number of fungi. Other antimicrobial agents such as isonitrites, sesquiterpenes and diketopiperazines are released by different species of *Trichoderma* [30, [31]. Jones and Hancock [32] stated that secondary metabolites of *Trichoderma* can reduce uptake of amino acids and glucose in the target fungus by 85%. It was also demonstrated that trichorzianines modify membrane permeability of liposomes and disturb ionic balance in the cells of target fungus [33]. Lorito *et al.* [34]; Fogliano *et al.* [35] found that peptaibols act synergistically with β -glucanases of *Trichoderma* and inhibit β -glucan synthase activity in the host fungus. The inhibition of this enzyme in the host prevents reconstruction of the host cell wall. Chet [36] interpreted that myco-parasitism by *Trichoderma* can be distinguished into four stages. The first stage is positive chemotropism which means that *Trichoderma* can detect its host from a distance. *Trichoderma* begins to branch as it approaches its target host. This phenomenon is very clearly visible in the microscopic image of dual culture on slide shown in this study. The next stage is molecular recognition event between parasite and host. This involves interaction between complementary molecules; likely to be lectin-carbohydrate interaction [37]. Lu *et al.* [38], suggested that particular compounds released by the host cell walls were responsible for inducing activation of mycoparasitic genes in *Trichoderma*. Similarly, Zeilinger and Omann [39] found that diffusible factors from host fungus are recognized by *Trichoderma* which activate transcription of mycoparasitism related genes. The authors also specified that cAMP signaling pathway was found to be involved in *T. atroviride* gene activation. During recognition events many alterations take place in the host cell such as plasma membrane retraction, cytoplasm aggregation, formation of numerous septae in the host and initiation of cell wall degradation [31, 40]. Such alterations have also been noticed in this research as shown in the microscopic slide dual culture. Further, in the third stage, the

parasite attaches and coils itself on host [41, 42]. The final stage is the lytic activity involving a range of enzymes to degrade fungal cell wall. The degradation activity is mainly due to chitinases, glucanases and proteases [43]. 1,4- β -N-Acetylglucosaminidase is among the first enzymes induced in *Trichoderma harzianum* in early stages of host recognition [44]. The expression of *Trichoderma* chitinases is very specifically regulated by the host, therefore, it is thought to play an important role in antagonism against pathogens [45]. Chitinolytic enzymes studied in *T. atroviride* showed inhibitory effect on the germination and hyphal elongation of several pathogenic fungi including *Fusarium* species. Also, many morphological changes were noted, in the target fungus such as swelling, branching, necrosis and vacuolization, by researchers [31]. Cellulolytic enzymes of *T. longibrachiatum* have also been implicated in biocontrol activity [46]. The demonstration of final effect of the mycoparasite *Trichoderma* on its host fungus largely depends on synergism shown among the assortment of enzymes produced and also on synergism shown between enzymes and antibiotics produced by the attacking myco-parasite. Di Pietro *et al.* [47] proved that *Trichoderma* enzymes and antibiotics showed 95% inhibition of pathogen spore germination when they acted in synergism but singly they were able to show only 20% inhibition. Schirmbock *et al.* [48] noted similar phenomenon with *T. harzianum* antibiotics trichorzianines and enzymes endochitinase, chitobiosidase and glucanase which showed better results in synergism rather than singly. Synergism between hydrolytic enzymes and antibiotic peptaibols of *T. harzianum* was found effective against *F. oxysporum* [34]. Also, *F. oxysporum* cell wall chitin is buried in β -glucans. Thus, synergistic activity of chitinase with beta-1,3-glucanase is essential. Lipases and proteases are also required at the same time to clear hindrances in cell wall degradation. As these enzymes diffuse and reach the host, degradation activity begins even before physical contact takes place [49]. *Trichoderma* have specific mechanisms to protect their own cell wall from their own lytic enzymes. One of such mechanisms involves activity of protein Qid3 [50, 51]. It has been interpreted in the results of this work that *Fusarium* also exerts a deleterious effect on the approaching mycoparasite *Trichoderma*. Other studies have also noted the same phenomenon where toxins such as fusaric acid produced by *Fusarium* species can adversely affect antagonistic activity of *Trichoderma* by down regulating *Trichoderma* mycoparasitism related genes. Therefore, toxins produced by *Fusarium* give this pathogen a competitive attribute [52]. In a study conducted by El-Hasan *et al.* [53] it was found that fusaric acid produced by *Fusarium oxysporum* and many other species of *Fusarium* is directly implicated in retarding mycelia growth and conidia production in *Trichoderma*. Further, in response to this effect of *Fusarium*, activity of 6-pentyl- α -pyrone from *Trichoderma* is generated to degrade and/or suppress synthesis of fusaric acid. In the present research, this can be one reason for initial halt in the progress of *Trichoderma* towards *Fusarium* after which *Trichoderma* moved ahead to parasitize *Fusarium* only when it was able to overcome inhibitory metabolites of *Fusarium*. In this research work, another feature that has been noted is excessive pigmentation by *Fusarium* when it encountered presence of *Trichoderma*. This activity is believed to be a response to the environmental changes in the vicinity of the fungus. Signal cascades are activated in fungi by such environmental changes which in turn alter their gene expressions [54, 55]. High production of pigment could be one such response to presence of *Trichoderma* and its metabolites in the vicinity. Carlile [56] reported that red- purple pigmentation in *F. oxysporum* was a result of carotenoids and a mixture of substituted dihydroxy naphthoquinones, the production of which was dependent on C/N ratio, light-dark conditions and pH variations. According to Naim and Sharoubeem [57], pigmentation in *Fusarium oxysporum* was correlated to source and concentration of nitrogen. Also, low concentration of phosphate helped production of color. Researchers have given nutritional and physiological variations as a reason for pigmentation but their role in self defense or antagonism may also be possible [58-59]. Therefore, biological control of pathogen is an outcome of bidirectional responses between the antagonist and the pathogen.

4. CONCLUSION

The goal of this work is that among several criteria defined for an ideal biocontrol agent, one important criterion is a fast acting agent. An isolate of *Trichoderma* which can overcome the inhibition posed by the pathogen and parasitize the pathogen in a short span of time will be an efficient biocontrol agent. In this study isolate 12 of *Trichoderma* showed quicker action than the others. After reaching the verge of inhibition zone posed by *Fusarium*, this agent took about two days to parasitize the pathogen. More isolates shall be screened to find a better agent in terms of rapid activity and the present isolates shall be subjected to genetic modifications. This also suggests that there may be variations in pattern and time duration of parasitism depending upon the pathogen encountered.

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