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MYCOPARASITISM AND ANTAGONISTIC EFFICIENCY OF *TRICHODERMA REESEI* AGAINST *BOTRYTIS* SPP.

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Abstract: A preliminary study was made to evaluate the antagonistic efficiency and mycoparasitic activity of *Trichoderma reesei* against *Botrytis* species, viz. *B. cinerea*, *B. allii* and *B. fabae*. The bioassay method to evaluate the efficacy of *Trichoderma reesei* was the reduction of mycelial growth of *Botrytis* spp. *in vitro*. Significant effect was observed against *B. cinerea* and *B. fabae*, however, no effect was found against *B. allii*. Hyphal and sclerotial parasitism of *Trichoderma reesei* against *Botrytis* spp. were also studied. Coiling of hyperparasite fungus, penetration into *B. cinerea* hyphae, and stunting of the latest were observed as typical signs of mycoparasitism. Coiling of *B. allii* and stunted hyphae of *B. fabae* were the only signs of hyphal parasitism observed. The sclerotia of *B. cinerea* were also parasitized but sclerotia of *B. allii* and *B. fabae* were not.

Key words: mycoparasitism, *Trichoderma*, *Botrytis*

Introduction

Usage of synthetic fungicides is generally applied method to control plant diseases, but the public concern over food safety and the development of fungicide resistance of plant pathogens has been increased demand finding alternative methods which potentially less harmful to human health and the environment. Biological control has been advanced as an alternative to synthetic fungicides, and considerable success has been achieved by utilizing antagonistic microorganisms.

Understanding the modes of action of antagonisms is essential to allow the use of antagonists under practical conditions and to enhance their role in biological control. Parasitism of fungi by various microorganisms has been recorded in many systems [1, 10, 21] and relies on the production of fungal cell wall degrading enzymes (CWDEs) [10]. *Trichoderma*, *Gliocladium* and *Pythium* species are among the best-known mycoparasites [4, 10]. Proliferation of *Trichoderma* on the margin between healthy and necrotic areas on rotting grape berries was observed by Dubos [8]. Microscopic observations revealed coiling and penetration of the mycelium of *B. cinerea* by the antagonist. *Pythium periplocum* has been found to be an aggressive mycoparasite of *B. cinerea* in culture, the parasitized mycelium of *B. cinerea* failed to infect grapes [17]. Parasitism of sclerotia has been also described. Parasitism of sclerotia of *B. cinerea* at 5°C was reported by Köhl and Schlösser [14], and production of sclerotia on grapevine was partially hindered by *Trichoderma* spp. applied in late summer [9]. Several CWDEs associate with antagonism. Isolates of *T. reesei* and *T. harzianum* capable of producing proteinase, mannase, laminarinase and chitinase were found by Labudova and Gogorova [15] and they postulated their role in mycoparasitism.

Antibiosis as a mean of biocontrol has been reported for many microorganisms [2, 20]. Sometimes it is difficult to predict the importance of inhibitory compounds in natural ecosystems that those are produced *in vitro* by antagonists. *Trichoderma* and *Gliocladium* species are common producers of antibiotics. *T. hamatum*, which produces inhibitory volatiles, reduced grey mould of snap bean pods and blossoms by 77-79% [16]. The peptaibol antibiotics, trichozianins

A1 and B1 from *T. harzianum*, and gliotoxin from *G. virens*, inhibited spore germination and hyphal elongation in *B. cinerea* [7, 18]. An isolate of *Penicillium chrysogenum* produced inhibitor products, which reduced conidial germination of *Botrytis fabae* and lesion development on faba bean [13].

The aim of the present work is to study the possible usage of *Trichoderma reesei* and its mode of action as a biocontrol agent against *Botrytis cinerea*, *B. allii*, and *B. fabae*.

Materials and Methods

Trichoderma reesei

The candidate antagonistic fungus isolate, *Trichoderma reesei* (isolated from soil in Papua New-Guinea) was stored in the Mycological Collection of Plant Protection Department, Centre of Agricultural Sciences, Debrecen University (MCDU).

Botrytis spp.

Tests were carried out with three *Botrytis* species: *B. cinerea* (isolated from bean seeds in Kafr El-Sheikh, Egypt in 2005), *B. allii* and *B. fabae* originated from MCDU.

Estimation of antagonistic efficiency of *Trichoderma reesei* against *Botrytis* spp.

The antagonistic effect was determined *in vitro* according to Ferreira et al. [11]. 5 mm agar in diameter disks covered by fungi were taken from the marginal growth of 5-day-old cultures of *Botrytis* species (*B. cinerea*, *B. allii* and *B. fabae*, respectively) and *Trichoderma reesei*. Disks of *Botrytis* spp. and *Trichoderma reesei* fungi were placed on the opposite side of Petri dishes containing potato dextrose agar (PDA) medium. The dual cultures in Petri dishes were incubated for 5 days at 25°C and three replicates were used in each test. Growth reduction of *Botrytis* spp. were determined as follow:

$$R = (A - B) / A \times 100$$

Where R = Reduction of growth in percentage

A = Distance of mycelial growth of *Botrytis* spp. away from *Trichoderma reesei* in mm

B = Distance of mycelial growth of *Botrytis* spp. towards *Trichoderma reesei* in mm

Mycoparasitism of *Botrytis* spp. by *Trichoderma reesei*

I. Hyphal parasitism

Trichoderma reesei was studied for mycoparasitic activity against *Botrytis* spp. The hyphal parasitism of *B. cinerea*, *B. allii* and *B. fabae* by *Trichoderma reesei* were studied using the following method: Small blocks of agar with fungal colonies from the margins of vigorously growing young cultures were placed 5 cm apart on cellophane over PDA. The plates were inoculated at 25°C. Colonies met approx. after 3-day-incubation period and then were examined under light microscope. For detailed observations, small squares of cellophane were cut from the area of mixed growth. These squares were mounted on microscope slides and were observed after staining with cotton blue.

II. Sclerotial parasitism

The sclerotial parasitism of *Trichoderma reesei* on individual sclerotia of *B. allii* and *B. cinerea* was tested on water agar (WA). Sclerotia were distributed on the surface of Petri dishes containing WA, and each sclerotium was inoculated with a 2 cm² piece of PDA from the margin of *Trichoderma reesei* culture. After 3 weeks incubation at 25°C, sclerotia were examined microscopically.

Results

Antagonistic efficiency of *Trichoderma reesei* against *Botrytis* spp.

Data presented in Table 1, and illustrated on Figure 1. revealed that *B. fabae* and *B. cinerea* were highly affected by *Trichoderma reesei*. Their mycelial growth were decreased by 40.2% and 30% respectively. However, no effect was found against *B. allii*.

Table 1: Reduction of mycelial growth of *Botrytis* spp. by *Trichoderma reesei* (%)

<i>Botrytis</i> species	Reduction of mycelial growth (%)
<i>B. cinerea</i>	30.0
<i>B. allii</i>	4.0
<i>B. fabae</i>	40.2

Mycoparasitism of *Botrytis* spp. by *Trichoderma reesei*

I. Hyphal parasitism

In double cultures *Trichoderma reesei* grew over the colonies of *Botrytis cinerea*. Parasitism of the hyphae of *B. cinerea* by *T. reesei* was very common. Appressorium formation as the first sign of penetration, as well as penetration of the host hyphae of *B. cinerea* by *T. reesei* was observed (Figs. 2 and 3). After penetration, the parasite developed malformed, wavy mycelium which usually pervaded the hyphae of the host, growing through the septa. Moreover, the hyphae of *B. cinerea* were stunted by *T. reesei* (Fig. 4). It was initiated by direct contact and coiling of *T. reesei* around the host hypha (Fig. 5). On the other hand, coiling of *B. allii* (Fig. 6) and stunted hyphae of *B. fabae* (Fig. 7) by *T. reesei* were the only signs of hyphal parasitism observed.

II. Sclerotial parasitism

Mycoparasitic activity of *T. reesei* on sclerotia of *B. cinerea* and *B. allii* was examined *in vitro*. Sclerotia of *B. cinerea* were parasitized but sclerotia of *B. allii* were not. Microscopic examination of free hand sections of infected sclerotia indicated that the mycoparasite invaded the medulla of sclerotia of *B. cinerea* as evidenced by the presence of the blue stained hyphae of *T. reesei* at various locations within the hyaline sclerotial tissue (Fig. 8).

Discussion

The current results help to confirm that *T. reesei* is a destructive parasite of hyphae and sclerotia of *B. cinerea* *in vitro*. Coiling, penetration and stunted hyphae were observed as signs of hyphal parasitism of *B. cinerea*. On the other hand, coiling of *B. allii* and stunted hyphae of *B. fabae* were observed as the only sign of hyphal parasitism by *T. reesei*. Coiling is not solely due to the contact stimulus, but some unknown factors may also be involved [6]. The hyphae running over the host are branched and adhere to the host hyphae, coiling around them and forming penetration pegs. Certain cell wall degrading enzymes, i.e. cellulase and kitinase, play important role in penetration process [5, 18]. Stunted hyphae may occur due to the effect of diffusing antibiotics produced by the parasite [12]. The adverse effect of the parasite on the host is presumably due to physiological changes brought about by the action of the parasite on the host metabolism [6]. The size of the host hyphae could also be a physical barrier in penetration phenomena. It was postulated that wider hyphae were easily penetrated by the narrower ones [6]. This was also confirmed by our present investigation. On the basis of the nutritional relationship of the parasite with the host, parasitism was classified into two main groups, necrotrophic and biotrophic [3]. Coiling and penetration [3, 6] are common to both interaction types, but degradation of cytoplasm and bursting of the hyphae [19] are typical of a necrotrophic relationship.

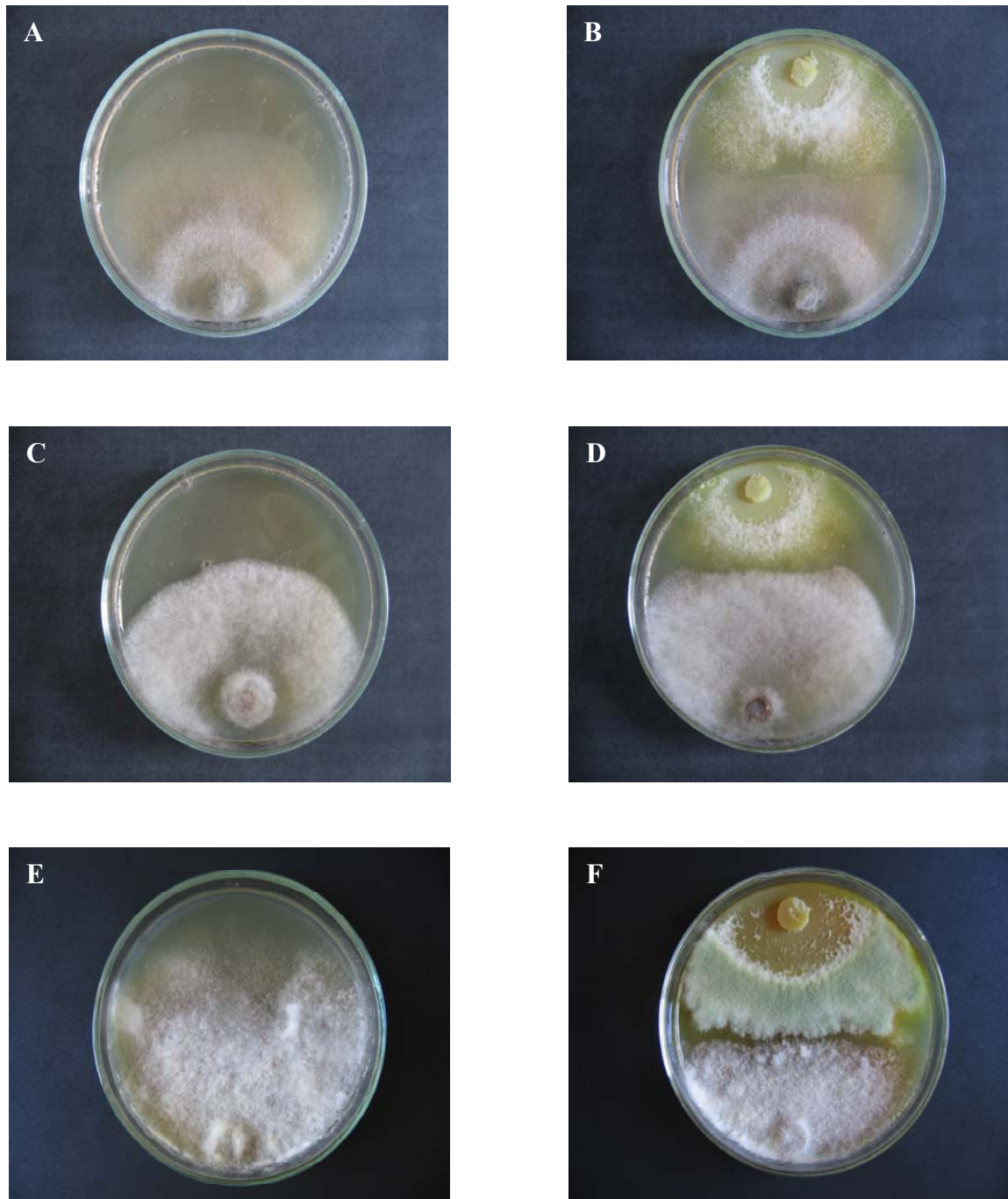


Fig. 1: Antagonistic effect of *Trichoderma reesei* against *Botrytis* spp. (hyphal growth of *Botrytis cinerea*, *Botrytis allii* and *Botrytis fabae* away from *Trichoderma reesei* (A, C, E), hyphal growth of *Botrytis cinerea*, *Botrytis allii* and *Botrytis fabae* towards *Trichoderma reesei* (B, D, F))

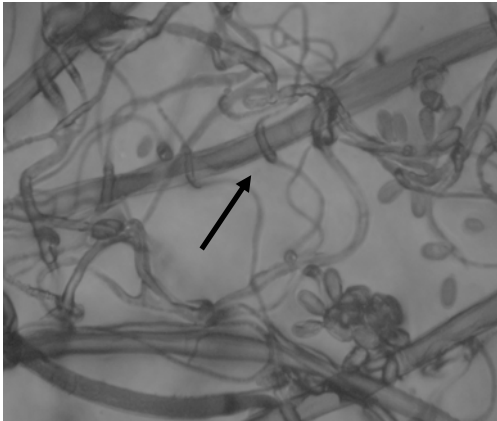


Fig. 2: Appressoria formation of infectious hyphae of *Trichoderma reesei* on hypha of *Botrytis cinerea*

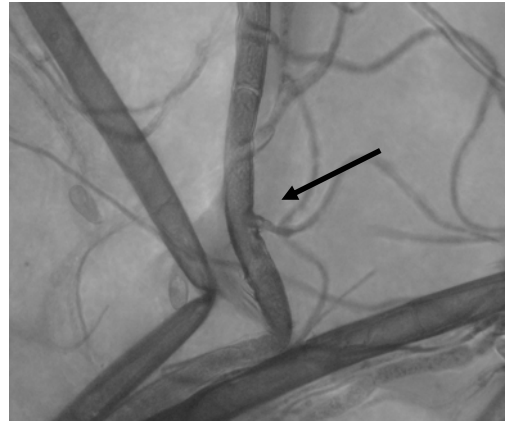


Fig. 3: A part of *Botrytis cinerea* hypha penetrated by *Trichoderma reesei*

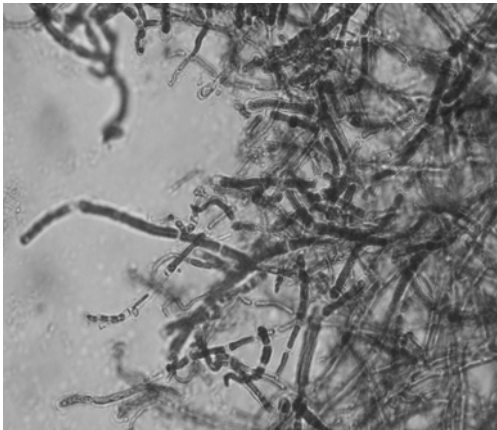


Fig. 4: Stunted hyphae of *Botrytis cinerea* by the metabolic effect of *Trichoderma reesei*

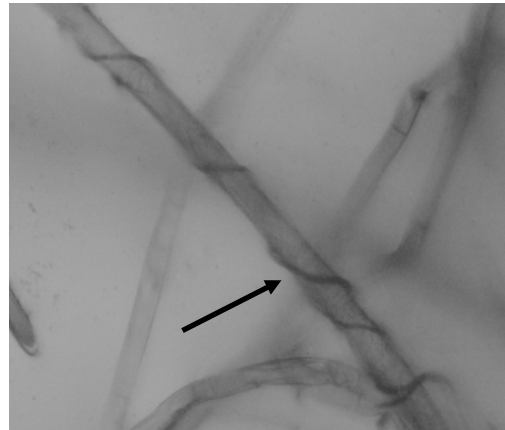


Fig. 5: *Trichoderma reesei* coiling around the hypha of *Botrytis cinerea*

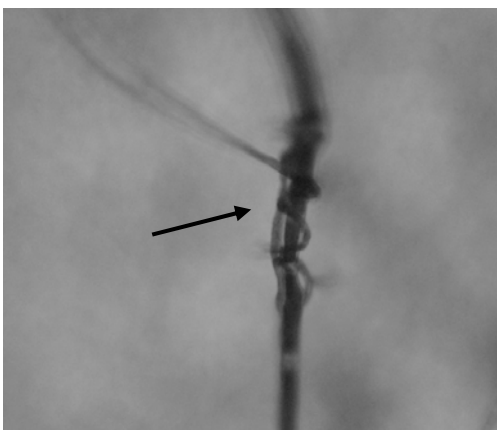


Fig. 6: Hypha of *Botrytis allii* is coiled by hypha of *Trichoderma reesei*

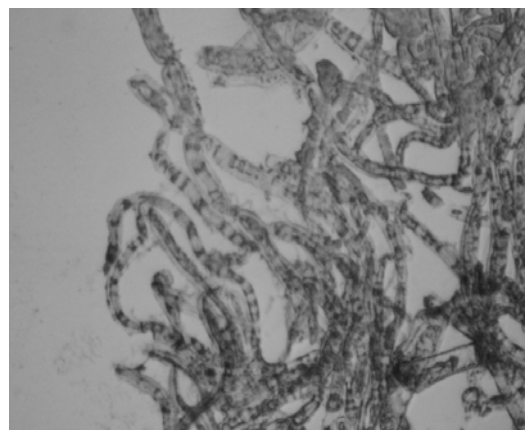


Fig. 7: Stunted hyphae of *Botrytis fabae* by the metabolic effect of *Trichoderma reesei*

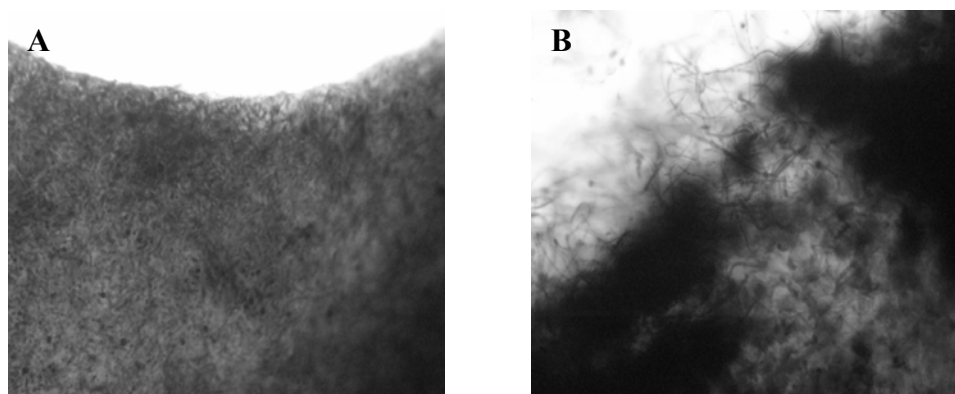


Fig. 8: Sclerotial parasitism of *Botrytis cinerea* by *Trichoderma reesei* (a thin section of healthy sclerotium (A), hyphal growth of *Trichoderma reesei* inside a section of infected sclerotium of *Botrytis cinerea* (B))

The infection of sclerotia and hyphal parasitism of *B. cinerea* by *T. reesei* indicates that this mycoparasite has considerable potential as a biological control agent by reducing the inoculum density of *B. cinerea*.

Future research should be encouraged and integrated into the whole range of modes and mechanisms of action of biological control agent if biocontrol is ever to become a practical option for agricultural purposes.

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MICOPARAZITISMUL ȘI EFICACITATEA ANTAGONISTĂ A CIUPERCII *TRICHODERMA REESEI* FAȚĂ DE *BOTRYTIS* SPP.

(Rezumat)

A fost efectuat un studiu preliminar de evaluare a capacității antagoniste și micoparazite a ciupercii *Trichoderma reesei* față de speciile de *Botrytis cinerea*, *B. allii* și *B. fabae*. Metoda de evaluare a eficacității ciupercii *T. reesei* a fost cea de apreciere a inhibării *in vitro* a creșterii miceliene a speciilor de *Botrytis*. Un efect puternic a fost observat asupra speciilor *B. cinerea* și *B. fabae*. Asupra speciei *B. allii* nu s-a constatat niciun efect. A fost, de asemenea, studiat parazitismul ciupercii *T. reesei* la nivelul hifelor și scleroților de *Botrytis* spp. S-au observat, ca aspecte de micoparazitism, încolăcirea hifelor de *Botrytis cinerea*, urmată de penetrarea și reducerea dimensiunilor acestora. Alte aspecte constatate au fost încolăcirea hifelor de *Botrytis allii* și reducerea dimensiunilor hifelor de *B. fabae*. *T. reesei* a parazitat hifele de *B. cinerea*, dar nu și pe cele de *B. allii* și *B. fabae*.

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