Detect causes of failure of *Trichoderma harzianum* in biocontrol damping off disease of eggplant ancited by *Rhizoctonia solani*

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تحدد الكيفية المكافحة *Trichoderma harzianum* الحيوية لمرض سقوط بادرات البذور المسبب عن الفطر

*Rhizoctonia solani*

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الخلاصة

*Trichoderma* وعزم الفطر *Rhizoctonia solani* عزمه من الفطر كانت مستخدمة لدراسة نجاح أو فشل المكافحة الحيوية للمرض المسبب *harzianum* عن الفطر *solani* في بادرات البذور (الصنف المحلي). تقييم المكافحة الحيوية ضد الفطر *R. solani* ومقارنة التنافس على الغذاء والنمو بين الفطر *R. solani* والفطر *T. harzianum* وكذلك تحديد تراكم المقاومة ورفع أنزيم البيروكسيد بفضل استخدام الفطر *harzianum*.

النتائج أظهرت عدم وجود اختلافات معنوية في ضرر المرض عن طريق استخدام الفطر *T. harzianum* كعامل مقاومة حيائية ضد الفطر *T. harzianum* السريعة (4.9 و 100% على التوالي). كذلك، أظهرت النتائج نمو ضعيف للفطر *R. solani* في وسط الجابكس دوکس اكر مع مادة CMC ودقيق الرز كمصعد وحيد *harzianum* للكربون مقارنة بالفطر *R. solani* مقارنة بمعالجة المقارنة (جابكس دوکس اكر مع السكروز). نتائج تحفيز المقاومة أظهرت عدم وجود زيادة بشكل معنوي في فعالية أنزيم البيروكسيد في النسجة جذور القطن مقارنة بفعالية أنزيم البيروكسيد في مقارنة مع فرقة الفطر *T. harzianum* المعاملة بباعوالفطر المذكور (7.6 و 6.16 وحدة أنزيم).
Abstract

The isolates *Rhizoctonia solani* and *Trichoderma harzianum* (isolate of tahadii biopesticide) were used to study success or failure of biocontrol of disease incidence caused by *R. solani* in eggplant (local cultivar), evaluation of biocontrol by *T. harzianum* against *R. solani*, comparison of competition on nutrients and growth between *R. solani* and *T. harzianum* and detect induced resistance and raise of peroxidase activity by *T. harzianum*.

Results showed non significant differences in disease incidence by using *T. harzianum* as biocontrol agent against *R. solani* compared with control (94.4 and 100% respectively). Also it showed weak growth of *T. harzianum* in czapek dox Agar with carboxy methyl cellulose (CMC) and rice meal as sole source of carbon compared with *R. solani* which appeared superior growth and significant differences of growth compared with control (czapek dox Agar with sucrose).

A result of induce resistance showed non significantly increasing of peroxidase activity in cotton root tissue of treated seeds with conidia of *T. harzianum* compared with peroxidase activity in cotton root tissue of untreated seeds. Therefore, the failure may be attribute to weak competition and induce resistance by *T. harzianum*.

Introduction

Most Fungi used for biological control of damping off and root rot are hyphomycetes and among those the genera *penicillium*, *Trichoderma* and *Gliocladium* have received most attention (Jensen and Wolffhechel, 1995).

The use of *Trichoderma* as a biocontrol agent of soil-born pathogenic fungi has been known for quite along time (Baker and Cook, 1974). *Trichoderma* was decreased significantly percentages of disease incidence caused by *R. solani* (Howell, et al. 2000, Hameed, 2002), *Fusarium* sp (Harman, et al, 1991) and other soil-born pathogenic fungi. There are a many mechanism to effect of *Trichoderma* on pathogen such as mycoparasitism, antibiosis, Induce resistance and competition for nutrients or space (Harman, 2000). Potential biocontrol of *Trichoderma* is not depending particularly on one mechanism such as competition, mycoparasitism, antibiosis (Howell, et al, 2000). Recent studies reported that may be...
combination among those mechanisms to obtain successful control (Hameed, 2002).

This study aimed to detect causes of failure of *Trichoderma harzianum* in biocontrol of disease incidence caused by *R. solani* in eggplant and mechanisms that involved in failure.

**Materials and Methods**

**Isolation of pathogen and test of pathogenicity**

Isolate of *R. solani* were isolated from infected seedling of eggplant in one nursery of Baqubah on plates containing PSA media. Soil surface of pottes which contain seedling of eggplant (age 14 days) were infested with growth *R. solani* on millet seeds (1 gm/pot.) and reisolation after appear infested seedling was done.

**Efficacy of *T. harzianum* in disease control of *R. solani* in eggplant**

*T. harzianum* of tahadii biopesticide was used as biocontrol agent by coated of eggplant seed with suspension of conidia concentre $5 \times 10^5$ spor/ml (Elad, et al, 1982). Sterilized soil was infested by 1 gm *R. solani* growth on millet seed per 1kg dry soil (sandy loam) and putted in pottes (Hameed, 2002). Three treatments with three replicates per each treatment were used. Pottes of first treatment contains infested soil were planting with 8 seed coated with suspension of *T. harzianum* per each pot, second treatment contains infested soil were planting with 8 untreated seeds per each pot and pottes of third treatment contains non-infested soil were planting with 8 untreated seeds per each pot. Survival of eggplant seedling were counted at day 28th and converted to percentages of disease incidence caused by *R. solani* depended control treatment (Howell, et al, 2000).

**Comparison of growth and competition on sole source of carbon between *T. harzianum* and *R. solani***

Two experiments were carried out in plates, first experiment carried out with four treatment and three replicates per each treatment for evaluation of growth of *R. solani* and *T. harzianum* in czapek dox Agar with Carboxy methyl cellulose (CMC) as sole source of carbon. Isolate of *T. harzianum* and isolate of *R. solani* were
inoculated individually as disk 5mm per each plate from colonies age 7 days on plates containing czapek dox Agar with CMC (1gm/litre) as carbon source instead of sucrose other plates containing czapek dox Agar with sucrose culture with T. harzianum and R. solani (control treatment) (Ahmed and Baker, 1987). Redial growth were measured after anecubated at 23 ± 2 c° for 24h.

Second experiment was carried out with six treatments and three replicates per each treatment for evaluation of growth of T. harzianum and R. solani in czapek dox with rice meal as sole source of carbon. Two isolate of fungi were inoculated individually as disk 5mm per each plate from colonies age 7 days on plates containing czapek dox with only 75gm/litre rice meal as sole source of carbon, with 75gm/litre rice meal and 30 gm/litre sucrose together and only sucrose (control). Redial growth were measured after anecubated at 25 ± 2 c° for 24h.

Detect induced resistance by isolate of T. harzianum

Peroxidase activity was using as indicator to induced resistance in cotton seedling roots (Howell, et al, 2000), therefore using to detect isolate of T. harzianum that able to induce resistance or not. A polystyrene plate contained 198 cavities was filled with sterilized mixture of peat moss and sand in a ratio of 1:1. Cotton seed of culviver coker 310 were coated dry prepartion of Trichoderma isolate. Untreated seeds were used as control. Eleven cotton seeds were planting individually in each cavity on 1cm depth with three replicates. Six days later, 0.5 gm of roots from each treatment were harvested and grinded with 2ml of buffer phosphate with pH 6 in ammortar (Howell, et al, 2000) and incubated in 10-ml test tube. The tubes then were centrifuged at speed of 6000 cycle/min for 10 min. supernant of each tube were used to test the activity of peroxidase enzyme based guaiacol test. The activity of peroxidase was measured by spectrophtometer through observation in light absorption at 420 nanometer/min/g fresh root tissues during 4 min. spectrophotometer was adjusted by reaction mixture without root extract (Whitaker and Berhand, 1972).
Results and Discussion

Effectiveness of *T. harzianum* in disease control of *R. solani* in eggplant seedling:

The results showed that isolate of *T. harzianum* couldn’t suppressing significantly percentage of disease incidence caused by *R. solani* in eggplant seedlings (Fig. 1).

![Graph showing disease incidence](image)

Fig. 1: Disease incidence of treated seed with *T. harzianum* and untreated seed planting in infested soil with *R. solani* - None significant differences between treatments.

However, many papers were reported that using *Trichoderma* isolates suppressed percentage of disease incidence of *R. solani* in cotton %83 in green house (Elad, et al, 1982), %79 in growth chamber (Howell, et al, 2000) and %85.3 in green house (Hameed, 2002). This experiment results were reflexed previous papers, however, we’ll discuss causes of failing of biocontrol in other section of our paper.

Comparison of growth and competition on sole source of carbon between *T. harzianum* and *R. solani*:

First experiment results were showed superlative growth of *R. solani* generally compared with *T. harzianum* which were measured (Fig. 2). Moreover, there are superlative growth of *R. solani* significantly in czapek dox Agar with carboxy methyl cellulose
(CMC) instead of sucrose compared with growth of *R. solani* in czapek dox Agar with sucrose (3.16 and 2.83 cm respectively).

![Bar graph](image)

**Fig. 2.** Radial growth of *T. harzianum* and *R. solani* after 24h in different media with carbon source
- L. S. D. p= 0.05 Value=0.322

Waste (1961) showed that the degree of success in competitive colonization by *R. solani* was correlated with its growth rate in pure culture, also found strict correlation between innate growth rate and saprophytism only in the absence of antagonism.

High growth of *R. solani* in czapek dox Agar with CMC may be attribute to well production of cellulose enzyme compared with *T. harzianum* present result agree with results of Ross (1960) was reported that isolates of *R. solani* can utilize cellulose as the sole source of carbon in vitro, grow well on cellulose fibers, and considerable amount of soluble cellulytic enzymes in culture filtrates.

Weak growth of *T. harzianum* compared with growth of *R. solani* and didn’t found significant difference growth of *T. harzianum* on czapek dox Agar with carboxy methyl cellulose (CMC) and on czapek dox Agar with sucrose as the sole source of carbon were indicate to weak ability to competition and saprophytism and little produced cellulase in culture. Previous study reported that the amount of cellulose produced by isolate of *T. harzianum* was directly correlated with saprophytism ability and rhizosphere competence (Ahmed and Baker, 1987).

Second experiment results were showed high growth of *R. solani* in czapek dox with rice meal as sole source of carbon (4.6cm)
compared with growth of *R. solani* in czapek dox with sucrose and rice meal together, and in czapek dox Agar (control) (3.5 and 3.66 cm respectively), also compared with growth of *T. harzianum* that didn’t differ significantly with growth of *R. solani* in czapek dox with sucrose or sucrose mixed with rice meal except control treatment of *T. harzianum* (czapek dox Agar with sucrose) which was weak (2.5 cm) (Fig. 3).

![Graph showing radial growth](image)

Fig. 3. Effects of rice meal (rm) as sole source of carbon or mixed with sucrose (su) on radial growth of *R. solani* and *T. harzianum* after 24h incubation - L. S. D. P=0.01 value= 0.692

The present results confirmed previous studies reported that the presence of energy materials, their kind, quantity and their availability are the primary factors directly determining saprophytic colonization and growth of *R. solani* and indirectly determining those by influencing other antagonists (papavizas, 1970). Moreover, the carbon-to-nitrogen (C/N) ratios determined of *R. solani* saprophytism and change of carbon-to-nitrogen balance which may be effected in growth of *R. solani* (papavizas and Davey, 1961).

Therefore, a superior growth of *R. solani* when used only rice meal in culture attribute to obtaining optimum balance in C/N ratio, also little growth of *T. harzianum* may be attribute to obtaining unoptimus balance, these are findings supported our conclusion that the fungus *T. harzianum* didn’t have competition mechanism.
Detection induced resistance by *T. harzianum*:-

The result of induce resistance experiment showed that isolate of *T. harzianum* didn’t able to induce resistance and raising of peroxidase activity in seedling roots of cotton (fig. 4). Although finding isolates of *Trichoderma* able to induce resistance and raising of peroxidase activity in seedlings roots (Howell, et al, 2000, Hameed, 2002). Howell, et al (2000) believed that induce resistance mechanism is achieved mechanism for biocontrol. Hameed (2002) reported that there is isolate of *Trichoderma* able to induce resistance and it had few antagonistic degree on plates but couldn’t biocontrol of disease caused by *R. solani*. Moreover, he was believe that to obtain successful biocontrol there are combination among the mechanisms of *Trichoderma* for effecting in disease incidence.

![Diagram](image)

**Fig. 4.** Effect of *T. harzianum* on peroxidase activity in cotton seedlings of coker 310 cultivar.
- Non significant difference between treatments.

In present study causes of failure of biocontrol by *T. harzianum* may be attribute to weak competition and didn’t have induce resistance mechanism. Meantime, *R. solani* could attacked seed of eggplant quickly before emergence of seedlings and exploited cellulose and starch comfomed with present study results which showed that *R. solani* grew very better in which media had cellulose (Carboxy methyl cellulose) and starch (rice meal) as sole source of carbon compared with *T. harzianum*. Although found isolate of *Trichoderma* didn’t able to competition but it’s fail in biocontrol (Howell, 1987). Therefore, it’s may be have assistance role to other mechanisms of *Trichoderma* as induce resistance mechanism. Previous study reported that one isolate of *Trichoderma* among induced isolates *Trichoderma* of resistance which were successful in biocontrol was failure in biocontrol, attributed to it’s had a weak
antagonism effect in Dual Culture (Hameed, 2002). Therefore, we think that causes of failure of biocontrol *T. harzianum* may be due to didn’t have induced resistance mechanism and weak competition with *R. solani* which appeared superior growth, well exploited of carbon source and severe varulance reflexed in high incidence of disease.

**References**


