



Biological Control of *Rhizoctonia solani* by Indigenous *Trichoderma* spp. Isolates from Palestine

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Abstract:

The effect of indigenous *Trichoderma* isolates against the soil-borne phytopathogen *Rhizoctonia solani* was investigated in dual culture and bioassay on bean plants. Application of the bioagent isolates as a conidial suspension (3×10^7) greatly reduced the disease index of bean plants caused by *R. solani* in different rates and the most effective *Trichoderma harzianum* isolate (Jn14) reduced the disease by 65%. In dual culture, the *T. harzianum* (Jn14) overgrew the pathogen *R. solani* in an average of 16.75 mm/day at 30 °C. In addition, the results showed that *T. harzianum* (Jn14) and *T. hamatum* (T36) were the most effective isolates at 25°C and inhibited *R. solani* mycelial growth by 42% and 78% respectively, due to fungitoxic metabolites production. The Effect of *Trichoderma* on bean seedlings growth was obvious; height was nearly doubled (160% - 200%), while fresh and dry weights increased by 133% and 217%, respectively. Germination of bean seeds in treated soil with *Trichoderma* isolates occurred about four days earlier than those in untreated soils. The results revealed however some variation between isolates which was due to genetic variation, mycelium-coiling rate, sporulation rate, fungitoxic metabolites, induced growth response and temperature effect.

Key words: Biological control, Damping off, *Trichoderma*, *Rhizoctonia solani*, Bean.

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المكافحة الحيوية لمرض عفن الجذور الريزوكتوني على نباتات الفاصوليا باستخدام عزلات فلسطينية من فطر التريكوديرما

المخلص:

خلال هذه الدراسة تم فحص مقدرة 47 عزلة محلية من فطر *Trichoderma* على مكافحة مرض عفن الجذور الريزوكتوني (*Rhizoctonia solani*) باستخدام طريقة التداخل الفطري (Dual Culture) ومعاملة نباتات الفاصوليا الكاملة (Bioassay). أظهرت نتائج إضافة العزلات كملق يحتوي ابواغ الفطر بتركيز (3×10^7 g soil) الى التربة لمكافحة المرض على نباتات الفاصوليا أن هناك تباين معنوي في مكافحة المرض حيث أدت أقوى العزلات (Jn14) الى تقليل شدة الاصابة بالمرض بنسبة 65%. أظهرت نتائج دراسة التداخل الفطري بين عزلات فطر التريكوديرما وفطر *R. solani* أن هناك تباين معنوي بين العزلات في التطفل على الفطر الممرض وكان معدل نمو أقوى عزلة في التطفل 16.75 ملم / يوم. ووجد من نتائج دراسة تأثير الحرارة على عملية التداخل بين أقوى العزلات والفطر الممرض بان العزلة (Jn14) كانت أقواها، وأفضل درجات الحرارة لنموها 25-30 درجة مئوية. وعند دراسة آلية عمل فطر التريكوديرما على الفطر الممرض؛ وجد أن فطر التريكوديرما لديه المقدرة على التطفل على الفطر الممرض وإنتاج المثبطات الفطرية عند تربيته على بيئة البطاطا المغذية السائلة (PDB). وأظهرت النتائج بان المثبطات المنتجة من العزلتين (Jn14 and T36) عند مزجها بنسبة 10% مع بيئة البطاطا المغذية الصلبة PDA قد قللت معدل نمو الفطر الممرض بنسبة 42% و 78% (على التوالي) على درجة حرارة 25°C. كما وجد أن فطر التريكوديرما يعمل على تكبير انبات بذور نباتات الفاصوليا المعاملة بمعدل 4 أيام من البذور الغير معاملة والى زيادة طول النباتات بنسبة 160%-200% وزيادة وزن النباتات بنسبة 133%-217%. وعزي التباين بين العزلات في مكافحة المرض الى مقدرتها على التطفل الفطري، سرعة نموها، انتاج الابواغ، درجة الحرارة ومقدرتها على انتاج المواد المثبطة، إضافة الى الاختلاف الوراثي والجيني بين العزلات.

Introduction:

Damping off caused by *Rhizoctonia solani* is an economically important disease on beans in Palestinian agriculture. Different fungicides and soil fumigants are currently used to control soilborne plant pathogens including *R. solani*. However, many of these compounds proved to be quite toxic to the environment and to the ground water. Methyl bromide is a good example for a very efficient soil fumigant that has a great

impact on the environment and has been recently phased out due to the public concern and international agreements. The use of antagonistic microorganisms against *R. solani* has been investigated as one of the alternative control methods. *Trichoderma harzianum* is well documented as effective biological agents for *R. solani* control in soil (Papavizas, 1985, Coley-Smith et al., 1991, Samuels, 1996, Prasun and Kan-

thadai, 1997, and Sivasithamparam and Ghisalberti, 1998, and Howell, 2003). Biological control can be specific and depends on soil, environmental factors, location, and season in addition to crop and pathogen. The biological control of the pathogen on bean plants by using *Trichoderma* spp. and/or combination with other techniques has been investigated. Application of small non-effective doses (1-2 µg/kg) of Pentachloronitrobenzene (PCNB) to soil along with a *Trichoderma* preparation (2g/kg), decreased the incidence of eggplant damping off caused by *R. solani* from 13 to 40%, while *T. harzianum* alone reduced disease incidence by 26% (Hadar et al., 1979). The combination of sublethal heat treatment dose and *T. harzianum*, enhanced control of disease on beans (by 90 to 100%) under greenhouse conditions (Elad et al., 1980). Aziz et al. (1997) found that the application of a wheat bran preparation of *Trichoderma lignorum* conidia (8×10^6 conidia/seed) at a rate of 15 and 20 g/500 g soil decreased the damping-off percentage by 12% and 6%, respectively, as compared to control. In addition, they found that the application of wheat bran preparation of *Trichoderma lignorum* (5×10^6 cfu/g) at a rate of 2.5 g/500 g of soil decreased the damping-off percentage by 45%. Furthermore, Noble and Coventry (2005) showed that the combination of *Trichoderma harzianum* and organic amendment can be used to control soil-borne pathogens including *R. solani*. The ability of *Trichoderma* to reduce the disease is well known and related to the antagonistic properties of *Trichoderma*, which involve parasitism

and lysis of pathogen and /or competition for limiting factors in the rhizosphere mainly iron and carbon (Chet, 1990). Another mechanism has been suggested by Kleifeld and Chet (1992) and related to *Trichoderma*-induced resistance in host plants to fungal attack. The increased growth response (IGR) of plants depends however, on the ability of the *Trichoderma* to survive, develop in the rhizosphere, and varies as well with the substrate (Kleifeld and Chet, 1992, and Yedidia et al., 1999). In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. Increased growth response has been demonstrated by several other investigators (Altomare et al., 1999; Anusuya and Jayarajan, 1998). They demonstrated the ability of *T. viride* and *T. harzianum* to solubilize insoluble tricalcium phosphate *in vitro*.

The objective of this study was to evaluate the potential of the bio-agent *Trichoderma* isolates recovered from Palestinian agricultural fields in controlling the soil-borne phytopathogen *Rhizoctonia solani*.

Materials and methods

Trichoderma isolates:

Forty-seven native isolates of *Trichoderma* were recovered previously from various agricultural soils in the West Bank; the antagonistic potential against *Botrytis cinerea* and *Sclerotium rolfsii* in dual culture and bioassay was inten-

sively studied (Barakat and Al-Masri, 2005, Barakat et al., 2006). These isolates were used in this study to evaluate their biocontrol potential against *R. solani* damping off of bean.

Preparation of *Rhizoctonia solani* inoculum:

The *R. solani* isolate (Rh1) used in the study was originally recovered from diseased bean plants (2000) and classified under antastomosis group 1 according to (Carling, 1996). Fifty milliliters of potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks was inoculated with two 7mm-diameter mycelial disks from 7- days- old PDA cultures of *R. solani* and incubated at 27°C for 10 days. After incubation, the upper solid layers of fungal growth were washed and air-dried with tissue paper layers. The amount needed from this preparation was (0.9 g dry preparation of *R. solani* / 0.5 kg soil). The air-dry mycelium was placed in autoclaved 250ml Erlenmeyer flask, shredded by Ultra thorax in distilled water and was then passed through a 2 mm sieve to obtain inoculum pieces of 1-2 mm in length.

Evaluation of antagonistic potential (Bioassay):

The screening for antagonistic potential was studied using the method of Mihuta and Rowe (1986). Forty-seven local isolates of *Trichoderma* were tested in growth chamber for antagonism to *R. solani* . Isolates were grown on

potato dextrose agar (PDA) at 25°C under continuous fluorescent light. After 7-10 days of incubation, conidia were harvested from cultures by flooding the plates with 10 ml of sterile distilled water and removed by sterile bent glass rod and poured into sterile test tubes and agitated for 15 sec. with vibrating agitator. The resulting suspensions were filtered through a layer of sterile tissue papers and the conidial concentration in the suspension was determined with a haemocytometer. Sterile distilled water was added to bring the concentration to 3×10^7 conidia / ml (Mihuta & Rowe, 1986). Four milliliters of the suspension (3×10^7 conidia / ml) was added to pots containing a 0.5-kg sand soil previously autoclaved at 121°C for 1 hr on three successive days.

The *Trichoderma*-inoculated soils were incubated for seven days at 25 °C and then mixed thoroughly with the 0.9 g *R. solani* preparation. Each plastic pot (10 cm diameter) was filled with non-autoclaved sand to 2/3 of pot volume and then seeded with six bean seeds (*Phaseolus vulgaris*). The final mixture containing the pathogen and the bio-agent was placed in the last 1/3 volume of pots which were previously seeded with bean seeds. The experimental design was completely randomized with five replicates (pots) for each *Trichoderma* isolate. The soil of the control treatment contained the pathogen (*R. solani*) without the *Trichoderma*. Plants were harvested after three weeks from seeding and incubated in the growth chamber at 25°C under a 12-hr photoperiod. All seedlings were uprooted, and the

hypocotyls were evaluated for infection by *R. solani* on a scale from 1 to 4: (1= symptomless, 2= small lesions with no hypocotyl constriction, 3= large lesions with some hypocotyl constriction, and 4= hypocotyl girdled).

These ratings were converted to a disease index (DI) value for each pot by using the formula: $DI = 30 * (1A + 2B + 3C + 4D) / N$

where A, B, C, and D represent the number of seedlings rated as 1, 2, 3, or 4 respectively; 30– the number of seeds planted; and N– the number of seedlings rated after three weeks.

Mode of Action

A) Dual culture interaction:

Dual culture interaction between the pathogen, *R. solani* and the *Trichoderma* isolates were studied using the following method (Dennis & Webster, 1971 b and Prasun & Kanthadai, 1997): 5-mm diameter mycelial blocks (7-days-old) cut from the margin of each *Trichoderma* isolate and of the pathogen colonies grown on potato dextrose agar (PDA) were placed 3 cm apart on the PDA surface. Five 90 mm diameter Petri dishes were incubated at 25 °C under continuous light and inspected daily for approximately 8-9 consecutive days for interaction. The fungus colony margins would meet 2-3 days after inoculation. The area of interaction of the bioagent and the pathogen was measured every 24 hours after contact. The overgrowth rate (mm/day) was measured during 10 days after contact. The experiment was completely

randomized design with five replicates (Petri dishes) used. The effect of temperature on dual culture interaction between the most effective *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q27, T33, T36, N38 and R42) and *R. solani* was repeated studied as described above under different temperatures (10, 15, 25, 30, 35 and 40°C) for 3-5 days in closed polyethylene bags lined with moist tissue papers (to prevent desiccation of the media), and observed regularly for ability of the fungus to restrict the growth, or to overgrow the other. The experimental design was completely randomized with four replicates (plates) for each treatment.

B) Hyphal interaction on thin film of agar:

This procedure was done according to that of Laing & Deacon (1991). Hyphal interaction was studied on sterile glass cover slips coated with 2% water agar (20g Difco agar / 1L distilled water). Each cover slip was immersed for 1-2 sec in autoclaved melted water agar at about 45 °C, allowed to drain and then placed on the surface of 2% solidified water agar in a 90 mm diameter Petri dishes, so that a thin film of agar set on the upper surface. Five mm disk of one-week-old growing colonies cut from the margins of each of *R. solani* and *Trichoderma* isolates were placed 3 cm apart on the agar surface and then incubated at 25°C. Cultures were inspected daily for interaction; *R. solani* and *Trichoderma* colony margins made contact across the coated coverslips in less

than three days. Each cover slip was removed carefully without damaging the mycelial contact and was inverted on a sterile microscopic slide (25.4 x 76.2). Microscopic examination was carried out using fresh direct mounts in Lactophenol cotton blue under medium and high magnifications (20 and 40x), respectively. Specimens were always sealed by nail varnish to prevent drying. Mycoparasitism was indicated by hyphal coiling and internal colonization of the host hyphae (*R. solani*) by the bioagent *Trichoderma*.

C) Production of toxic metabolites (Antibiosis):

The ability of *Trichoderma* spp. to inhibit the mycelial growth of *R. solani* through production of fungitoxic metabolites at different temperatures was tested according to the method mentioned by Dennis and Webster (1971 a and b). Fifty ml of potato dextrose broth (PDB), pH 6 in 250 ml Erlenmeyer flasks were inoculated with 7mm-agar disks from 7-day-old PDA cultures of three *Trichoderma* isolates J8, Jn14, and T36 and incubated at 25°C without shaking. After 10 days of incubation, the cultures were filtered through a Millipore membrane filter (0.45 µm) and autoclaved at 121°C for 15 minutes. The culture filtrate (1.2 ml) was placed in Petri dishes (90-mm diameter) and approximately 12 ml of PDA was added and mixed with the filtrate (10% v/v). The filtrate-amended PDA plates were then centrally inoculated with 7-mm mycelial plugs of *R. solani* after solidi-

fication. Plates were incubated at 15, 20, 25, 30, and 35 °C; unamended PDA plates served as the control. The linear growth rate of *R. solani* was measured after 48 hour and the growth rate was calculated as cm²/day. The experimental design was completely randomized with four replicates (plates) for each treatment.

Plant's increased growth response (IGR):

The ability of *Trichoderma* to protect the emergence of seedlings, and to improve plant's growth was tested. Fifteen *Trichoderma* isolates (H2, H3, H4, J8, J9, Jn14, Jn18, Jn21, Q26, Q27, T36, T37, N38, R42, and B47) were used. The isolates were grown on Petri plates (90-mm in diameter) containing PDA for 10 days at 25°C under illumination. Conidia were harvested from cultures, washed several times in water, and suspended in 0.001% Tween 20 (Polyoxyethylenesorbitan, Sigma-Aldrich. Com.) (Chang et al., 1986). Conidial suspensions were added to the peat moss growth substrate at the concentration of 5x10⁶ conidia per gram of soil and incubated for 14 days at 25°C. After 14 days, six bean (*Phaseolus vulgaris*) seeds were sown per pot and five replicates were employed. Plants were grown in growth chamber at 25 2 °C for 4 weeks. The various measurements of plant's growth responses were made including number and time of emergence of seedlings. The emergence was evaluated after 3 and 7 days. Plant heights were measured from soil surface to

apical buds after 4 weeks. Concerning fresh and dry weights, plants were washed under running tap water to remove soil from roots; plants were then dried at 80 °C in drying oven after recording fresh weights. After 72hr, plant dry weights were determined (Shenker et al., 1992).

Statistical analysis:

Data of all experiments were analyzed statistically using One-Way Analysis of Variance (ANOVA) to test for significance; Fisher test was used for mean separations by SigmaStat Software (1999).

Results:

Evaluation of antagonistic potential in bioassay:

Trichoderma isolates reduced disease index of bean plants caused by *R. solani* in different rates (LSD= 19.588; $P \leq 0.05$); means of disease index ranged from 38.2 to 111 (Fig. 1). Thirty nine *Trichoderma* isolates (Jn14, R42, Jn21, T33, T36, H2, N38, J8, Q28, J9, H3, H4, T37, N39, Q26, Jn17, Jn18, B47, Q24, Q29, J10, Q27, Q30, R43, Jn20, Q25, Jn11, Jn19, Jn23, H7, T35, T31, R41, H5, T32, H6, Jn15, Jn12, and Jn16), significantly reduced disease index by 18.2%- 65.6%, compared to the control. Disease index was reduced on bean seedlings by 65.6%, 60.5%, 56%, 54.9%, 54.8%, and 53.4%, respectively, when the most effective *Trichoderma* isolates (Jn14, R42, Jn21, T33, T36, and H2) were used compared to the

control. The isolates (T34, N40, Jn13, B46, B44, B45, Jn22, and H1) were less effective; they reduced disease index by 1.5%-16.4% compared to the control.

Mode of action

Dual culture interaction:

The ability of *Trichoderma* to inhibit the mycelial growth of *R. solani* in dual culture was determined on PDA medium (Fig. 2). A clear zone of interaction was formed on PDA media at the point of contact between the mycelium of *R. solani* and *Trichoderma*. Results of interaction between the *Trichoderma* isolates mycelia and *R. solani* mycelia showed statistically significant differences in the overgrowth rate between the different isolates (LSD= 0.867; $P \leq 0.05$) (Fig. 2). Means of overgrowth rate (mm/day) of *Trichoderma* isolates on *R. solani* ranged from 2.4 for the *Trichoderma* isolate H5 to 9.9 for the *Trichoderma* isolate Jn17 (*Trichoderma atroviride*). Results showed that the most effective *Trichoderma* isolates against *R. solani* were (Jn17, J8, J9, T36, Jn18, Jn14, Jn21, and H1).

The effect of different temperatures on the interaction between the most effective *Trichoderma* isolates mycelia and the *R. solani* mycelia was evaluated. Results showed significant differences between isolates (LSD= 1.635; $P \leq 0.05$) in this respect (Fig.3). The interactions between the two fungi were highly dependent on temperature. The mean overgrowth rate ranged from 0.02 mm/day for the isolate Q28 (*Trichoderma hamatum*) at 15 °C and 16.75 mm/day

at 30 °C for the isolate Jn14 (*Trichoderma harzianum*) (Fig. 3).

Hyphal interaction on thin film of agar:

The *Trichoderma* isolates (H2, J8, Jn14, Jn17, Jn21, T33 and T36) showed identical mode of action during interacting with *R. solani* on water agar films. After contact, the hyphae of *Trichoderma* grew along the pathogen hyphae; sometimes the main hyphae coiled around the host or produced short branches that tightly surrounded the host hyphae. Dense coiling around host hyphae and internal growth within the host mycelium had commonly been seen during interaction between *R. solani* and *Trichoderma*; disintegration of the host cell wall was observed as well.

Microscopic examination had also revealed that the isolate Jn14 (*T. harzianum*) was very efficient in coiling and interned colonization of *R. solani* hyphae. Coiling of *T. harzianum* (Jn14), *T. hamatum* (T36), and *T. Pseudokoningii* (Jn21) hyphae around *R. solani* hyphae was noticed on 40x microscopic magnification. Host hyphae internal colonization by the *Trichoderma* isolates Jn14, T33, and J8 was very clear and obvious. The types of hyphal interactions were observed in this study; coiling around the host hyphae; penetration of the host hyphae and lack of cytoplasm in host cells; and subsequent lysis of the infected hyphae.

Production of toxic metabolites (Antibiosis):

The growth rate of *R. solani* was re-

duced significantly in amended media at 20 °C and 25 °C (LSD = 0.942; $P \leq 0.05$) (Fig. 4). The variations between isolates were obvious at 25 °C. *R. solani* growth rate inhibition percentages were 42%, 78%, and 50.5% when growth media were amended with 10% of PDB containing metabolites produced by the isolates T36, Jn14, and J8, respectively. Results showed that Jn14 metabolites was the most effective at all temperatures (15 °C, 20°C, and 25 °C), and reduced *R. solani* mycelial growth by the percentages of 76%, 66%, and 78%, respectively (Fig. 4).

Plant's increased growth response (IGR):

The results showed that there was a significant increase for each of the parameters measured (plant's emergence, heights, and fresh and dry weights) in bean seedlings, 4 weeks after sowing compared to the non-treated seedlings (Table 1). Bean seedlings treated with *Trichoderma* isolates J8 and T36 increased in height by 160 to 200%, respectively. In addition, seedlings treated with the isolates Jn14 and Jn21 increased in fresh weights in the range of 133 % to 217%, respectively. There were no significant differences, however, in respect to plant dry weights within treatments. Germination of bean seeds planted in soils treated with *Trichoderma* isolates mentioned above started about four days earlier than those planted in untreated soil. Germination of seeds planted in *Trichoderma* (Jn 14 and T36) treated soils increased in the range of (16.7% - 55.6%), compared to the control.

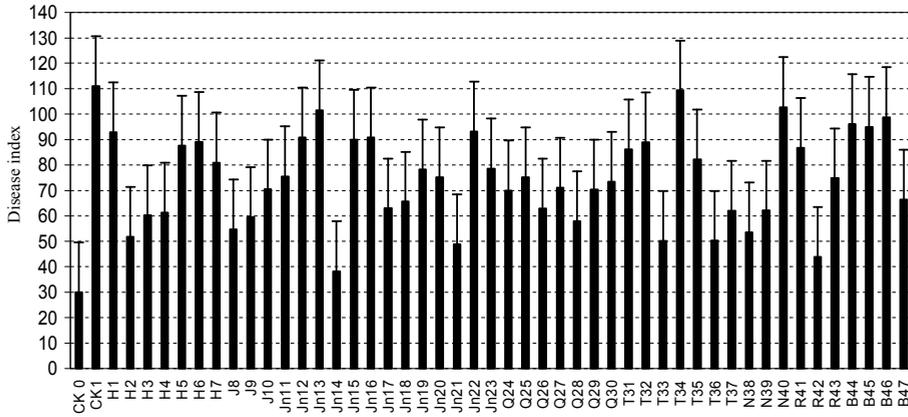


Figure 1. The effect of *Trichoderma* isolates on disease index caused by *R. solani* on bean plants (LSD=19.6).

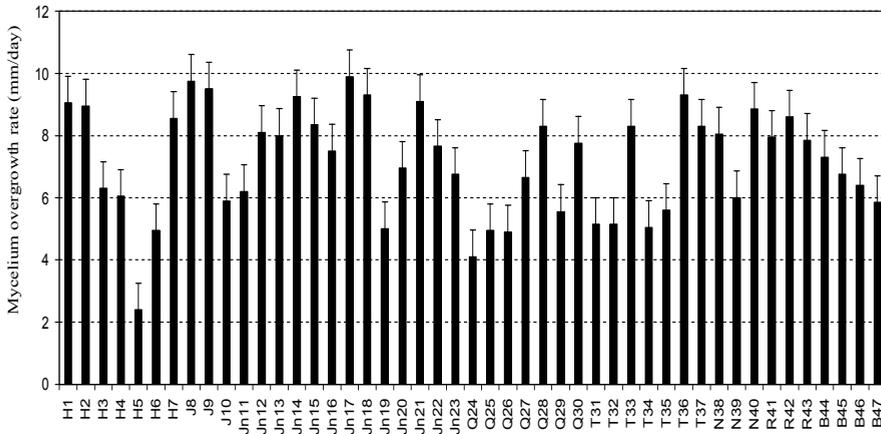


Figure 2. Mycelium overgrowth rate (mm/day) of local *Trichoderma* isolates over *Rhizoctonia solani* in dual PDA culture incubated at 25 °C (LSD=0.86).

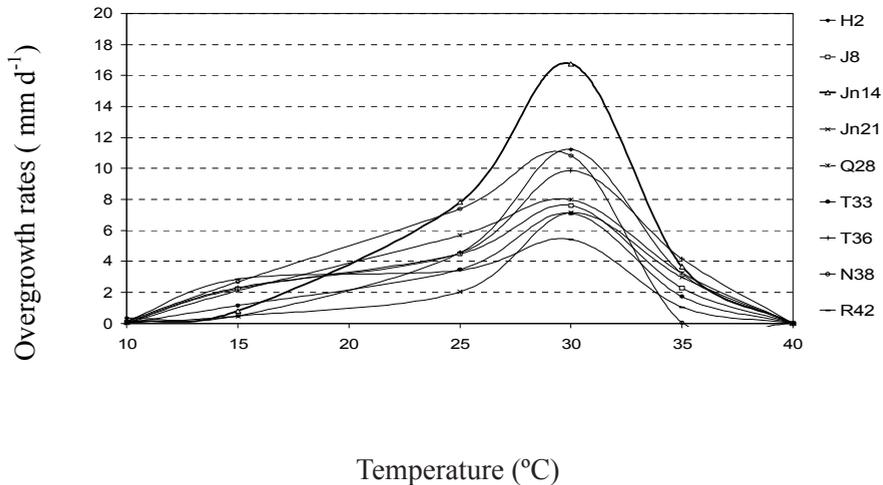


Figure 3. Overgrowth rates of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, and R42) over *R. solani* growing on PDA medium in dual culture at different temperatures (10, 15, 25, 30, 35 and 40 0C) (LSD=1.635).

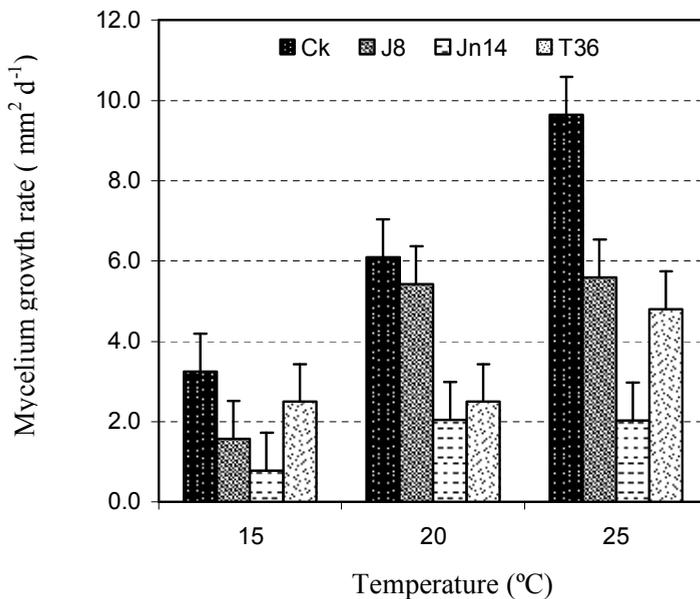


Figure 4. Mycelium growth rate (mm²d⁻¹) of *R. solani* growing on PDA medium amended with metabolites produced by the *Trichoderma* isolates (J8, Jn14, T36) and incubated at different temperatures (15, 20, and 25 °C) (LSD=0.942).

Table 1. The effect of *Trichoderma* isolates on bean plant's growth

Isolate	Emergence		Plant height	Plant weight (g)	
	3 days	7days		Fresh	Dry
CK	0.00* c	3.6 b	18.3* c	5.9 c	0.59 c
H2	1.6 c	4.6 a b	29.9 b	10.4 a b	1.0 b
H3	1.5 c	4.75 a b	31.0 b	1.06 a b	1.07 a b
H4	1.6 c	4.4 a b	34.7 a	11.1 a	1.11 a
J8	1.75 c	4.25 a b	29.8 b	8.9 b	0.89 b
J9	1.0 c	4.2 a b	29.8 b	8.8 b	0.88 b
Jn14	3.4 b	5.6 a	36.7 a	12.9 a	1.28 a
Jn18	2.0 c	4.2 a b	29.7 b	11.2 a	1.12 a b
Jn21	1.8 c	5.2 a	32.1 b	8.4 b c	0.84 b
Q27	1.6 c	4.8 a b	32.5 b	11.8 c	1.18 a b
Q28	1.6 c	4.8 a b	33.4 ab	11.9 a	1.19 a b
T36	2.2 c	5.6 a	36.8 a	12.7 a	1.27 a
T37	0.6 c	4.6 a	29.7 b	9.5 b c	0.95 b
N38	2.2 c	5.2 a	34.7 a	11.0 a	1.10 a b
R42	2.4 b c	5.2 a	34.7 a	9.8 b c	0.98 b
B47	1.2 c	4.6 a b	34.0 a	10.9 a b	1.09 a b
LSD (P≤ 0.05)	0.998		4.19	2.23	0.22

* Mean of five replicates; values followed by the same letter within columns are not significantly different according to Fisher LSD test.

Discussion:

Results showed that the dual culture contacts between *Trichoderma* isolates and *R. solani* occurred after 2 days of growth. All *Trichoderma* isolates grew over the *R. solani* colonies and degraded its mycelium. The study further revealed that the *Trichoderma* isolates (H2, J8, Jn14, Jn17, Jn21, T33 and T36) parasitized the hyphae of *R. solani*. *Trichoderma* hyphae of the isolates (Jn14, Jn21, and T36) grew over those of the patho-

gen and formed branches that coiled around them. Dense coiling of the *Trichoderma* isolate (H2) around *R. solani* hyphae was observed. Light microscopy revealed the penetration and growth of *Trichoderma* isolates (Jn14, T33, and J8) inside the hyphae of *R. solani*. Similar results were obtained earlier on *Trichoderma harzianum* (Jn14) against *Botrytis cinerea* (Barakat and Al-Masri, 2005) and *Sclerotium rolfsii* (Barakat et al., 2006). Other observations have been reported as well for *Trichoderma harzianum* and *Sclerotinia sclerotiorum*

interaction by Inbar et al. (1996). The antagonistic ability of *Trichoderma* isolates, however, is highly variable (Chet, 1990), as was shown in this study in which only 11.54% of the *Trichoderma* isolates tested were effective in controlling *R. solani* in the bioassay studies done in the growth Chamber. The most effective isolates were (Jn14, Jn21, T33, T36, H2, and R42).

Application of *Trichoderma* as a conidial suspension reduced disease index caused by *R. solani* by 65.6%. The ability of *Trichoderma* to reduce diseases caused by soilborne pathogens is well known and related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and /or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan and Chet, 1986). Another mechanism has been suggested by Kleifeld and Chet (1992) and related to *Trichoderma*-induced resistance in host plants to fungal attack. Furthermore, Aziz et al. (1997) reported the effect of plant exudates on the inhibition of conidia germination *in vitro* and on the suppression of Rhizoctonia damping-off of bean *in vivo* when *Trichoderma lignorum* was applied. In the presence of bean exudates, the reduction in Rhizoctonia damping-off of bean by *Trichoderma lignorum* was obvious. Increased growth response of several plants including vegetables, following the application of *Trichoderma* to pathogen-free soil has been documented (Baker, 1989; Chang et al., 1986; Kleifeld and Chet, 1992). In this study, bean seeds which were planted in *Trichoderma* treated soils germinat-

ed earlier by 4 days than those planted in nontreated soils in addition to a better emergence rate. In relation to this, Yedidia et al. (1999) suggested that a 30% increase in cucumber seedling emergence was observed up to 8 days after sowing when soil was amended with *T. harzianum* propagules. This was explained by (Kleifeld and Chet, 1992) who referred to the ability of *Trichoderma* to inhibit minor pathogens in the rhizosphere which might induce seed rots and preemergence damping off. Furthermore, seedlings grown in *Trichoderma* treated soils recorded higher values of plant heights and weights. Some investigators reported that the increased growth response caused by *Trichoderma* isolates resulted in large increase in the root area and root lengths and may be related to the effect on root system. Yedidia et al. (1999) suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of fungal-plant association. In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones.

The effect of temperature on the interaction between the bioagent and the pathogens was evaluated as well. *Trichoderma* isolates overgrew the pathogen (*R. solani*) in dual culture at the temperatures 15-35 oC. The overgrowth rate of *Trichoderma* isolate (Jn14) reached a peak at the temperature of 30 oC. However, the mean overgrowth rate of *Trichoderma* isolate (Jn14) reached a peak at 25 oC. Similar results were demon-

strated by Prasun and Kanthdai (1997). In addition to possible mycoparasitism, results of this study revealed that the growth rate of *R. solani* was reduced due to the production of fungitoxic metabolites by *Trichoderma* isolates at different temperatures. The growth rate of *R. solani* was reduced significantly at 20 °C and 25 °C by the *Trichoderma* isolate (Jn14). Similar results were observed by Prasun and Kanthadai (1997) who reported that *Trichoderma* (isolate Td-1) produced higher concentration of fungitoxic metabolites at higher temperatures and effectively suppressed the growth of *S. rolfsii* at or below 33 °C.

As a conclusion, this study showed that some *Trichoderma* species and isolates can be very efficient in controlling *R. solani* damping off of various vegetable plants using several mode of actions against the pathogen. Further studies are needed on these promising fungi to identify potential compounds produced and evaluate other possible mode of actions before going to field studies.

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