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# ANTAGONISTIC SUCCESSION OF *TRICHODERMA* AGAINST RHIZOCTONIAL DAMPING- OFF ON TOMATO IN COMPOSTED MEDIA

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## ABSTRACT

The results revealed that the incidence of Rhizoctonial damping-off of tomato was 65% and 67% in both rotations. Substrates of pine leaf litter and mushcom 2 suppressed infection reaching 59 and 60%. Mushcom1 restricted disease occurrence to 53%. In contrast, formulated *Th + B. subtillus* revealed a noticeable disease reduction reaching 33.16%, due to nutrients incited from mushroom thallus. The highest occurrence of damping-off (92 and 94 %) was found in control (sandy loam soil) during rotations. However, partial suppressive of *Trichoderma* spp. against *R. solani* was detected in different substrates. Mortality was 90% in control (non-amended soil). Finally, a comparable reduction of disease observed on tomato grown in mushcom 1 and mushcom 2 during rotations particularly when amended with *T.h. + B. subtillus*.

**Keywords:** *Rhizoctonia solani*, Tomato, Compost, *Tichoderma* spp.

## INTRODUCTION

*Trichoderma* pers. Ex., refer to Deuteromycotina, Hyphomycetes, Phialasporace, Hyphales , Dematiaceae has gained immense importance, since its ability as biological control, contrary to many phytopathogens. Bio-control mechanisms must be independently operated in any microbial interaction (Joshi *et al.,* 2010). *Trichoderma* is a fast growing, secondary opportunistic invader; sporulation is strong, produce antibiotics and cell wall degrading enzymes (Francesco *et al*., 2008). The success of strains as bio-control agents is due to their high reproductive ability, survive adverse conditions, utilize of nutrients efficiently, rhizosphere modifying capacity, aggressiveness against plant pathogenic fungi and improving plant growth. Therefore, *Trichoderma* spp. are the most investigated fungal bicontrol agents are available commercially as biopesticides (Harman, 2000).

*Trichoderma* species *T. viride, T. harzianum, T. longibrachiatum, T. hamatum, T. koningii, T. polysporum,* and *T. pseudokoningii* are very useful against several plant pathogenic fungi such as *Sclerotinia sclerotium, Fuzarium oxysporum*, *Pythium ultimum*, and *Rhizoctonia solani* (Rojo *et al.,* 2007). Worthily, the distribution of each species is influenced by different soil properties including: soil pH, chemical components, salt and organic matter content and presence of soil microflora (Kredics *et al.,* 2003). However, *Trichoderma* isolated from soil, decayed wood and different plant organic matters and grow rapidly in culture and the conidia produce in huge number that have varying shades of green characteristic fungus colonies are amber, yellow, or yellow-green, buff in the reverse side of colonies and many species produce in submerged mycelium prodigious quantities of thick-walled chlamydospores (Gams and Bisset, 1998).

Recently, bio agents of *Trichoderma* spp. were used to improve the efficacy of organic amendments against some diseases such as red rot of sugarcane (Singh *et al.*, 2008 a, b). Improvements in uptake of nutrients and growth due to application of *Trichoderma* were also noticed (Yadav *et al.*, 2008). In India about 50-55% canes were found free from red rot disease due to the application of *Trichoderma* multiplied culture (TMC) and / or culture filtrate (metabolites) obtained from *T. harzianum* Th37 (Singh *et al.*, 2009).

Application of *Trichoderma* associated with its performance and organic matter (compost) application realized more benefits for soil structure including: improving of physical condition of soils such as water holding capacity, water filtration, preventing soil aggregation and crusting, thus improve soil aerations, root penetration and the soil buffering capacity, reduce effects of soil alkalinity and acidity, moreover enhancing soil texture and increasing nutrients availability (Panahian *et al.*, 2012). The present work aimed to estimation efficacy of the local strains of *Trichoderma* cultures and commercial formulation for the control of damping-off of tomatoes grown in composted media.

## MATERIALS AND METHODS

1- Preparation of Substrates:

Substrates used for sowing tomato seeds included: Mushroom's compost1 (Mushcom 1) that consist of wheat straw, chicken manure 30%, urea (trace), calcium carbonate 15%, Gypsum 3% and Mushroom's compost 2 (Mushcom 2) composed of chopped wheat straw, wheat bran 5% and Gypsum 5%. Other substrates including chopped pine leaf litter, and Sandy loam soil 1:4 (v/v) as control.

2- Pathogen's Inoculum:

Virulent isolate of *R. solani* (Kuhn) was obtained from the laboratory of plant pathology, Department of Plant Protection, College of Agriculture, Univ. of Duhok, Iraq. Pathogen's Inoculum was prepared by using, millet seeds-sand medium (broken maize or barley 5.0g, sand 100g, tap water 20.0 ml) which was put into 600 g glass bottles and autoclaved then inoculated with mycelia discs (5mm) a week old grown on PDA culture of *R. solani* (one disc/bottle) before incubation at $28^{\circ}$c for 10 days. The bottles content were thoroughly mixed, during incubation period.

3- Preparation of antagonistic fungi:

Commercial product (Bio health of *T. harzianum* plus *Bacillus subtillus* 10% with Humic Acids 75% and Seaweed Extracts 5%) manufactured by Humintech GmbH, HecrdterLandstr., Dusseldorf, Germany. used at 10g/kg seeds.

Another isolates of *T. harzianum* (Riafi) strain (Kh. 20) and *T. viride* were obtained from Plant Protection Dept., College of Agric. & Forestry, Univ. of Mosul, Iraq.

The examined isolates of *T. harzianum* grown in two liter conical flasks containing 250g Mushroom's compost, 250g wheat barn 250g and millet seeds and 250 ml autoclaved PDA medium, incubated for 25 days, and thoroughly mixing at 5 days interval to certify inoculum distribution. Flasks content were transferred to aseptic plastic, left to air dry then blended thoroughly and kept in sterilized polyethylene container at room temperature until used at 0.5%. Colony forming unit's adjusted at $3\times10^{7}$cfu/g as described (Sallam *et al.*, 2008). Spore suspension of *T. viride* at $3\times10^7$cfu/g prepared from fresh cultures grown at 28°c for 10 days. Twenty ml was drenched, container with 15 cm diameter used.

Untreated substrate with bio-control used as control. An experiment replicated three times with three pots for each.

4- Efficacy of Formulated and *Trichoderma* Cultures in Suppressing Damping - off:

The composted substrates of Mushcom 1, Mushcom 2, Pine leaf litter and control (sandy loam soil) were autoclaved and blended with 2% (w/w)=100g inoculum of *R. solani*. Infested substrate (1kg) was poured into pots (15 cm) and incubated at 28°c room temperature subjected to darkness for seven days before planting. Tomato seeds (Mustakbel cv.) were surface disinfested in 2% Naocl for 3 min., washed three times in sterilized distilled water, and dried between filter papers.

Inoculated (10 seeds) were sown in potting sterilized soil infested with any of tested bio-control agents as described before. Untreated seeds were sown in infested soil used as control. Four replicates were placed in lath house and watered as required. Data were recorded after 20 days for damping-off. After taking data from the first rotation, the container medium was replanted with tomato seeds and disease incidence was rated again after another 20 days. Samples from control and amended treatments were collected for opportunistic fungi counts based on dilution plating $10^{-4}$ .Total fungi were enumerated colony forming units (cfu) for each rotation.

Statistical Analysis: Data were subjected to analysis of variance (ANOVA) and pooled together after test of homogeneity of variance ($P\leq0.05$). Data were analyzed using statistical analysis systems software (SAS version 8, Institute, Inc., and means compared by Duncan test).

## RESULTS AND DISCUSSION

1- Effect of Container Substrates on Rhizoctonial Damping - off:

Occurrence of Rhizoctonial damping-off of tomato did not noticeably affected in both two rotations. This result demonstrated that *R. solani* colonized container media at 20 days rotation period interval, therefore, the incidence of damping off was 65% and 67% in both rotations respectively (Fig.1). Substrates of pine leaf litter and Mushcom. 2 were significantly increased the suppression of pathogen to 59 and 60% respectively.

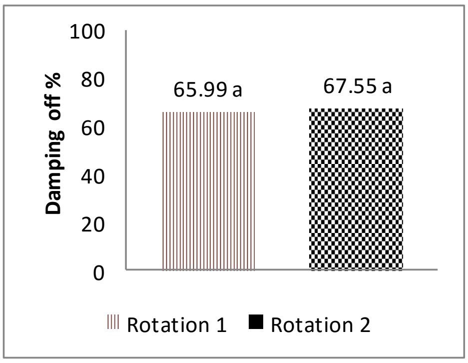


Fig.1: Incidence of Rhizoctonial damping-off of tomato in both rotations.

The component of Mushcom. 1 of calcium carbonate, gypsum, and chicken manure 30% considered use a key composition for formulation of container media suppressive to *R. solani* when inhibits the incidence of damping-off to 53% compared to 93% in the control sandy loam soil (Fig.2). Apparently, the suppression of a pathogen in the amended composts of wheat straw and chicken manure differs in compost age and dynamics of microflora population (Kuter *et al.,* 1988; Nelson and Hoitink, 1983).

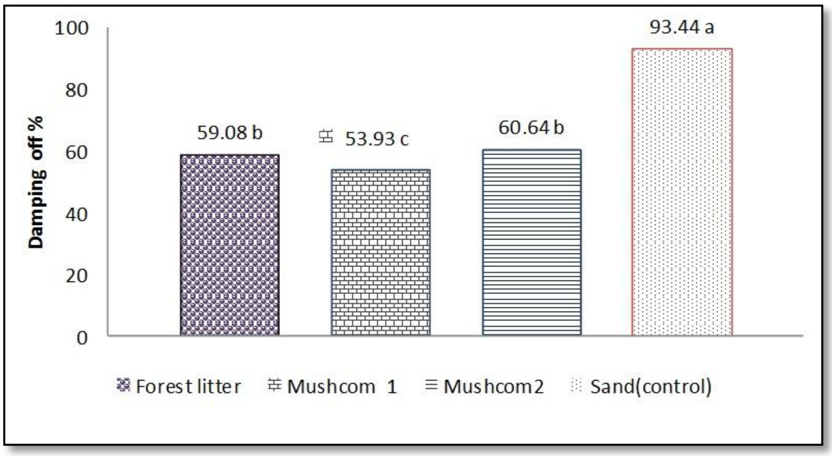


Fig.2: Incidence of Rhizoctonial damping-off of tomato in different substrates.

2- Soil Amendments:

Soil amendments of commercial product *T. harzianum* plus *Bacillus subtillus* at 10g/kg seed resulted a significant reduction of seedling mortality (33.16%) (Fig.3). However, Mushcom and its substrate are unique slow nutrient releasing substrates to *Trichoderma* because of the presence of chitin rich mushroom mycelia mat and slowly degrading lingo-cellulose straw (Sing *et al.*, 2014). Production of container media that were consistent manner suppressive to *R. solani* required not just applying of antagonists, but also the introducing of the antagonist into environments that suitable for antagonism theory (Chung and Hoitink, 1990; Craft and Nelson, 1996).

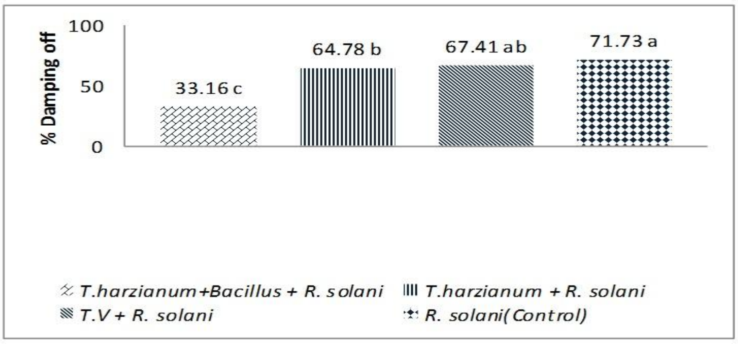


Fig. 3: Effect of several amendments on the occurrence of damping-off.

3- Effect of Organic Substrates on the Damping- off:

The occurrence of *R. solani* on tomato was high (92-94%) when grown in sandy loam soil during two rotations (Table 1). However the media of Mushcom. 1 and 2 decreased disease incidence to (37 and 42%) respectively. The components of organic matter in formulated cultures may have direct harmful effects on soil-borne pathogens, but some composted media and urban wastes may increase the substrate pH and concentration of $NH_4^+-N_2$ reached maximum 10 days after amendment this results also confirmed by (Lee *et al.*, 1997), observed that *R. solani* colonization released $NH_4^+- N_2$ at high concentration from degradation of the rich media.

Table (1): Effect of substrates during two rotations on the occurrence of damping-off.

| Substrate / Rotation | Forest litter | Mushroom substrate 1 | Mushroom substrate 2 | Sand ( Control) |
|---|---|---|---|---|
| Rotation 1 | 56.77 d | 69.94 c | 42.58 e | 94.67 a |
| Rotation 2 | 61.4 d | 37.91 e | 78.7 b | 92.22 a |

3- Effect of Amended Substrates on the Damping- off:

Results of (Table 2) revealed a partial inhibitory effect of *Trichoderma* spp. For *R. solani* on tomato grown in different composted treatments particularly when cultivated in Mushcom. 1 though the diseased seedlings were (43–50%) compared to (90–96%) in the control treatments. Both substrates of mushcom consisted more cellulosic materials of wheat straw and bran thus, become highly preferred and efficiently utilized by *Trichoderma* due to its ability in higher secretion of chitinase, legninase, hemicellulase and cellulase enzymes (Kaviyarsan and Siva, 2007; Ali *et al.,* 2011).

Table-2: Effect of substrate and amendments on the occurrence of damping-off.

| Substrate / Amendment | Forest litter | Mushroom substrate 1 | Mushroom substrate 2 | Sand ( Control) |
|---|---|---|---|---|
| *T.harzianum+Bacillus + R.solani* | 52.18 d-g | 50.43 fg | 60.03 b-f | 90 a |
| *T.harzianum+ R. solani* | 63.05 b-d | 43.88 g | 57.77 c-f | 94.43 a |
| T.v + *R. solani* | 64.43 bc | 50.9 e-g | 61.65 b-e | 92.67 a |
| *R. solani*(Control) | 56.67 c-f | 70.48 b | 63.1 b-d | 96.67 a |

The sites of necrotic tissues caused by *R. solani* were noticeable when reduced tomato root growth cultivated in the control sandy loam soil. However, strains of *Trichoderma* improved more activity of protease against such different pathogens as *R. solani* (Szekeres *et al.*, 2004), salt tolerance for control of *F. oxysporum* (Mohamed and Haggag, 2006) and of pesticide polyresistant strains (Hatvani *et al.*, 2006).

Result suggests that *Trichoderma* used in this trial didn't play major role in preventing the damages made by *R. solani* due to the fact that most plants exhibit moderate signs of necrotic lesions. Therefore specific interactions may be developed between the host and bio-control agent, and this was observed on the tomato (Santander *et al.,* 2003).

Data represented in (Table 3) demonstrated that the variance of inhibitory impacts of amended substrates on the incidence of disease during both rotations were clarified a comparable reduction on tomato grown in different amended Mushcom 1 in both rotations. The favorable substrate has been shown to encourage *T. h.* and *B. subtillus* to produce high levels of cellulase enzyme that hydrolyzes the cell walls of several such pathogenic fungi as *F. oxysporum* and *R. solani* (Rini and Sulochana, 2007). On the other hand the viability of *Rhizoctonial sclerotia* decreased after 30 days of incubation by reducing their germination to more than 70% when investigated by (Mello and Faull, 2000; Montealegre *et al.,* 2010).

Table (3): Effect of substrate and amendments on the occurrence of damping-off during two rotation.

| Rotation | Amendments / Substrate | *T.harzianum+ Bacillus + R.solani* | *T.harzianum+ R.solani* | T.v + *R.solani* | *R.solani* (Contro) |
|---|---|---|---|---|---|
| Rotation 1 | Forest litter | 38.83 ij | 66.100 e-h | 70 e-g | 52.13 h-i |
| | Mushroom substrate 1 | 70.87 e-g | 60 f-h | 68.93 e-g | 79.97 b-e |
| | Mushroom substrate 2 | 42.17 ij | 39.87 ij | 36.63 j | 51.67 hi |
| | Sand ( Control) | 90 a-d | 96.67 a | 95.33 ab | 96.67 a |
| Rotation 2 | Forest litter | 65.53 e-h | 60 f-h | 58.87 gh | 61.20 f-h |
| | Mushroom substrate 1 | 30 j | 27.77 j | 32.87 j | 61 f-h |
| | Mushroom substrate 2 | 77.90 c-e | 75.67 d-f | 86.67 a-d | 74.53 d-g |
| | Sand ( Control) | 90 a-d | 92.20 a-c | 90 a-d | 96.67 a |

## CONCLUSION

We conclude that disease incidence coincides by re-colonization of opportunistic fungi and *B. subtillus* after both rotations of tomato seedlings with a rapid decline of the soil microbial in the second one. Therefore, the suppression of *R. solani* and other soil-borne pathogens required not only the application of antagonists, the soil conditions for colonization each of antagonist concurrent microbial dynamics are crucial for the efficient environment to the antagonism and mycobicota succession in the soil. Results suggest that noticeable reduction in disease incidence depended on both bio-control and saprobes colonization and several literatures clarified the endophyticfungi of *Aspergillus* and *Penicillium* spp. might serve as the main components responsible for pronounced antifungal properties involved in protecting the host against invasion of such virulent pathogens as *R. solani* (Wang *et al.,* 2008; Xiao-Jun *et al.,* 2012).

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# التأثير التضادي لفطر الترايكوديرما *Trichoderma* ضد مرض موت البادرات الرايزكتونية Rhizoctonial damping-off على الطماطة النامية في وسط العضوي

وزير علي حسن و ابراهيم عيسى طاهر و خديجة احمد سيدو و علي سامي علي
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## خلاصة

كشفت النتائج أن نسب الإصابة بمرض موت البادرات الرايزكتونية Rhizoctonial damping-off في الطماطم كانت ٦٥ % و ٦٧ %، في مرحلتي الزراعة. نسبة اللأصابة في اوساط الزراعة المستخدمة كل من أوراق الشجر الصنوبر و mushcom2 وصل إلى ٥٩ و ٦٠%. وفيMushcom1 اقتصر حدوث المرض على ٥٣ %. في المقابل، اضافة *T.h. +B. subtillus* قد ادت الى انخفاض نسبة الأصابة بصورة واضحة لتصل إلى ٣٣,١٦ %، أعلى نسبة الأصابة وصل الى ٩٢ و ٩٤ % في معاملة السيطرة (التربة الرملية المزيجية)، ومع ذلك كان هناك تأثير تضادي لفطر *Trichoderma* ضد فطر الممرض *R. solani* في اوساط المختلفة و ان معدل موت البادرات في معاملة السيطرة كان ٩٠ %. وأخيرا لوحظت انخفاض الاصابة بهذا المرض على برادات الطماطم المزروعة في mushcom 1 و mushcom 2 خلال دورتين زراعيتين بصورة خاصة عند خلطها مع *T.h. + B. subtillus*.