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SAPROPHYTIC COMPETITION OF SOIL MICROFLORA AND *Rhizoctonia Solani* IN THE ROTATING AMENDED COMPOSTS PLANTED BY TOMATO

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ABSTRACT

The succession competitiveness of predominant saprophytic fungi, and bacteria colonized different amended composts, inoculated with propagules of Rhizoctonia solani were investigated after 20 and 40 days of the first and second rotation of tomato seedlings. Fungal communities of Alternaria alternata , Aspergillus flavus, A. niger, A. terreus, Fusarium sp., Penicillium spp., and a bacterium of Bacillus subtilis were isolated from substrates of pine leaf litter, mushroom's compost1, and control of (sandy loam soil). Microbial population occurred in a range of 1.88-2.4×10 ^{4} cfu gm $^{-1}$, this density decline in the mushcom 2 to 0.52 and 0.75×10 ^{4} cfu gm $^{-1}$ during both rotations, respectively.

Amended composts with formulation of T. harzianum (T.h) and B. subtilis 10% at 10gm/kg tomato seeds were instigated the saprobes competition during both rotations when insulated with 2.3 and 2.4×10 ^{4} cfu gm^{-1}. T. viride which was also heartening a comparable development of competitive saprobes particularly in the second rotation which observed with 1.87 and 2.15×10 ^{4} cfu gm $^{-1}$.

The highest frequency for each of Penicillium spp.,with 16.3 ×10 ^{4} cfu gm $^{-1}$and B. subtilis with 18.7 ×10 ^{4} cfu gm $^{-1}$ were detected in the first rotation colonizing substrates of pine leaf litter and (mushcom 1) amended with T. h. & B. sublilis. Therefore, these species are capable to develop and compete the pathogen in space and nourishment even under conditions of nutrient deficiency, hence, colonized of Penicillium spp in non-amended sandy loam soil remarkably with 5.33×10^{4} cfu gm^{-1}.

KEYWORDS**:** *R. solani*, compost, *T. harzianum, T. viride, B. subtilis* and microbial activity

INTRODUCTION

Rhizoctonia diseases occur throughout the world. They cause losses on almost all vegetables and flowers, several field crops, turf grasses, and perennial ornamentals, shrubs and trees. The most common symptoms on most plants are damping-off of seedlings and root rot, stem rot or stem canker of growing plants (Agrios, 2005).

Suppression of soil-borne pathogens as *R. solani* has been reported to be typically related to the level of total microbial activity present in the compost (Hoitinketal, 1997). Regardless of application biocontrol agent such as *Trichoderma spp*. and *Bacillus subtilis* or other antagonists, disease suppression by composts is mainly attributed to the opportunistic microbial population linked to the source of the organic matter in the compost, and this potential of suppressive compost can be restored by reintroducing a mixture of microorganisms, a specific organisms, or amendments (Dukare etal., 2011and Noble, 2011). Furthermore, nutrients for antagonistic or beneficial organisms provided by organic matter or waste products released by other organisms (Haggag, 2002).

However, opportunistic fungi strains are the major components that grow in stacked masses of plant material, piles of agriculture such as mushrooms compost, forestry products and other accumulations of organic matter where the aerobic environment facilitates the basic conditions for their development and colonization during plant growth or after harvesting (Dhahir, 2013).These strains of *Aspergillus, Penicillium, Cladosporium, Alternaria, Curvularia, Fusarium,* bacterial genera of *Bacillus*, *Pseudomonas*, and more genera in the Ascomycetes and Deuteromycetes with a minimum at or above 20C° and a maximum temperature range of up 62 C° for their growth and representative of soil microflora that can grow at above 45C° (Ashraf etal., 2007; Mouchacca,1997).

The main reason how *B. subtilis* grew much faster than other microorganisms indigenous to the mature compost and antifungal or antibacterial

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substances, this obvious presumably through death of these competitive organisms at higher temperature .(Gunphae et al. 1990).

Indeed, thermophilic and mesophilic fungi have a powerful ability to degrade polysaccharide constituents of biomass like cellulose and consider the potential source of cellulolytic enzymes with scientific and commercial interest, these fungi can make the process more economical due to their thermostable enzymes, high rate of cellulolytic enzymes and ability to saccharify under non aseptic conditions (Maheshwari et al., 2000; Sohail et al., 2009).

Most of *Trichoderma* strains are mesophilic and can't protect germinating seeds from soil-borne diseases caused by cold-tolerant strains of pathogens during cold spring (Samuels, 1996). On the other hand, *R. solani* surviving in the peat-mix could not be avoided though the competition of microbiota in the substrate (Dhahir, 2013). Chung and Hoitink (1990) revealed that *R.solani* propagules were not eradicated from tree bark compost when applied with their mesophiles isolates of *Humicola spp.* even application of *T. harzianum*, and this suggest the aim of addition such preservatives as sugar and other compounds to keep the spores of formulated isolates of *Trichoderma* and may increase their growth in the consumption (Panahian etal., 2012).

Finally, the concurrent utilization of the available carbon sources by thermophilic fungi might be an adaptive strategy for opportunistic growth in nature under conditions of low nutrient availability and thermal fluctuations in the environment. For more understanding of additional sustainable antagonism of the soil microbes, this work aimed to assess the succession competitive of most common saprophytic fungi with the inoculum potential of *R. solani* after two rotations of tomato seedlings planted in the pine leaf litter and mushrooms compost amended with each of commercial product of T. *harzianum* plus *B. subtilis*10% and *T. viridae.*

MATERIALS AND METHODS

Composts origin:

Substrates tested for sowing tomato seeds were:

1. A mixture of manufactured Mushroom's compost 1 (Mushcom. 1) and sandy loam soil 1:4 (v/v) , that include of wheat straw , chicken manure 30 % , urea (trace) , calcium carbonate 15% and Gypsum 3%.

2. Mushroom's compost 2 (Mushcom.2) consisted of chopped wheat straw , wheat bran 5 % and Gypsum 5 %.

3. Chopped pine leaf litter.

4. Control treatment of sterilized sandy loam soil.

Source of antagonistic fungi, bacteria and tomato seeding:

Commercial product (Bio health WSG= Water Soluble Granular of *T. harzianum* and *Bacillus subtilis*10 % with Humic Acids 75% and Seaweed Extracts 5%) used at 10 gm / kg seeds manufactured by Humintech GmbH, Hecrdter Landstr., Dusseldorf, Germany. Another isolates of *T. harzianum*(Riafi) strain and *T. viride* were provided by Plant Protection Dept., College of Agric. & Forestry, Univ. of Mosul, Iraq. The isolate of *T. harzianum* was grown in one liter conical flasks containing 250 gm Mushroom's compost, 250 gm wheat Barn 250 gm and millet seeds and 250 ml autoclaved Potato Dextrose medium, incubated for 25 days , and thoroughly mixing at 5 days interval to warrantee inoculum distribution. Contents of flasks were poured into plastic plates under aseptic conditions, left to air dry then mixed thoroughly before kept in sterilized polyethylene container at ambient temperature until using at 0.5 %, spore suspension was adjusted at 3×10^7cfu gm^{-1}according to (Sallam et al., 2008).

Spore suspension of *T. viride* at 3×10^7cfu gm^{-1}prepared from cultures grown at28 ^{0}c for 10 days. 20 ml conidial suspension was drenched with each of substrate filled in pot of 15 cm in diameter. The composted substrates of 1kg filled into pots and inoculated with 2% of pathogenic *R. solani* incubated at 28^0c under darkness for seven days before planting. Control treatments included sandy –loam soil with inoculum of *R. solani*. Ten seeds of tomato were sown in each pot. Each treatment replicated four times, and irrigated as required. After 20 days, diseased tomato seedlings harvested and the container medium was reseeding and harvested again after another 20 days. Samples from control and amended substrates were collected for competitive opportunistic fungi and bacteria counts.

Population of microbial community colonized different substrates

After two week of harvesting diseased seedlings each of tomato rotation, soil samples of about 20 gm were taken from different amended

substrates within the root zones and placed in refrigerator 4^0C. Samples were quantified for opportunistic fungi and bacteria of *B.subtilis* using serial soil dilutions to 10^{-4} of sterile water. Nine plates divided into three replicates for each medium were used. Total fungi and bacteria were computed as colony forming units (cfu) for each rotation.

Statistical Analysis: Systems software (SAS version 8, Institute, Inc.) were used for data analysis, subjected to analysis of variance mmmmmmmmm(ANOVA) and pooled together after testing of homogeneity of variance ($P \leq 0.05$). Means of the treatments were compared by Duncan Multiple Range Test at 0.05 levels.

RESULTS AND DISCUSSION

This work revealed that microbial biomass is the characteristic of micro organizations that participate in the biochemical cycle and constitute alive part of the soil organic matter. Accordingly, after 20 days for each of the first and second rotation of tomato seedlings, fungal communities of *A. alternata*, *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium* sp.,*Penicillium* spp., and a bacterium of *B. subtilis* were isolated. Population density of these soil microflora were colonized substrates of pine leaf litter and mushcom1 considerably with a range of 1.88 and 2.40×10^4 cfu gm^{-1} in both rotations. Similarly, comparable microbial community of 2.23 and 2.4×10^4 cfu gm^{-1} was also developed in the control sandy loam soil revealed their ability to develop under poorly substrates environment. In contrast, compost mushcom 2 lead to significant reduction in the microbial occurrence since, their occurrences were restricted to 0.52 and 0.75×10^4 cfu gm^{-1} in both rotations, respectively (Fig.1), this may be due to mushcom 2 richness of cellulosic contents of wheat bran which fervor by most saprobes compared to other substrates, suppress pathogenic fungi of *R. solani* through competitive inhibition and the products of natural antibiotic, facilitative aerobe can survive extreme heat, and its ability to biodegrade hydrocarbons. (Kinselia et al., 2010).

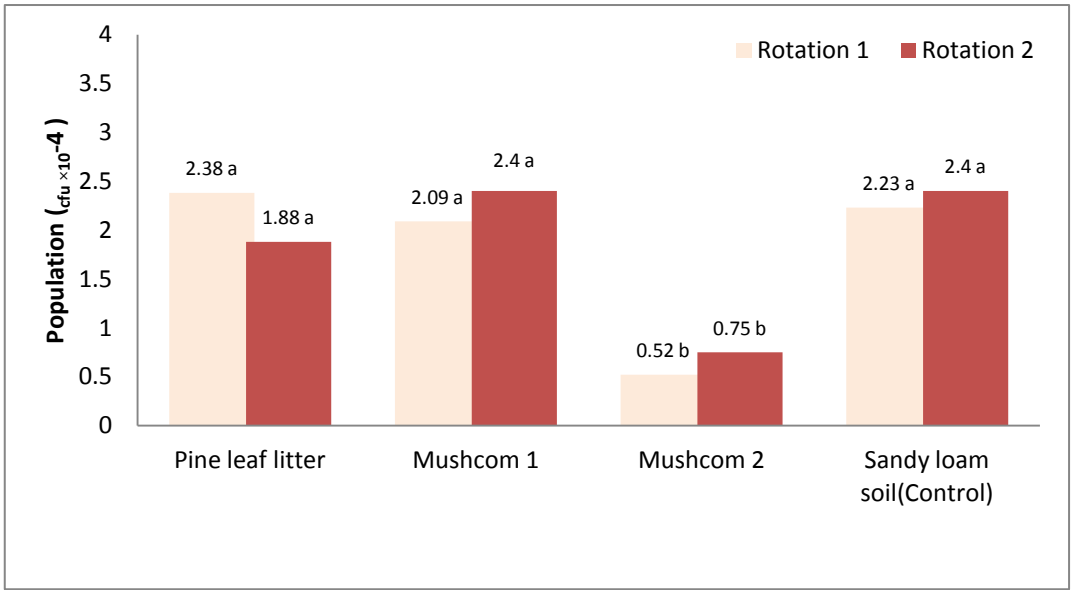

Fig.(1): Effect of different substrates on the soil microbial population in both rotations.

Worthily, competition occurs between pathogenic and saprobes in the rhizosphere is a major concern for space and nutrients (Viterbo et al., 2007). Thus, competition can be divided into saprophytic for nutrients in the soil and infection sites on and in the roots (Fravel et al., 2003). Amendments of *T. h + B. subtilis*. Formulation were also encouraged the saprophytic colonization with 2.33 and 2.40×10^4cfu gm^{-1} in both rotations, respectively (Fig.2).

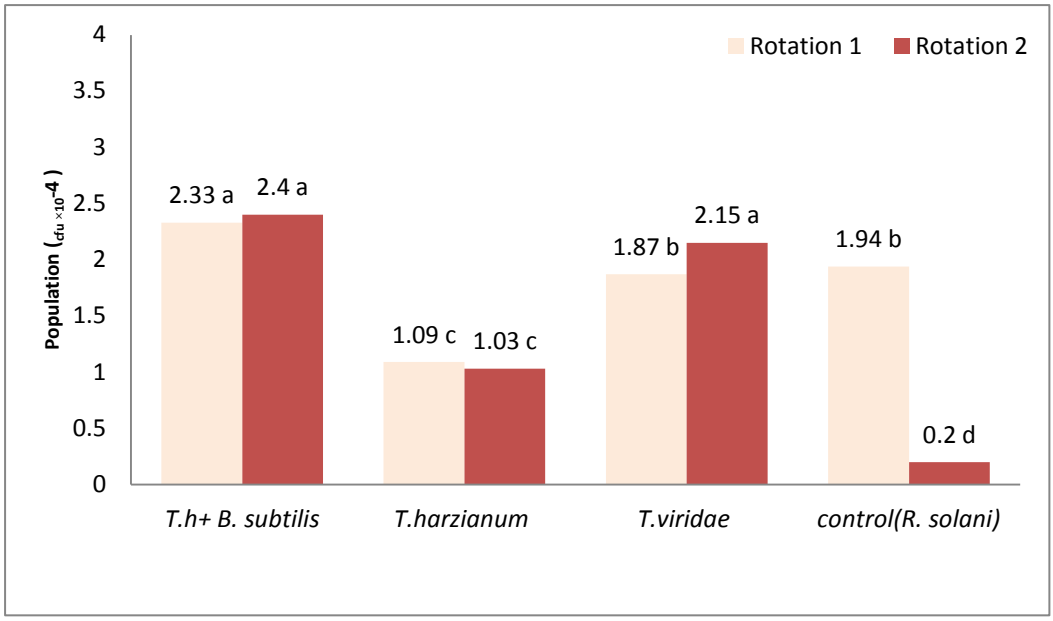

Fig.(2):- Effect of different amendments on the microbial population in both rotations.

These results confirmed the tolerance of *T. harzianum* to ammonia induced mycostasis leading to successful use of this species as abroad spectrum biocontrol agent (Papavizas , 1985) . Furthermore, *Trichoderma* and *Bacillus* don t adversely affect the beneficial microorganisms in the rhizosphere and facilities the biocontrol of plant disease (Singh et al., 2008). *T.viridae* was also participated clearly in the creating a favorable condition for saprobes colonization with 1.87 and 2.15×10^4cfu gm $^{-1}$ compared to their limited presence with 1.09 and 1.03×10^4cfu gm $^{-1}$ in the substrates amended with *T. harzianum*. Inoculum of *R.solani* (control) didn't prevent saprobes incidence particularly in the first rotation when developed to 1.94×10^4cfu gm $^{-1}$, whereas reduced to 0.2×10^4cfu gm^{-1} in the second one.

On the other hand, in spite of several soil-borne pathogens have been reduced using composts made of different raw material agrees with what has been indicated by Borrero et al., (2004) and Litterick et al.,(2004), their capacity to suppress *R. solani* that affects both seedlings and adult plants of many species, remains limited (Hassan et al., 2015; Hoitink and Boehm , 1999; Scheuerella et al., 2005).Assessment of saprobes population in the substrate samples were clarified in the (Fig. 3). High population of *Penicillium* 6.85×10^4 cfu gm^{-1} in the rotation 1 was restricted to 0.04 cfu in the rotation 2, whereas *B. subtilis* was the predominant bacterium in the rotation 2 when colonized the examined substrates with 5.72×10^4 cfu gm^{-1}, since it colonized the roots metabolizing nutrients of phosphates and nitrates into more bio available forms often used as biological soil amends and in composition facilities since *B. subtilis* survives the thermophilic phase.(Kinselia et al., 2010).

Several reports demonstrated that endophytic fungi of *Aspergillus* and *Penicillium* spp. might serve as the main components responsible for pronounced antifungal properties involved in protecting the host plant against attack of

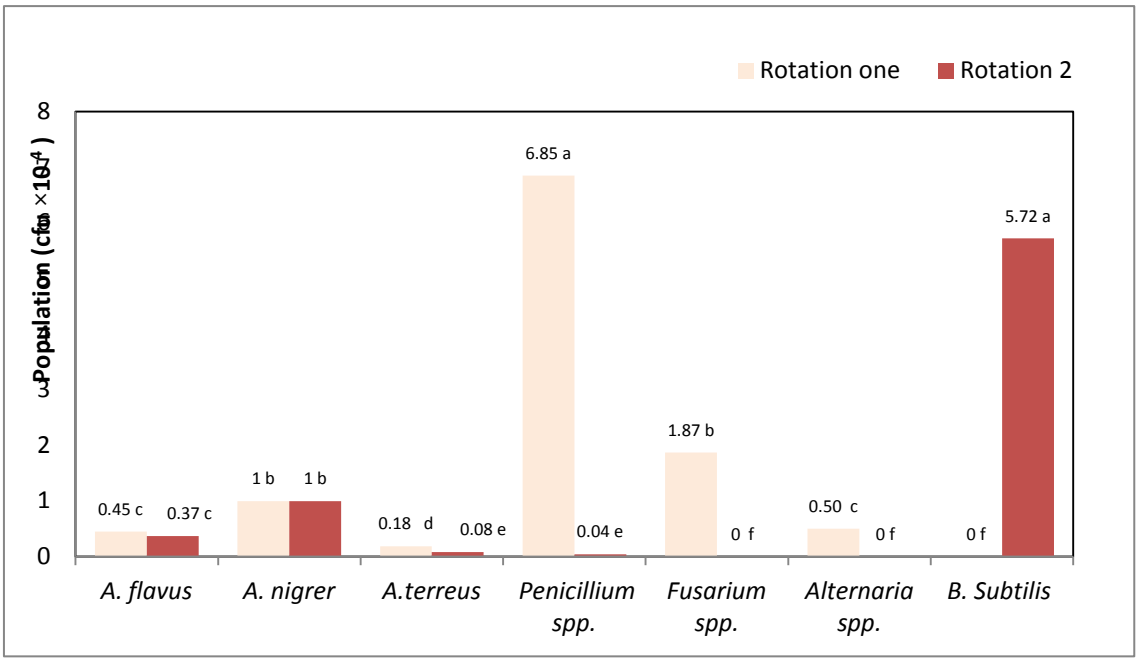

Fig.(3):- Microbial population ($_{cfu\times10}^{-4}$)in each gm isolated from compost samples in both rotation

Virulent soil borne pathogens as *R. solani* (Wang et al., 2008; Xiao-Jun et al., 2012).

Literatures emphasized that control of such soil – borne pathogens as *R. solani* and *Fusarium* root rot was more effective in the soil amended with mushroom's compost , leaf litter , and manures with *Trichoderma*, these amendments stimulated ashift in the soil microbial population dynamics in the rhizosphere particularly fluorescent such as bacteria *Bacillus spp.* (Stevens et al. , 2003) , and each of *Rhizopus stolonifer* , *Aspergillus* spp. , *Penicillium* , *Humicola* spp. , and others (Hassan and Yousif, 2013).Therefore, in addition to the most common biocontrol agents of *Trichodema* spp., several opportunistic fungi have been reported to be antagonist particularly in the organic composts against plant pathogens of *R. solani* , *Pythium spp*. and *Phytophthora spp*. successfully *Aspergillus niger* , *Chaetomium spp*. , *Penicillium spp*. and *Ulocladium atrum* (Kaewchai et al., 2009), as well as Bacterial genera of *Bacillus* , *Pseudomonas* and *Streptomyces* were also documented and widely applied in the biological control of *R. solani* (Sabaratnam;Traquair ,2002 and Yu et al., 2005). Results suggested that microbial community of *Aspergillus* and *Penicillium* have a greater bio-diversity potential source of cellulase , and remarkable ability to saccharify under non- aseptic condition of substrates and of low nutrient availability and thermal fluctuations in the environment (Dhahir , 2013 and Maheshwari et al., 1987).The influence of amended substrates on the microbial dynamics associated with each of *R. solani* and *Trichoderma* spp. in the first rotation were represented in (Table1)

Table (1): -Influence of substrates & amendments on the microbial population in the first rotation.

Substrates	Amendments	Population (10^4 cfu gm^{-1})						
		A. flavus	*A. niger*	*A.terreus*	*Penicillium* spp	*Fusarium* sp.	*A.alternata*	*R. solani*
Pine leaf litter	*T.h + B. subtilis*	1.33 hij *	0.667 ij	0.333 ij	16.33 a	0.0 j	0.0 j	-
	T.harzianum	0.0 j	0.0 j	0.0 j	0.0 j	7.33 cde	0.0 j	-
	T.viride	0.0 j	0.0 j	0.0 j	10.0 b	3.66 fgh	0.0 j	-
	R. solani (Control)	0.66 ij	0.0 j	0.66 ij	16.33 a	0.0 j	0.0 j	+
Mushcom Compost 1	*T.h + B. subtilis*	1.33hij	0.0 j	0.33 ij	18.667 a	0.0 j	1.33 hij	-
	T.harzianum	0.0 j	0.0 j	0.0 j	0.0 j	8.33 bcd	0.0 j	-
	T.viridae	0.0 j	8.33 bcd	0.33 ij	3.00 ghi	0.0 j	0.0 j	-
	R. solani (Control)	0.66 ij	1.33hij	0.0 j	6.00 def	0.66 ij	0.0 j	+
Mushcom compost 2	*T.harzianum+ B. subtilis*	0.0 j	0.66 ij	0.0 j	0.66 ij	0.0 j	6.66 cde	-
	T.harzianum	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	-
	T.viridae	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	0.0 j	-
	R. solani (Control)	0.66 ij	0.0 j	0.33 ij	3.66 fgh	0.0 j	0.0 j	+
Sandyloam soil (Control)	*T.h + B. subtilis*	1.00 hij	1.33 hij	0.0 j	5.33 efg	0.0 j	0.0 j	-
	T.harzianum	0.0 j	1.00hij	0.0 j	0.66 ij	9.00 bc	0.0 j	-
	T.viridae	1.33hij	1.00hij	0.0 j	16.33 a	1.00 hij	0.0 j	-

*Means followed by the same letters are not differ significantly ($P \leq 0.05$).

Substrates of control forest litter and (Mushrom 1) amended with *T.h + B. subtilis* were colonized forcibly with *Penicillium* spp. , ranging between 16and18.7 cfu gm^{-1} , these substrates were resemble to sandy loam soil (control) amended 18.7 cfu mg^{-1} , these substrates were resemble to sandy loam soil (control) amended with *T.v* in the comparable existence of *Penicillium* . Moderate occurrence of *Fusarium* sp. was found in the *T.v* amendments of different examined substrates.

However, the crucial factor of the microbial competition includes concurrent utilization of the available carbon sources by soil microorganisms and this may be an adaptive strategy for opportunistic growth in nature. The aggressive competition of *Penicillium* spp. was apparent in the first rotation even under unfavorable conditions of non- amended sandy loam soil i. e lack of nutrients. Deuteromycetes such as *Penicillium* species are very common soil borne saprophyte fungi and are known to be potent antagonists of such pathogens as *Fusarium* (Sabuquillo et al., 2005).The competiveness of microbial dynamics were apparent in their great wide spread colonization during rotation 1, since the pathogenic *R. solani* was isolated in just non-amended substrates of sandy – loam soil.

In the second rotation (Table 2) *B. subtilis* was mostly recovered substrates in spite of amendments when re isolated by 45 % in the sandy loam soil amended with *T.h+B. subtilis*, considerable density of this bacterium 13.66 and 14×10^4 cfu gm^{-1} was found in the forest litter and (Mushcom 1) amended with *T.v* ,the presence of other opportunists of *Aspergillus* spp. were rarely or not found.

Amendments with organic matter of compost improve plant health; the mechanism of diseases suppression coincide with many variable within examined substrate that need to be simultaneously monitored. During second rotation when the most nutrients depleted, *R. solani* was forcibly recolonized non- amended substrates of sandy loam soil and other treatments which lost their contents of microbial competition of most opportunistic fungi in spite the occurrence of *B.*

Table(2):- Influence of substrates & amendments on the microbial population in second rotation.

Substrates	Amendments	Population ×10^{4}cfu gm $^{-1}$					
		A. flavus	***A. niger***	***A.terreus***	***Penicillium* spp**	***B.subtilis***	***R.solani***
Pine leaf litter	*T.harzianum + B. subtilis*	0.33 d *	0.00 d	1.00 c	0.00 d	1.00 c	-
	T.harzianum	0.00 d	0.00 d	0.33 d	0.00 d	0.00 d	+
	T.viridae	0.33 d	14.66 b	0.00 d	0.00 d	14.00 b	-
	R. solani (Control)	1.00 c	0.00 d	0.00 d	0.33 d	4.66 c	++
Mushroom's substrate 1	*T.harzianum + Bacillus*	0.00 d	0.33 d	0.00 d	0.00 d	0.00 d	+
	T.harzianum	0.33 d	0.00 d	0.00 d	0.00 d	0.00 d	+
	T.viridae	0.00 d	0.00 d	0.00 d	0.00 d	13.66 b	-
	R. solani (Control)	0.66 d	0.00 d	0.00 d	0.00 d	0.00 d	++
Mushroom's substrate 2	*T.harzianum + B. subtilis*	0.33 d	0.00 d	0.00 d	0.00 d	0.00 d	-
	T.harzianum	0.66 cd	0.33 d	0.00 d	0.33 d	0.00 d	-
	T.viridae	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	+
	R. solani (Control)	1.00 c	0.00 d	0.00 d	0.00 d	12.33 b	++
Sandy loam soil (Control)	*T.harzianum + + B. subtilis*	0.00 d	0.00 d	0.00 d	0.00 d	45.00 a	-
	T.harzianum	0.66 cd	0.33 d	0.00 d	0.00 d	1.00 c	-
	T.viridae	0.00 c	0.33 d	0.00 d	0.00 d	0.00 d	+
	R. solani (Control)	0.66 cd	0.00 d	0.00 d	0.00 d	0.00 d	++

*Means followed by the same letters are not differ significantly ($P \leq 0.05$).
- no colonized, + slight colonization, ++ density colonization

CONCLUSION

We conclude that microbial community colonized amended substrates particularly of mushroom's compost 1 with commercial product of *T.h&B. subtilis* was richness in the nutrient necessary to sustenance and development of saprophytes competition against propagules of *R. solani* in their dynamics space and nutrients. This was clarified when computing damping off of tomato seedlings which reduced remarkably during the first rotation (Hassan et al., 2015).

The decline of the microbial colonization in the second rotation may be attributed to the relative depletion of nutrients available in the container composted media necessary for microbial dynamics. The future challenge is the identification for specific parameters for predicting the suppressiveness of each organic amendment in combination with each pathogen species.

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هەڤرکییا جە ند کییانەوەرێن ئاخێ ین هور دگە ل ئێشکەر ***Rhizoctonia solani*** د خولین جا ندنا زبلێن ئورگانیك یی بهێزکری و چاندی باجان سورکی

پوختە

ئەڤ ڤەکولینە هاتە ئەنجامدان ل سەر هەڤرکی یا ل دویف ئێك یا کەرووپا وبەکتریا ئەوێن بەربەلاڤ وئاکنجی دناف جۆرێن جورا وجۆر یێن زبلێ ئورگانیکی یێ بهێزکری ب ڤاکسینێ ئێشکەر ***Rhizoctonia solani*** ب ڕێکا ڤەدەرکرنا وان پشتی دوو خۆلێن چاندنێ بو شتلێن باجان سورکێ ب ***20*** ڕۆژا و***40*** ڕۆژا بو خۆلا ئێکێ ودوویێ ل دویف ئێك دا. ***Bacillus subtilis, Fusarium sp., Penicillium spp., Alternaria alternarnata, Apergillus flavus, A. niger, A. terreus*** هاتنە ڤەدەرکرن ژ زبلێ بەرمایێن بەلگێن کاژا ودوو زبلێن زێدەکرنا مەشرومی ژناخا خیزی وزمیجی. چراتی یا هەبوونا کەرووا گەهشتە ***1,88 -2,4 4 10 X*** کۆلۆنی/ گم. وئەڤ چراتی یە کێم بوو وگەهشتە ***0,52 -0,75 4 10 X*** کۆلۆنی/ گم دناڤ زبلێ مەشرومی ژمارە ***(2)*** دەدەمێ هەردوو خۆلێن چاندنێ.

زبلێن بهێزکری ب هەر ئێك ژ کەرەستێ بازرگانی یی بەرگیرێ زیندوویی ***T. harzianum*** + بەکتریا ***B. subtilis10%*** ب ڕێژەیا ***10*** گم/ ککم ژتۆڤێ باجانکێ یێن کاریگەربوون بۆ پشتەڤانیا هەڤرکی یا روممی. هاتنە ڤەدەرکرن ب چراتی یا ***2,3*** و ***2,4 4 10 X*** کۆلۆنی/ گم ل دویف ئێك دا. بکارئینانا ***T. virid***بوویە ئەگەرێ زێدەبوونا شینبوونا مترممانا نەخاسمە دخۆلا دووێ وگەهشتە ***2,15 4 10 X*** کۆلۆنی/ گم.

بلندترین دووبارەکرن بێن کەروویێ ***Penicillium spp.(16,3 X 10 4*** کۆلۆنی**/** گم**)** وبەکتریا ***B. subtilis***، ***18,7X 10 4*** **)** کۆلۆنی**/** گم**)** دناڤ زبلێ بەرمابێن کاژا وزبلێ زێدەکرنا مەشرومی ژمارە **(*1*)** یێ بهێزکری ب ***T.hharzianum+ B. subtilis*** ول سەر وی بناغەی ئەڤ جۆرە دکارن ل سەر شینبوونێ وهەڤرکی یا ئێشکەری ل سەر زادی وجهی حەتا دکاودانێن کێمبوونا کەرەستێن خوارنێ لەورا هاتنە ئاکنجی کرن دناڤ ئاخا خیزی وزمیجی دا بشێوەکێ بەرچاڤ**.**

التنافس الرمي لبعض احياء التربة الدقيقة مع الفطر الممرض *Rhizoctonia solani* في الدورات الزراعية للاسمدة العضوية المدعمة و المزوعة بالطماطة

الخلاصة

تم البحث عن المنافسة المتعاقبة للفطريات و البكتريا الرمية السائدة والمستوطنة لانواع مختلفة من الاسمدة العضوية المدعمة والمعداة بلقاح الممرض *Rhizoctonia solani*بعزلها بعد دورتين زراعيتين لبادرات الطماطة بواقع *20* و *40* يوم للدورة الاولى و الثانية على التوالي.

عزلت الفطريات *Alternaria alternata* , *Aspergillus flavus، A. niger,A. terreus، Fusarium sp.*، *Penicillium spp.*، والبكتريا *Bacillus subtilis* من سماد مخلفات اوراق الصنوبر, و نوعين من سماد اكثار المشروم, و من التربة الرملية المزيجية . تراوحت كثافة تواجد الفطريات *1,88 -2,4*×*104*مستعمرة/غم ,و انخفضت هده الكثافة الى *0,75-0,52*×*104* مستعمرة/غم في سماد المشروم رقم (*2*) خلال دورتي الزراعة.

كانت الاسمدة المدعمة بكل من المستحضر التجاري للمقاوم الحيوي *T. harzianum(T.h)* +بكتريا *B.subtilis* *10* % بمعدل *10* غم/كغم بذور طماطة مشجعة للمنافسة الرمية في كلا الدورتين فقد عزلت بكثافة *2,3* و *2,4* ×*104* مستعمرة /غم.ادى استخدام *T. viride*الى زيادة نمو المترممات و خاصة في الدورة الثانية بواقع *2,15* ×*10^4* مستعمرة /غم .

ظهرت اعلى كثافة للفطر *Penicillium spp*. *16.3*×*10^4* مستعمرة /غم) و بكتريا *B.subtilis*(*18,7*×*10^4* مستعمرة /غم) في سماد مخلفات الصنوبر وسماد اكثار المشرورقم *(1)* المدعمة بالمستحضر*T.h+ B. Subtilis*, و بناء على ذلك فان هذه الانواع قادرة على النمو و منافسة الممرض في الغداء و المكان حتى في ظروف نقص المغذيات و لهذا فقد استوطنت التربة المزيجية الرملية بشكل لافت.