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Soil Amendment and Using Bare Root Trees Treatments as Alternative Fungicides for Controlling Root Rot Diseases on Citrus Transplanting

Faten S. Mansour* and H.S. El-Shimy**
**Centeral Lab. of Organic, and **Fruit and Woody Trees Dis. Dep., Plant Pathology Research Institute,Agricultural Reasearch Centre, Cairo, Egypt.*

FUSARIUM SOLANI, Rhizoctonia solani and *Phytophthora nicotianae* proved to be the dominant fungi isolated from roots of citrus seedling in El- Sharkia , El-Behera, El- Kalubia, El- Menofia and El- Gharbia governorates, meanwhile *Fusarium oxysporum, Pythium* spp. and *Phytophthora citrophthora* show less frequent. Pathogenicity test provided that the most aggressive fungi on citrus were *F. solani* and *R. solani* followed by *P. nicotianae*.var. *parasitica*.

Citrus seedlings treated with four formulations of bio-products (Bare root, *Trichoderma harzianum* on bagasse, *T. harzianum* drenched in soil)

In orchards trails, citrus bare- root were coated with *Trichoderma harzianum* formulated on sugar cane bagasse at the rate of 10% (w/w) of soil. This treatment highly reduced root rot incidence caused by *F. solani*, *R. solani* and *P. nicotianae*. with 73.9,76.4 and72.2% respectively, ether coating nor drenching root treatments reduced root rot diseases. On the other hand, coating seedling root by drenching with *T. harzianum* or drenching with clean- root+ Bio- product caused moderate effect in reducing root rot diseases incidence. Under field conditions, amended soil with *T. harzianum* + bagasse at the rate 10% (w/w) of soil reduced root- rot disease after 20 days. Moreover, plant heights, dry weight, stem thickness, new leaves emergence and total leaf area were positively related to bagasse + *T. harzianum* and clean-root+ bio- product treatments.,. Thereby total plant biomass, plants treated with such two compounds had relatively greater shoot, root ratio than the untreated control. It could be noted that particle using of soil amendment with agricultural wastes formulated with bio control agents and / or coating by drenching seedlings root treatments to control soil borne plant pathogens as a substitute of chemical fungicides is possible without any risk to human, animal and the environment.

Keywords: *F. solani, R. solani* and *P. nicotianae*, Citrus, Root rot, Bare root.

Citrus occupies the greatest plant area among growing fruit trees in Egypt. Citrus root rot is worldwide problem, causing the potential destruction of orchards and reducing fruit quality and yield of citrus. It is estimated that 60% of citrus orchards are affected by the disease. The pathogens commonly associated with root rot of citrus are *Fusarium solani, Rhizoctonia solani, Phytophthora* spp., *Phytophthora* root rot is the most serious soil born disease of citrus, *Fusarium solani* is always considered as a" mild Pathogen" that has more chronic effect in contrast with the actual effect of *Phytophthora,* the damage caused by *F. solani* is probably underestimated. Often, this pathogen is only associated with" dry root rot" of citrus while the extensive feeder root rot caused by *F. solani* is overlooked. However, much data exists which demonstrate *F. solani* to be an important pathogen of citrus, forming part of the root rot complex, interacting with other pathogens such as the citrus nematode *Tylenchulus semipenetrans* and causing sever root rot, when citrus trees are under stress. Further more, this fungus has been implicated as possible causal agents of citrus blight and has been shown to produce orange of phytotoxins in the vascular system of citrus trees. Being involved in both root rot, related decline of citrus and in citrus blight, *Fusarium solani* is undoubtedly of major importance in terms of productivity of citrus (Michael, 1995).

Controlling soil born pathogen depends mainly on fungicidal applications, that causing hazards to the human health and environment (Rauf, 2000). Soil amendment and bio-priming seedlings treatments are gaining importance in management of many plant pathogens as another alternative to chemical fungicides in recent times. Also, citrus budlings are dug in the nursery for transplanting to the orchard either as balled trees (traditional method) or as bare-rooted (Miller, 1968 and Walter, 1973). Balled budlings may usually held longer after digging and before planting without injury as the balls supply the root system with adequate moist. However, the soil in the ball must be of the same or similar texture to that in the orchard to insure adequate irrigation of the young trees. Growers who have used the bare- root method believe that it has several advantages (Miller, 1968). It is ideal for detection and elimination of bud lings with a diseased or malformed root system. A large amount of the root system can be permitted since their length is not limited to the size of the ball. At times, there may be a variation in texture between soil in the nursery and the location, a nursery bundling grown in clay soil and planted in sandy soil will be more difficult to irrigate. These soil differences are eliminated with bare- root plants. Furthermore, weeds usually growing in the nursery can be eliminated and not moved as with balled bud lings. Seedlings drenching with bio-control agents *(Pseudomonas fluorescence)* as bio-primed (coating) on sweet corn yields, was the most effective treatments for controlling root rot diseases as shown by (Callen *et al.,* 1991 & Jahn & Puls, 1998).

Soil amendment with agricultural wastes formulated with bio-control agents was recommended for controlling soil borne pathogens instead of fungicides. These methods introduced efficient disease control and increased survival

seedlings. Mitra and Nadi (1994), Nemec *et al.* (1996) and May & Kimati (1999) noted that amended planting mixes with formulation of commercial bio-control agents such as *T. harzianum, B. subtilis* and *Streptomyces sp.* reduced root rot and growing rot diseases on tomato, bell pepper, celery and citrus. Bio-priming as seedlings drenching that integrates the biological and physiological aspects of disease control was recently used as alternative method for controlling many seed and soil borne pathogens (Harman & Taylor, 1988, Harman *et al.*,1989 and El-Mohmedy, 2004).

The present study aimed to evaluate the efficacy of soil amendment with *T. harzianum* formulated on sugar cane bagasse and /or bio-priming by drenching root seedling treatment in controlling citrus transplants root rot pathogens under orchard and field conditions.

Material and Methods

The causal organisms

Samples of citrus seedlings showing root rot disease symptoms were collected from different governorates El-Sharkia, El-Behera, El-kalubia, El-Menofia and El-Gharbia. All samples were subjected to isolation samples of each infected part where separately thoroughly washed under running tap water, dried between folds of filter papers then cut into small pieces and surface sterilized using 0.1% mercuric chloride solution for 1 min. Sterilized pieces where rinsed several times with sterilized distilled water, then dried between two folds of sterilized filter paper. Under aseptic conditions, sterilized pieces were transferred onto the surface of potato dextrose agar (PDA) medium in Petri-dishes. Plates were inverted and incubated at 22° trails for the causal organisms. The purified isolated fungi were identified according to cultural and microscopically characters described by Barnett and Hunter (1972); Nelson *et al.*, (1983). The percentage and the frequency of each isolated fungus were recorded in each location Disease severity was determined as a percentage of the infection seedlings which were collected from the above different orchards areas.

Pathogenicity test

Representative isolates of the high frequency isolated fungi *Fusarium solani, Rhizoctonia solani* and *Phytophthora nicotianae* were selected to study their pathogenic ability. Plastic Pots (25cm diameter) containing sterilized sandy loam soil artificially inoculated with inoculums of each fungus, which was grown on sandy- barley medium (1:1 w/w of soil and 40% water) for two weeks at 25 ± 1°C. Ten pots were used for each fungus, check treatment [control] was prepared without addition the tested fungi. Valencia orange (*citrus sinensis*) nursery bud lings were chosen to be the parental lines of this investigation. Ninety nursery bud ling plants were dig up every year from the citrus nursery, surface sterilized seedlings of citrus at eighteen month old were transplanted into 25° Cpots. The percentage of died plants were calculated after 15, 45 and 60 days from transplanting.

A. Orchards experiment

In this trials the efficacy of amended soil with bio-enhanced bagasse (*Trichoderma harzianum* formulated on sugar cane bagasse) and bio- priming seedlings (dipping citrus seedlings root system in *T. harzianum* suspension) in controlling citrus root rot pathogens were evaluated.

This experiment was carried out in plastic pots (25 cm) containing individually artificially infested soil with citrus seedlings root rot pathogens. Different soils and seedling root treatments, were applied to evaluate their efficacy in controlling root rot disease pathogens.

Preparation of bio-enhanced : Sugar cane bagasse was ground to fine powder, 250g of this powder was mixed with sandy soil (4:1v/v or w/w) in autoclaved bags. 2.0g of ammonium sulphate, 5.0g of super-phosphate, 5g of potassium sulphate and 400ml water were added to 1000g of bagasse . All bags were sterilized for 1hr in autoclave at 121°Candinoculated with spore suspension of *T. harzianum* ($3X10^6$ spore/ml) then incubated at 25°C±1°C for 14 days, then used as bio-enhanced bagasse for direct delivery into the soil according to El-Mohamedy (2004).

Citrus seedling root were initially washed with tap water to remove clay and sand, the washed roots were drenching according to Harman and Taylor (1988). In Carboxy Methyl Cellulose [CMC] 1% in Erlenmeyer flask on rotary shaker set at 150 rpm CMC 1% or CMC1% supplemented with spore suspension of *T. harizianum* ($3x10^6$ spore/ml) were subsequently to drenching citrus seedlings root. Direct drenching seedlings with spore suspension of *T. harizianum* ($3x10^6$ spore/ml) and use as drenching seedlings root coating with *T. harizianum.*

Sand loam soil was artificially inoculated with *F. solani*, *R. solani* and *P. nicotianae* as mentioned before in plastic pots (25cm diameter) and the following treatments were used.

Citrus seedlings were transplanted in inoculated soil previously amended with bio- enhanced bagasse at the rates 5 and 10% (w/w of soil) 14 days of before transplanting.

Drenching citrus seedlings were transplanting in infested soil.

Drenching or coating with T. harzianum solution : Citrus seedlings were deepen (15min) in spore suspension of *T. harzianum* ($3x10^6$ spore/ml) at 5% and 10% then transplanted into artificially inoculated soil.

Clean roots bio- product : Citrus seedlings were immersed with clean roots Bio-product against roots diseases produced by Central Lab of Organic Agriculture (A.R.C) at the recommended dose (5g/1L) then drenching seedlings in the solution after that seedlings were transplanting in infested soil. It's prefer to keep seedling in polyethylene bags for further studies for don't root dry.

B. Field experiment

Tow field experiments were carried out during 2007 and 2008 seasons under field conditions in naturally heavily infested soil 5% (W/W) soil weight, with citrus root rot pathogens at Abo- kepear (Sharkia). The effective treatments from the previous experiment were chosen to evaluated there efficacy under field conditions. Field experiment consisted of 20 plot in (5m x 5m) space between each pot, comprised of one pits/holes 80^{cm} x 80^{cm} x 80^{cm} which were conducted in randomly complete block design with five replicates (plots) for each particular treatment as well as control (check treatment).

Citrus seedlings were transplanted in all treatment at one seedling/ pit. All plots were sown on the first of October or March, 2007 and 2008. The percentages of root rot disease incidence of citrus seedlings was recorded after 20 days and 40, 60 days from sowing date .percentage of both diseased and healthy plants (survival) were determined and statistically analysed according to the method described by Snedecor and Cochran (1967). Fresh weight of pods, shoots, leaves, and roots of citrus plantlets were evaluated at the end of each growing season.

Plant high (cm) and total leaf area (cm^2) were measured at the end of the experiment (fourteen weeks after sowing). Total left area (cm^2) were measured as described by Ahmed and Morsy (1999).

At the third week of October (first season) and last week of March (second season) all leaves of each plant were counted .also, their length and width (cm) were measured for determining total leaf area (cm^2)/plant according to Ahmed & Morsy (1999) and average leaf area (cm^2) was calculated .Stem length and thickness (5 cm above crown) was also measured in cm. Thereafter, all plants were excavated and separated into leaves, stems and roots. All plant organs were cut into small pieces and dried at 100°C up to constant weight. The total dry weight (g) of each plant was calculated from the sum of the total leaves. Stem and root dry weight .Specific leaf weight per unit leaf area (g/dm^2), was also calculated.

Results

Eighty three fungal isolates were obtained from diseased root of citrus and classified as follow: *Fusarium* spp., *Phytophthora nicotianae*, *Rhizoctonia solani*, *Fusarium oxysporum* *Phytophthora citrophthora* and *Pythium* spp..

Results in Table 1 indicate that the most dominant fungi were *F. solani* (29.0%) followed by *P. parasitica* (24.0%), *R .solani* (19.0%) , *F.oxysporium* (13.0%), *P.citrophthora* (9.0%), *and Pythium spp.* (6.0%), meanwhile *F. oxysporum*, *Phytophthora citrophthora* and *Pythium* spp. were less frequent.

TABLE 1. Frequency of isolated fungi from roots rhizosphere of citrus seedlings showing root rot symptoms at different locations.

Location	Frequency of isolated fungi %						
	F. solani	*R. solani*	*P. nicotianae*	*F. oxysporum*	*Pythium* spp.	*P. citrophthora*	Total
El-Sarkia	25.5	18.5	25.5	13.5	8.0	8.5	30.5
El-Behera	35.0	18.0	30.0	6.0	5.3	5.3	23.0
El-Kalubia	36.5	18.0	18.0	18.0	1	9.0	15.3
El-Menofia	24.0	7.5	25.0	15.5	14.5	13.4	18.0
El-Gharbia	23.0	33.5	22.3	11.2	0	10.0	12.5
Total	29.0	19.0	24.0	13.0	6.0	9.0	100

Samples were collected from 12 month to 14 month old citrus seedlings.

Orchards experiment

Pathogenicity test : Pathogenicity test proved that all tested fungi were able to cause root rot infection on citrus seedlings with different degrees of disease incidence.

Results in Table 2 show that, *F. solani, R. solani,* and *P. P. nicotianae* were the most isolates caused root rot disease of citrus plants. *F. solani* caused a significant effect , in roots of citrus trees in the field causing greet losses on productivity of citrus orchards , after 15,45 and 60 days with 57.5, 55 and 52.5% respectively, meanwhile, *R. solani* and *P. P nicotianae.* obtained moderate effect if compared with *F. solani.* The lowest percentages of survival plants were recorded with *F. solani* (12.5%) followed by 5.5% and 3.0 % with *R. solani* and *P. nicotianae,* respectively, compared with control it was 87.0% survived plants.

TABLE 2. Pathogenic ability of isolated fungi to induce root rot infection on citrus seedlings sown in artificially infested soil in Orchards.

Fungi isolate	Root rot incidence %			
	15 days	45 days	60 days	Survival plats %
Fusarium solani	17.0^{c}	22.5^{b}	55.0^{d}	5.5^{d}
Rhizoctonia solani	10.0^{b}	20.0^{b}	57.5^{c}	12.5^{c}
Phytophthora nicotianae	12.0^{b}	32.5^{c}	52.5^{b}	$3.0.5^{d}$
Control	3.0	5.0	5.0	87.0

Figures with the same letter in same column are not significant differed (P = 0.05).

Efficacy of soil amendment and drenching seedling treatments of root rot disease incidence

Results in Table 3 and 4 indicate that all treatments reduced significantly the percentage of root rot diseases caused by *F. solani*, *R. solani* and *P. P. nicotianae*. The most effective treatments in reducing disease incidence were bagasse + *T. harzianum* (10%), clean root +bio-product, and bagasse + *T. harzianum* (5%), which reduced disease incidence, caused by *Fusarium* 78.1, 63.6 and 67.3% respectively. The same treatments reduced *Rhizoctonia* root rot by 78.2, 69.1 and 74.5%, and *Phytophthora* rot by 69.1, 67.3 and 67.3%, respectively.

Moderate effects were shown when citrus seedlings drenching in spore suspension of *T. harzianum* at 5% or 10%. These treatments reduced root rot disease incidence in infested soil with *F. solani* 52.8 and 56.7% while in infested soil with *R. solani* by 45.5 and 67.3% as well as by 58.2 and 60.0% in *P. nicotianae* infested soil.

TABLE 3. Effect of soil amendment and root treatment on root rot diseases in citrus seedling in orchard.

	% of survival plants after 15 days					
Treatments	***F. solani***	**Reduction % of *F.solani***	***R. solani***	**Reduction % of *R.solani***	***P. nicotiana***	**Reduction % of *P. nicotiana***
Bagasse +*T. harzianum* 5%	20^{c}	63.6	15^{c}	69.1	18^{bc}	67.3
Bagasse +*T. harzianum* 10%	12^{d}	78.1	12^{c}	78.2	15^{c}	69.1
drenching root in *T. harzianum* solution 5%	26^{b}	52.8	30^{b}	45.5	23^{b}	58.2
Drenching root in *T. harzianum* solution 10%	24^{b}	56.7	18^{c}	67.3	22^{b}	60.0
Clean – root bio- product	18^{c}	67.3	14^{c}	74.5	18^{bc}	67.3
Control	55^{a0}	0.0	55^{a}	0.0	55^{a}	0.0

Figures with the same letter in same column are not significant differed (P = 0.05).

All treatments, except drenching seedling root in *T. harzianum* solution, clearly show a significant reduction in root rots incidence as well as increasing the percentages of survival citrus seedlings (Table 4).

TABLE 4. Root rot disease incidence and survival plants (%) of citrus seedlings grown in artificially infested soil amended with different bio agents treatments.

Treatments	% Root rot incidence (45 days)								
	F. solani	Reduction % *of F.solani*	*R.solani*	Reduction % *of R.solani*	*P. nicotianae*	Reduction % *of P. nicotianae*	% survival plants		
							F. solani	*R. solani*	*P. nicotianae*
Bagasse+*T. harzianum* 5%	16^{c}	62.2	12^{c}	64.2	16^{c}	55.5	64^{d}	72a	66^{d}
Bagasse+*T. harzianum* 10%	12^{c}	73.9	8^{c}	76.4	10^{c}	72.2	76^{e}	80c	76^{c}
Drenching root in *T. harzianum* 5%	42^{a}	8.7	30^{a}	11.7	34^{a}	5.5	18^{b}	20b	22^{a}
Drenching root in *T. harzianum* 10%	32^{b}	30.4	26^{b}	23.5	28^{b}	22.2	42^{c}	44c	48^{b}
Clean – root bio- product	18^{c}	60.8	14^{c}	58.8	20^{bc}	44.4	64^{d}	72a	62^{b}
Control	46^{a}	0.0	34^{a}	0.0	36^{a}	0.0	8.00	10c	16^{a}

Figures with the same letter in the same column are not significant differed (P = 0.05).

Amended soil with 10% or 5% of bagasse + *T. harzianum* and / or clean – root treatments were significantly reduced root rot disease of *F. solani* with 73.9, 62.2 and 60.8% for the three treatments respectively. The same treatments also reduced root rot diseases caused by *R. solani* in 76.4, 64.2 and 58.8%; meanwhile, with *P. nicotianae* the treatments reduced root rot by 72.2, 44.4 and 55.5% respectively. On the other hand, no significant differences obtained between seedling root drenching in *T. harzianum* solution at 5% and 10%, they reduced *Fusarium* root rot by 8.7and 30.4% and *Rhizoctonia* root rot by 11.7and 23.5%, while the reduction was 22.2 and 5.5% for *Phytophthora* root rot. Soil amended with bagasse + *T. harzianum* at rat 10% reduced *Rhizoctonia* root rot on citrus plants with values significantly difference from other treatments 76.4%. *Trichoderma* solution show least effect in reducing root rots incidence, with values similar to the control. Soil amendment with bagasse + *T. harzianum* (5 and 10%) and clean – root bio- product treatments show the highest percentages of citrus survival plants as compared to other treatments.

Field experiments

The highly effective treatments in reducing root rot pathogens of citrus seedlings under orchards conditions, bagasse + *T. harzianum* 10%, clean- root bio- product and seedling drenching with *Trichoderma* solution at 10% as comparison treatment were applied during 2007 and 2008.

All treatments reduced significantly disease incidence, being bagasse + *T. harzianum* 10% the best, with a reduction values up to 71.4% after 20 days during 2007 and up to 68.1% after 20 days during 2008. A reduction in root rot incidence after 40 days and 60 days were also recorded (Table 5).

TABLE 5. Effect of different soil and seedling treatments on root rot disease incidence of citrus plants under field condition during 2007 and 2008 seasons.

Treatment	20 days	Reduction %	40 days	Reduction %	60 days	Reduction %	Survival plants (%)
2007							
Bagasse+ *T. harzionum* 10%	6.7^{c}	71.4	5.3^{d}	79.2	5.0^{d}	76.5	83.0a
Clean – root	8.5^{c}	63.8	8.0^{c}	68.6	8.5^{c}	60.0	75.0^{a}
Drenching in *T. harzionum* 10%	15.0^{b}	36	13.8^{b}	45.8	138.^{b}	35.2	57.3^{b}
Control	23.5d		25.5^{d}		21.3^{a}		29.7^{c}
2008							
Bagasse+ *T. harzionum* 10%	8.6^{d}	68.1	9.0d	71.4	7.0^{c}	66.6	75.3^{a}
Clean – root	11.8^{c}	56.2	13.5^{b}	57.1	10.7^{b}	49.0	64.0^{b}
Drenching in *T. harzionum* 10%	18.0^{b}	33.3	19.8^{b}	37.1	15.8^{b}	24.7	46.3^{c}
Control	27.0^{a}		31.5^{a}		21.0^{a}		20.5^{d}

Means with same letter in same column are not significantly different. (P = 0.05).

Seedling drenched with Clean-root bio-product show the least reduction of root rot incidence as compared to other treatments. A significant differences were obtained between *Trichoderma* solution amendment or clean – root treatments.

Plant height, stem diameter, number of leaves (total leaf area) of citrus seedlings were greatly increased under bio- agents amendments, particularly with the use of bagasse + *T. harzianum* (Table 6). In both seasons *T. harzianum* solution at 10% was less effective. In contrast no significant decrease was observed in the individual leaf area and specific leaf weight by the use of both clean – root bio- product and bagasse + *T. harzianum* as compared with untreated plants or those treated with *T. harzianum* solution at 10%. In other words the control plants had smaller leaf area/plant but had slightly heavier leaf weight and larger leaf blades. Generally, the highest proportion of dry weight (39.8%) was found in leaves, followed by the stem (31.7%) and roots (24.6%) in average of both seasons for the control treatment (Table 6 and 7).

Plant treated with bagasse +*T. harzianum* as well as Clean root +bio-product at 10% produced more dry matter than control (Table 7). Also, similar significant effect was obtained by the use of *T. harzianum* solution of 10% complex in the first season only. Plants treated with bagasse+*T. harzianum* and clean-root bio gave greater amount of dry matter of leaves and of stem. However, no significant increase was observed in root dry weight by used bagasse+*T. harzianum* supplying in both seasons. It may attributed to the increased photosynthetic efficiency of leaves which mainly reflected from increasing number of leaves/plant. Therefore, the increase of total leaf area/plant treated with bagasse+*T. harzianum* was due to new leaves emergence rather than larger leaves as increasing in area. As a result they developed a greater number of leaves/plant and ultimately, accumulated greater dry weight. This led to develop a stronger stem and root system that mainly generated by more carbohydrates partitioned to them. The vise versa in control plants. Plant growth and shoot root ratio were enhanced by bagasse + *T. harzianum* and clean-root. In contrast, root, shoot ratio were decreased by such treatments. In average of both seasons, shoot, root or root, shoot ratio were 3.03 or 0.04 and 3.04 or.31 for plants treated with bagasse + *T. harzianum* and clean-root (bio-product), respectively, as compared with 2.75 or 0.34 for control plants. It is suggested that the control plants may divert more photosynthesis towards roots as less utilized in shoot growth and this results in high root, shoot ratio also. The slight increase of leaf weight in control plants may support this suggest.

TABLE 6. Effect of (Bagass + *T. harzianum* 10%, clean- root, *T. harzianum* 10% solution) on leaf area, number of leaves, Plant height and stem diameter of citrus plantlet. c.v "Valencia".

Treatment	Total leaf area/ plant $(cm)^2$	Av. Leaf area $(cm)^2$	Av. No of Leaves/ plant	Av. Stem thickness (cm)	Av. Stem length (cm)	Specific leaf weight
Season (2007)						
Bagass + *T. harzianum* 10%	606.1^{ab}	54.00^{a}	20.0^{a}	0.95^{a}	65.00^{a}	**0.63^{a}**
Clean - root	650.3^{a}	58.60^{a}	20.8^{a}	0.91^{a}	52.70^{ab}	**0.61^{a}**
***T. harzianum* olution10%**	545.8^{b}	60.00^{a}	18.1^{a}	0.75^{ab}	51.53^{ab}	***0.65*^{a}**
Control	445.8^{a}	62.81^{a}	14.3^{b}	0.71^{b}	46.60^{b}	**0.65^{a}**
Season (2008)						
Bagass+ *T. harzianum* 10%	705.05^{a}	66.63^{a}	23.0^{a}	0.94^{a}	57.50^{a}	**0.67^{a}**
Clean- root	701.8^{a}	65.8^{a}	21.5^{a}	0.84^{abc}	53.00^{b}	**0.66^{a}**
***T. harzianum* solution 10%**	583.6^{b}	66.01^{a}	17.4^{b}	0.74^{c}	50.24^{b}	***0.70*^{a}**
Control	564.7^{b}	66.16^{a}	17.0^{b}	0.78^{bc}	50.8^{b}	**0.67^{a}**

Means with same letter in same column are not significantly different (P = 0.05).

TABLE 7. Effect of Bagass + *T. harzianum* 10%, clean- root, *T. harzianum* solution at 10% on biomass and dry matter Partitioning of citrus transplants. c.v "Valencia".

Treatment	Av. Dry matter of root (g)	%	Av. Dry matter of stem (g)	%	Av. Dry matter leaves (g)	%	Total dry matter/ plant (g)
First season (2007)							
Bagass+*T. harzianum* 10%	4.83[a]	24.3	7.66[a]	36.0	7.90[a]	38.8	21.3[a]
Clean – root	5.00[a]	23.8	7.07[a]	33.8	8.35[a]	40.0	20.28[a]
***T. harzianum* solution10%**	4.61[a]	24.9	5.75[ab]	31.9	7.11[ab]	46.3	16.99
Control	3.75[a]	24.6	4.60[b]	31.7	4.98[b]	39.8	14.90[b]
Mean	4.55	24.4	6.27	33.4	7.09	41.22	18.36
Second season (2008)							
Bagass+ *T. harzianum* 10%	5.90[a]	25.0	8.09	35.0	9.22[a]	40.2	23.30[a]
Clean – root	5.31[a]	24.5	7.26[a]	31.9	9.50[a]	44.4	24.23[a]
***T. harzianum* solution 10%**	5.53[a]	26.5	7.24[a]	30.9	9.24[a]	41.8	22.23[a]
Control	4.94[a]	23.6	6.04[b]	34.0	7.84[b]	41.3	18.81[b]
Mean	5.42	24.9	7.16	32.95	8.95	41.9	22.14

Means with same letter in same column are not significantly.

Discussion

Fusarium solani, Phytophthora nicotianae and *Rhizoctonia solani* were the dominant causal agents of citrus root rotin El-Sharkia , El- Behera, El- Kalubia, El-Menofia and El-Gharbia governorates. Meanwhile, *F. oxysporum, P. citrophthora* and *Pythium* spp. were less frequent. The most pathogenic fungi on citrus seedling were *F. solani and R. solani* followed by *P. nicotianae.* Many researchers reported that *F. solani, R. solani, P. nicotianae, F. oxysporum, Pythium* spp. and *P. citrophthora*, are the main pathogens causing root rot diseases of citrus (Kathryn C. Taylor 1995 and Michael E. Matheron, 1995).

Root rot disease on citrus caused by *F. solani, R. solani* and *P. nicotianae* were significantly decrease either under artificially infested soil in orchards or in naturally infested soil in Abo-Kebeer (Sharkia) province amending the soil with *T. harzianum* formulation on sugar cane bagasse Moustafa *et al* (1990). Such treatment cause highly increase in shoots, leaves of citrus seedlings during 2007 and 2008 seasons. Soil amendment with agricultural wastes alone or in combination with bio- control agent was recommended for controlling soil born pathogens and increasing shoots and leaves of many crops (Elad *et al.,* 1986Stem rot of ground nut reduced by *T. harzianum* formulation (wheat bran sand soil mixture was added to soil (30 g/ Kg soil) (Mitra & Nandi 1994, Ceuster *et al.,* 1999, May & Kimati, 1999, Liu & Huany, 2000 and El-Mohamedy, 2004). noted that stem rot of grand nut reduced by up to 83% when *T.harzianum* formulation in wheat bran sand soil mixture was add to soil (30g/Kg soil). Control of root rot pathogens through amended soil with organic materials formulated either bio- control agents may be attributed to:1) increasing the activity of indigenous micro flora resulting suppression of pathogens population on through competition or specific inhibition (Adams, 1990), releasing degradation compounds such carbon dioxides, ammonia, nitrites, saponine or enzymes which are generally toxic to the pathogens (Lakshmanan & Nair, 1984 and Neler *et al.,* 1985), inducing plant defense mechanisms , cellulose and glucanase are prevalent to high concentration in soil as a result of biodegradation of cellulose and lignin (Windham *et al.,* 1986 and Wang, 1999). It is clear to notice that amended soil with bagasse *T. harzianum* 10% or clean – root treatment gave the best result in reducing root rot on citrus plants under field conditions compared with *T. harzianum* solution treatment. This reduction resulted in increasing stand and survival plants which reflected.

Drenching seedling root in which specific biological control agents are incorporated into the seedling root process, can be very effective in suppressing many disease caused by seed and soil borne pathogens (Miller, 1968 and Moustafa *et al.,* 1990). Moreover, drenching seedling root has great promise for enhancing the efficacy, Shelf life and consistent performance of biological control agents (Jahn & Puls, 1998 and Jensen *et al.,* 2002).

In this study the application of *T. harzianum* to citrus seedling roots during transplanting process shows high reduction in root rots incidence under orchards conditions. Meanwhile, drenching roots in *T. harzianum* solution or in clean-root bio- product caused a least reduction in root rots incidence as compared to drenching or soil amendment treatments. This may be due to fail bio- protection on seedling or in rhizosphere at sufficient level for disease control and releasing high level of exudates during the grow of new roots. Drenching of seedling roots caused highly significant reduction in root rot incidence under field conditions during 2007 and 2008 seasons. Furthermore, plant growth and shoot, root ratios were enhanced by bagasse + *T. harzianum* and clean- root bio- product, in contrast, root, shoot ratio was decreased by *T. harzianum* solution at 10%, as compared to control treatment. These results are in agreement with Sritharan, (1992). It may be attributed to the increasing photosynthetic efficiency of leaves which mainly reflected from number of leaves/plant. Monselise and Lenz (1980) reported that the increase of specific leaf weight might be mainly attributer to increased starch accumulation in the leaves. Higher levels of starch in leaves may be explained on the basis that more photosynthesis than could e utilized in growth were produced (Sritharan, 1992).

Control of root rot disease on drenching citrus seedling root, due to reduced by up to80.0% exudation of nutrients from the primed root seedlings up on inhibition of water. Moreover, the direct antagonistic ability of *T. harzianum* against citrus root rot pathogens. Combination between drenching seedling root and bio- control agents has improved the rate of root growth and reduced root rot disease incidence (Nemec *et al.*, 1996).

Soil amendment with *T. harzianum* formulation on sugar cane bagasse and/ or drenching citrus seedling root, they caused highly decrease in root rot disease incidence, increase leaves and shoots and roots and enhancement the persistence availability of *Trichoderma* propagates in rhizosphere soil of citrus plants. So, it could be suggested that such soil and seedling treatments could represented an environmentally eco friendly strategy for controlling soil borne pathogens as substitute of chemical fungicides.

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معاملة التربة وشتلات الموالح بعد استخدام طريقة الملش ببدائل المبيدات لمقاومة أمراض أعفان الجذور وإعادة زراعتها

حامد سيد الشيمي* و فاتن سيد منصور**
*قسم بحوث أمراض الفاكهه و**المعمل المركـزي للزراعة العضوية - معهد بحوث أمـراض النباتات - مركز البحوث الزراعية - القاهرة - مصر .

أثبت العزل والتعريف للفطريات المصاحبة لشتلات الموالح في مناطق مختلفة مثل "الشرقية -البحيرة -القليوبية -المنوفية -الغربية "وكذلك من مناطق جديدة أن أعـفان الجـذور لشتـلات الموالـح نـاتجة عن المسببات الآتيـة *Fusarium*] : *Phytophthora nicotiana,Pythium solani, Rhizoctonia solani* , spp. *and Phytophthora citrophthora*.وهي التي تسبب أعفان جذور الشتلات في الموالح.

- أظهرت دراسات الحصر المرضي أن الفطريات السابقة هي أكثر الفطريات إنتشارا على شتلات الموالح وخاصة صنف البرتقال الصيفي .

- تم دراسة فاعلية استخدام معاملة التربة بتركيبة من أكياس تحتوي علي سكر القصب وبعض العناصر كوسط لتنمية وحمل *Trichoderma harzianum* بالتركيزات ٥٪ ، ١٠٪ من *Trichoderma harzianum* وكان فاعلية التركيز ١٠ ٪ أكبرفي خفض نسبة الإصابة بفطريات التربة بعد فترة زمنية ٢٠ ، ٤٠ وحتي ٦٠ يوم من تاريخ الزراعة.

- وفي اختبار أخر لفاعلية معلق جراثيم *Trichoderma harzianum*بتركيزات ٥ ٪ ، ١٠٪ في مقاومة فطريات التربة المسببة لاعفان جذور شتلات الموالح كان التركيز ١٠ ٪ من معلقة الجراثيم له أثر فعال نسبيا في خفض نسبة الإصابة لفطريات التربة.

- بينما كان لاختبار المركب (clean-root) كلين روت وهو مركب تجريبي لمقاومة أعفان جذور النباتات أثر واضح في مقاومة الفطريات المسببة لأعفان جذور الموالح ويأتي في المرتبة الثانية بعد ال *Trichoderma harzianum* المنماة علي بيئة أكياس السكر.

- تم اختبار أكثر المركبات فاعلية في خفض نسبة الإصابة أعفان الجذور *Trichoderma harzianum* منماه علي أكياس السكر ١٠٪ . الكلين روت ومعلقة جراثيم علي تطور نمو شتلات الموالح ، كما وجد أن طول الشتله ، سمك الشتلة ، تكشف الأوراق الحديثة ، المساحة الكلية لأوراق نبات ارتبطت ايجابيا بمعاملتي الـ *Trichoderma harzianum* + ١٠٪ كياس سكر القصب ، الـ كلين روت بالإضافة إلي ذلك وقد لوحظ زيادة في المادة الجافة للأوراق والساق وبالتالي النبات ككل.

- كما أن النباتات المعاملة بكلا المركبين قد أحرزت نسبة (أفرع :جذور) أعلي مقارنتة بنسبة تلك الغير معاملة . وقد كان معلقة الجراثيم *Trichoderma harzianum* عند التركيز ١٠٪ ذو تأثير قليل وغير معنوي من هذا المجال.

تشير هذه الدراسة إلي إمكانية استخدام هذه المعاملات الآمنة وخاصة + *Trichoderma harzianum* أكياس سكر القصب في مقاومة الأمراض الكامنة في التربة وزيادة قدرة النبات علي النمو.