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CONTROL OF FUSARIUM ROOT ROT DISEASE ON MANDARIN BY SOIL AMENDMENT WITH *TRICHODERMA HARZIANUM* GROWN ON BAGASSE (SUGAR CANE WASTE)

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ABSTRACT

Soil amendment with bioenhanced bagasse (bagasse colonized by *Trichoderma harzianum*), enhanced bagasse, *T. harzianum*, Topsin-M and bioenhanced bagasse + Topsin-M for controlling Fusarium root rot disease on mandarin under green house and field conditions was studied. In vitro, amended Dox medium with 2.5 and 5.0% (w/v) of powdered bagasse resulted in enhancement the antagonistic of *T. harzianum* ability against *F. solani* and increased chitinase and cellulase activities of *T. harzianum* compared with control (un amended medium). In green house, Fusarium root rot disease incidence on mandarin seedlings was high significantly reduced when bioenhanced bagasse, bioenhanced bagasse + Topsin-M (0.5 g/L) and Topsin-M (1 g/L) treatments were dilervied into artificially infested soil as twice soil applications compared with control treatment. Moreover, such treatments caused high reduction on population density of Fusarium propagules in rhizosphere soil, but Trichoderma propagules counts were highly increased. Under field conditions amended soil around stems of diseased mandarin trees by bioenhanced bagasse, bioenhanced bagasse + Topsin-M and Topsin-M as twice application per season results in recovering great number of diseased trees and decreased the disease severity on others. Population density of *Fusarium spp.* were highly decreased, where population density of *Trichoderma spp* were increased in rhizosphere soil of trees that treated by bioenhanced bagasse alone or in combined with Topsin-M. Such treatments caused increased fruit yield by 130.2 and 116.3% compared with 67.4% of Topsin-M treatment.

It could be suggested that using soil amendments such bagasse colonized by *T. harzianum* alone or in combined with a reduced rates of fungicides for controlling root rot pathogens on citrus can be success fully used replacing traditional fungicides and avoid environmental pollution.

Keywords : Citrus - Soil amendment - Root rot - Control - Bio agent - Mandarin

INTRODUCTION

Fusarium root rot disease of citrus caused by *Fusarium solani* (Mart.) was reported to attack all citrus varieties (Conzulex *et al.*, 1997; El-Mohamedy, 1998; Catara and Polizzi, 1999). In Egypt it has been estimated to affect 11.6% of mandarin trees and caused 39.6% loss in fruit yield (El- Mohamedy, 1998) *F. solani* induce two syndrome of root rot on citrus. First , dry root rot is confined to the crown and scaffold roots and the second, feeder and fibrous root rots are associated with gradual decline of the canopy, leaf curl (wilting), defoliation, dieback, fibrous roots turn soft and appears water soaked, slough their cortex easily by hand (Kore and Mane, 1992; Praksasm *et al.*, 1992 and Verma *et al.*, 1999).

Control of this disease depends mainly on fungicides application (El-Mohamedy, 1998 and Verma *et al.*, 1999). Meanwhile fungicides always desirable due to high coast, probability of development of resistant strains and potential hazards to the environment.

Among the recent recommended trials for controlling soil borne pathogens other than fungicides, biological and soil amendment means individually or in combination were recommended. Such means comprise elimination of pathogens density in the soil and maintaining soil condition, favorable for root development and enhancement the competitive ability of bio agents against pathogens. There fore, these methods introduced efficient disease control and increasing yield of many crops (Huang, 1991; Tumey and Meng, 1994; Fang and Tsao, 1995; Walker and Morry, 1999; May and Kimati, 1999 and Ceuster *et al.*, 1999).

Using agricultural wastes, domestic food wastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil for controlling soil borne pathogens on some crops were recorded (Hari and smosekhar, 1998; Godwin and Arinze, 2000; Liu and Huany, 2000; Prasad and Ragashwaran, 1999 and 2000). Sugar can bagasse degraded by *Trichaderma* spp. was used as soil amendment to improve growth and yield of rice and pea (Mitra and Nandi, 1994).

Nemec *et al.*, 1996 noted that amended planting mixes with formulation of commercial bio control agents such as *T. harzianum, Bacillus subtilis, Gliocladium virens* and *Streptomyces spp.* reduced root rot and Grown rot diseases on tomato, ball pepper, celery and citrus.

Although many researchers have studied biological control of root disease pathogens, few studies have been made to control Fusarium root rot disease on citrus by antagonistic fungi. The purpose of this research was to evaluate the efficiency of amended soil with bagasse colonized by *Trichoderma harzianum* (bioenhanced bagasse) on controlling Fusarium root rot disease on mandarin and on population density of both the pathogen (*Fusarium solani)* and bio agent (*T. harzianum)* in rhizosphere soil. An attempt has been made to convert sugarcane bagasse into a bio manure for land application using *T. harzianum*.

MATERIALS AND METHODS

Fungal isolates:

Fusarium solani (Mart) Appal & Wr. Emeed. Snyd & Hans Was previously isolated from naturally infected roots of citrus trees affected by root rot disease. This isolate was recorded to cause root rot on different citrus rootstocks in previous studies (El-Mohamedy, 1998).

Trichoderma harzianum (Rifai) was previously isolated from rhizosphere soil of citrus trees by Dep. Plant. Pathology, NRC, Cairo.

Laboratory studies:

Antagonistic and enzymatic activity of *T. harzianum:*

Antagonistic ability of *T. harzianum* against *F. solani* was carried out on Dox agar medium amended with different rates of bagasse, i.e., 1.25, 2.5

and 5.0% (w/v) using duel culture technique (Ferreira *et al.*, 1991) Five Peteri dishes were used as replicates for each treatment. All plates were incubated at 25 °C for 5 and 10 days. Reduction in linear growth of *F. solani* was calculated .

Chitinase and cellulase activity:

Chitinase and cellulase activity in filtered cultures of *T. harzianum* grown on Dox medium amended with 1.25, 2.5 and 5.0% (w/v) of bagasse were determined after 14 and 21 days from incubation Chitinase activity was expressed as mM N-acetyl glucose amine equivalent reduced 1 mL of filtered culture / 60 min according to methods described by Ried and Ogryd – Ziak (1981) and Monreal and Reese (1969). Cellulase activity was done by methods described by Nelson (1944) as percent of increasing in reducing sugars released ug /min.

Preparation of bioenhanced and enhanced bagasse mixture:

Sugarcane bagasse (industrial wastes of sugarcane)was ground to fine powder , 250 g of this powder was mixed with sand soil (4 : 1) in autoclavable polyethylene bags, 2.0 g ammonium sulphate ,5.0 g super phosphate , 5.0 g potassium sulphate and 400 ml water per 1000 g bagasse were added to each bag .All bags were sterilized for 1 hr in autoclave at 121C.

The mixture in a half of these bags were inoculated by spore suspension of *T. harzianum* 3 x 10^6 spore / mL (this mixture = bioenhanced bagasse) and the other left as ckek treatment control (this mixture = enhanced bagasse). All bags were incubated at 25°C for 14 days at room temperature , then used as bio enhanced bagasse (bagasse colonized by *T. harzianum*) or enhanced bagasse for direct delivery into the soil.

Green house experiments:

The experiments were carried out to evaluate the efficacy of different soil amendments (bioenhanced or enhanced bagasse) as well as fungicide treatment on controlling Fusarium root rot disease on mandarin seedlings, inoculum density of Fusarium and Trichoderma in rhizosphere soil of seedlings. Plastic pots (20 cm diam.) containing sand loam soil (2 : 1) were infested by *F. solani* according to El-Mohamedy, (1998) were used. Mandarin (Baladi cv.) seedlings (3 years old) grafted on sour orange rootstock were inoculated by the same pathogen and replanting in infested soil according to Strauss and Labuschagen, (1995). Five seedlings were used as replicates for each treatment. The following treatments were evaluated.

- Bioenhanced bagasse (bagasse – sand medium colonized by *T. harzianum*) at the rate 5% (w/w) of soil infested soil.
- Enhanced bagasse (bagasse – sand medium) at the rate 5% (w/w) of soil infested soil.
- *T. harzianum* at the rate 5% (v/w) of infested soil.
- Fungicide (Topsin-M 70%) g /L.
- Bioenhanced bagasse 2.5% + Topsin-M 0.5 g/L.
- Control (non treated infested soil)

After 15 days from soil infestation, all mandarin seedlings were replanting .All soil treatments were applied at replanting time of seedlings as one application, and 30 days from replanting seedling as second application. The number of diseased seedlings was recorded and development of disease symptoms (severity) on seedlings was determined and rated on scale from 0-4 rates (0 = healthy plants 4 = dead plants) according to Morgan and Timmer (1984) and El-Mohamedy (1998). Percentages of the disease infection and severity after 90 days from replanting of the seedlings were recorded and calculated.

Field experiments:

The experiments were carried out at El-Kata (Giza Governorate) during 2002 and 2003 seasons on orchards of mandarin (10-year old Baladi cv. grafted on sour orange rootstock) with the history of root rot disease .The following soil amendment treatments were used:

- Bioenhanced bagasse 1 kg/tree.
- Topsin .M 70% 20 g / tree.
- Bioenhanced bagasse 500 g / tree + Topsin. M 10/g tree.
- Control (non-treated diseased trees).

Forty-eight diseased mandarin trees showing typical symptoms of Fusarium root rot disease were selected, 12 diseased trees with different rates of disease severity (1, 2 and 3) were used as replicates for each treatment as well as control treatment. All soil amendment treatments were applied to soil around mean stem of tree twice per season, first, in spring (March, 2003) and the second in summer (June, 2003). After 60 and 120 days from the adding second application a number of recovered trees and the development of disease severity on diseased trees was recorded. The percentages of disease severity were calculated as maintained before.

Count of Fusarium and Trichoderma propagules in rhizosphere soil.

Plate count technique (Allen, 1961), using Peptone PCNB agar medium (Nash & Snyder, 1962).and PDA medium supplement with 250 ppm chloromycetin (Papavizes & Lumsden, 1982) was used to determine total counts of Fusarium and Trichoderma in rhizosphere soil of mandarin seedlings or trees respectively. Five plates were used as replicated for each treatment. Total count of each fungus were expressed as cell forming units (CFU) per gram dry soil.

Effect of soil amendment treatments on mandarin yield:

All fruits of each tree in each treatment were picked and weighted, the average of fruit yield per tree as well as per faddan was calculated.

A number of 50 fruit from each treatment were weighted and the average weight of fruit was determined.

Statistical analysis:: Tukey test for multiple comparison among means was utilized (Neler *et al.*, 1985) .

RESULTS

Effect of *T. harzianum* on linear growth of *F. solani* medium :

Dox agar media amended with different rates of bagasse were used to evaluate the antagonistic ability of *T. harzianum* against *F. solani*. Data in Table (1) indicate that amended Dox media with 1.25, 2.5 and 5.0% (w/v) of bagasse caused enhancement the antagonistic ability of *T. harzianum*. As, the growth of *F. solani* was completely inhibited compared to 80% reduction with control (non-amended media). Meanwhile, the fungal growth was reduced by 61-68% compared with 55% with control treatment after 5 days of incubation.

Table (1): Growth reduction (%) of *F. solani* as affected by *T. harzianum* on Dox medium amended with different rates of bagasse..

Bagasse rates*	Growth reduction %	
	5 day	10 day
1.25%	61.0a	100b
2.5%	70.0b	100b
5.0%	68.0b	100b
Control (non amended medium)	55.0a	80.0a

Figures with the same letters in the same column are not significantly differed (P=0.05)
* Each 100 ml medium was amended with 1.25,2.5and 5.0 g bagasse .

Chitinase and cellulase activity:

Dox media amended with 1.2, 2.5 and 5.0% (w/v) of bagasse and chitin or cellulose powered were used to determine chitinase and cellulase activity in filtered culture of *T. harzianum*. Data in Table (2) showed that increasing rate of bagasse in media resulted in increasing of chitinase and cellulase activity in filtered cultures of *T. harzianum* compared with control treatment (un amended media with bagasse). High activities of both chitinase and cellulase were recorded when 2.5 and 5.0% of bagasse were added to Dox media. These values were 6.6, 7.1 of chitinase and 725.4, 781.4 of cellulase compared with 4.9 and 530.8 of control treatment respectively after 30 days from incubation at 25°C.

Table (2): Effect of different rates of bagasse on chitinase and cellulase activity of *T. harzianum* grown on Dox medium.

Bagasse rates*	Chintiase activity**		Cellulase activity***	
	15 day	30 day	15 day	30 day
1.25 %	4.7	5.3	283.2	606.2
2.5 %	5.9	6.6	504.8	725.4
5.0 %	6.1	7.1	501.6	781.4
Control	4.2	4.9	320.2	530.8

* Each 100 ml medium amended with 1.25,2.5and 5.0 g bagasse
** Chitinase activity was expressed as mM N-acetyl glucose amine equivalent reduced 1 mL of filtered culture / 60 min
*** Cellulase activity was expressed as reducing sugars released ug /m

Effect of different soil amendments on Fusarium root rot disease incidence:

The Effect of amended artificially infested soil (in greenhouse) or naturally infested soil (in field) with different soil amendments as well as fungicide treatment on percentage of infection and severity of Fusarium root rot disease on seedlings and trees of mandarin was studied. Population density of Furarium and Trichoderma propagules counts in rhizosphere soil was also determined.

Data in Table (3) showed that both disease infection and severity on mandarin seedlings with all tested soil amendments were significantly reduced compared with control. Fusarium root rot disease on mandarin seedlings was highly significant reduced when bioenhanced bagasse, bioenhanced bagasse + Topsin.- M and Topsin-M treatment were delivered into artificially infested soil as twice application compared with control (untreated infested soil). As, the percentages of disease infection and severity were 20, 20, 40% and 10, 15, 25% compared with 100 and 80% of control treatment respectively. Meanwhile, enhanced bagasse and *T. harzianum* treatments gave a considerable disease control compared with control treatment especially with twice application.

Table (3): Effect of different soil amendments treatments on Fusarium root rot incidence (%) on mandarin seedlings planting in artificially infested soil in green house.

Soil amendments	No of application	Fusarium root rot incidence %	
		Disease infection	Disease severity
Bioenhanced bagasse (5%)	One	60 ab	35 abc
T. harzianum (5%)		80 ab	55 abc
Enhanced bagasse (5%)		80 ab	65 ab
Topsin-M (1 g/l)		80 ab	50 abc
Bioenhanced bagasse (2.5%) + Topsin-M (0. 5 g/l)		60 ab	40 abc
Bioenhanced bagasse (5%)	Two	20 b	10 c
T. harzianum (5%)		60 ab	40 abc
Enhanced bagasse (5%)		80 ab	55 abc
Topsin-M (1 g/l)		40 ab	25 abc
Bioenhanced bagasse (2.5%) + Topsin-M (0. 5 g/l)		20 b	15 c
Control (infested untreated soil)		100 a	80 a

Figures with the same letters are not significant (P =0.05)

Data in Table (4) indicate that all tested soil amendments treatments were significantly reduced the population total counts of *F. solani* compared with control. Bioenhanced bagasse, bioenhanced bagasse + Topsin-M and Topsin-M treatments show high significant reduction in population density of *F.solani* in rhizosphere soil of treated seedlings. These counts were 1.2, 1.6 and 2.0 x 10^5 cfu/g dry soil compared with 2.8 x 10^5 cfu/g soil of control treatment. Meanwhile, when these treatments were applied as one application the total counts were 2.8, 2.2 and 2.8 x 10^5 cfu/g dry soil of the same pathogen. On the other hand, amended soil with bioenhanced bagasse alone or in combined with Topsin-M resulted in increasing mass multiplication of *T. harzianum* around roots of treated seedlings compared with adding *T. harzianum* alone to the soil, where the propagules counts reach to 5.8 and 5.2 x 10^4 cfu/g dry soil with such treatment compared with 4.2 x10^4 cfu/g dry soil of *T..harzianum* at twice applications . Twice soil applications of bioenhanced bagasse, bioenhanced bagasse + Topsin-M and Topsin-M treatments were highly effective in controlling Fusarium root rot disease on mandarin seedling in green house experiments. Moreover, they decreased population density of Fusarium and caused increasing in population density of Trichoderma around roots of seedlings. So, these treatments were chosen for controlling the disease on mandarin trees under field conditions.

Table (4): Effect of different soil amendments treatments on total counts of Fusarium and Trichoderma propagules in rhizosphere soil of mandarin seedlings planting in artificially infested soil in greenhouse.

Soil amendment	No of application	Fusarium propagules count cfu x 10^5/g dry soil	Trichoderma propagules count cfu x 10^4/g dry soil
Bioenhanced bagasse (5%)	One	2.8 bc	5.4 ab
T. harzianum (5%)		3.0 bc	4.0 b
Enhanced bagasse (5%)		3.2 b	-
Topsin-M (1 g/l)		2.8 bc	-
Bioenhanced bagasse (2.5%) + Topsin-M (0. 5 g/l)		2.2 cde	4.8 ab
Bioenhanced bagasse (5%)	Two	1.2 e	5.8 a
T. harzianum (5%		2.8 bc	4.2 b
Enhanced bagasse (5%)		3.0 bc	-
Topsin-M (1 g/l)		2.0 cde	-
Bioenhanced bagasse (2.5%) + Topsin-M (0. 5 g/l)		1.6 de	5.2 ab
Control (infested untreated soil)		**2.8 a**	-

Figures with the same letters are not significant (P =0.05)

Control of Fusarium root rot disease an mandarin trees:

The experiments were carried out under field conditions to evaluate the effect of different soil amendments mentioned before on recovering naturally infested mandarin trees with Fusarium root rot disease., Population total counts of Fusarium and *Trichoderma* propagules counts in rhizosphere soil and also fruit yield of trees were estimated.

Data in Table (5) show that all soil amendments treatments reduced both the number of infected trees and percentages of disease severity on these trees. But there are no significant differences between all tested treatments and control (untreated diseased trees).

Table (5): Fusarium root rot disease development on mandarin trees as affected by different soil amendment treatments under field conditions.

Soil amendments	Fusarium root rot devilment					
	No. of diseased trees after application			Disease severity (%) after application		
	0 day	60 day	120 day	0 day	60 day	120 day
Bioenhanced bagasse 1kg /tree	12 a	8 a	3 b	50 a	37.5b	14.6 b
Topsin-M 20 g/tree	12 a	10 a	7 b	50 a	43.8ab	37.3 b
Bioenhanced bagasse 500 g + Topsin-M 10 g / tree	12 a	8 a	5 b	50 a	33.3 b	25.0 b
Control (untreated diseased trees)	12 a	12 a	12 a	50 a	66.7 a	77.1 a

Figures with the same letters are not significant ($P = 0.05$)

But the severity of disease on these trees was reduced after 60 days from soil application. Meanwhile, after 120 days from amended soil with bioenhanced bagasse, bioenhanced bagasse + Topsin-M and Topsin-M resulted in recovering 9, 7 and 5 trees and reduced disease severity from 77.1% of control treatment to 14.6, 25.0 and 37.3% of the same treatments respectively.

Data in Table (6) indicate that all tested soil amendments were significantly reduced the number of Fusarium propagules counts in rhizosphere soil of treated trees, while it increased in untreated diseased trees (control). Bioenhanced bagasse or bioenhanced bagasse + Topsin-M treatments show highly effective in inhibition of Fusarium activity (density) around roots of trees followed by Topsin-M treatment. These treatments reduced the counts of Fusarium propagules counts from 11.4 x 10^3 cfu / g soil (untreated diseased trees) to 4.6, 6.2 and 6.8 x 10^3 cfu / g soil respectively. Moreover, these treatments cause increasing in population density of *Trichoderma spp* around roots of treated trees except in the case of Topsin-M treatment.

Table (6): Fusarium and Trichoderma propagules counts in rhizosphere soil of infested mandarin trees affected by different soil amendment treatments under field conditions.

Soil amendments	Fusarium propagules count cfu x 10^3/ g dry soil			Trichoderma propagules count cfu x 10^3/ g dry soil		
	0 day	60 day	120day	0 day	60 day	120 day
Bioenhanced bagasse 1kg /tree	6.8 a	4.4 b	6.2 bc	1.5 a	7.9 a	8.2 a
Topsin-M 20 g/tree	6.8 a	5.7 b	6.8 b	1.4 a	2.0 b	2.2 c
Bioenhanced bagasse 500 g + Topsin-M 10 g / tree	6.8 a	4.6 b	4.6 c	1.8 a	4.2 a	5.0 b
Control (untreated diseased trees)	6.8 a	9.6 a	11.4 a	1.6 a	2.8 b	2.9 c

Figures with the same letters are not significant ($P = 0.05$)

As, the total propagules count of *Trichoderm spp* in rhizosphere soil of treated trees with bioenhanced bagasse or bioenhanced bagasse + Topsin-M were 8.2 and 5.0 x 10^3 cfu / g dry soil compared with 2.9 and 2.2 x

10^3 cfu / g dry soil with untreated control and Topsin-M treatments respectively.

Data in Table (7) show that averages of fruit yield per tree and also fruit weight were increased due to amended soil of diseased trees with bioenhanced bagasse, bioenhanced bagasse + Topsin-M and Topsin-M treatments. These values were 61.2, 58.4, 45.0 kg/tree and 140, 128, 110 g/fruit respectively compared with 27.0 kg/tree and 85 g/fruit of untreated diseased trees (control). Total fruit yield per / feddan was increased by 130.2, 67.4 and 116.3% due to amended soil of infected trees with the same treatments, respectively. Moreover , no significant differences were found between yields of trees that treated by bioenhanced bagasse alone or in combined with Topsin -M and health trees .

Table (7): Effect of different soil amendment treatments on fruit yield of mandarin trees affected by Fusarium root rot disease under field conditions.

Soil treatments	Av . fruit / tree. (kg)	Av. Fruit weight (g)	Fruit yield	
			Ton / feddan	Increase %
Bioenhanced bagasse 1kg /tree	61.2 bc	140bc	9.9bc	116.3
Topsin-M 20 g/tree	45.0 b	110b	7.2b	67.4
Bioenhanced bagasse 500 g + Topsin-M 10 g / tree	58.4 bc	128b	9.3bc	130.2
Control (diseased trees)	27.0 a	85 a	4.3a	
Control (healthy trees)	64.2 bc	152 bc	10.3b	

Figures with the same letters are not significant (P =0.05)

DISCUSSION

Fusarium root rot disease caused by *Fusarium solani* (Mart.) is one of the most serious diseases attacked mandarin trees especially that cultivated in new reclaimed lands in the desert (El-Mohamedy, 1998). Control such disease has been greatly concerned in Egypt, especially after an increasing of citrus cultivation in these lands.

Trichoderma spp. formulations on wheat- bran, CMC ,sorghum grains, agricultural wastes and domestic food wastes were used and delivery for bio control into soil and soil amendments for controlling soil borne pathogens on some crops were investigated and recorded by many researchers (Mitra and Nandi, 1994 ; Sawant *et al.*, 1995 ; Nemec *et al.*, 1996; Prasad and Ragashwaran., 1999 ; Liu and Huany, 2000 and Godwin and Arinze, 2000).

In this study, amended Dox medium with different rates of bagasse (industrial waste of sugarcane) resulted in an increasing of the antagonistic ability of *T. harzianum* against *F. solani*. Moreover, chitinase and cellulase activity were increased in filtered cultures of *T. harzianum* grow on amended media compared with control (non amended media). In this regard, Hari and Somosekhar, 1998; Ravelo *et al.*, 2000 and Wang, 1999 noted that the highest mass multiplication and enzymatic activity of *Trichoderma* spp. were recorded when the fungus grow on bagasse substrate. High concentration of free nutrients such glucose in fresh crop residues repress the production of enzymes required for parasitism by biocontrol agents.

In green house experiments, amended artificially infested soil with bioenhanced bagasse (bagasse sand medium colonized by *T. harzianum*) , enhanced bagasse, bioenhanced bagasse + Topsin-M (0.5 g/L), Topsin-M (1 g/L) or *T. harzianum* treatments caused significant reduction in percentages on infection and severity of Fusarium root rot disease on mandarin seedling compared with control (untreated seedling). twice soil application with bioenhanced bagasse, bioenhanced bagasse + Topsin-M or Topsin-M were highly effective treatments in controlling the disease. Moreover, population density of propagules counts of Fusarium were highly decreased, where population density of Trichoderma propagules were highly increased in rhizosphere soil of seedling treaded with bioenhanced bagasse only or in combined with the reduced rate of Topsin-M. The reduction in both disease infection and severity on seedlings may be attributed to the highly decreased in population density of the pathogen in the soil and increased the activity of *T. harzianum* (bio agent) propagules in the soil Table(4) . Moreover the antagonistic ability of the bio agent against the pathogen and its enzymatic activity (chitinase and cellulase) were highly increased Tables(1 and 2) . *Trichoderma spp.* enhanced the growth and induced systemic resistance of plants (Kloepper *et al.,* 1980; Windham *et al.,* 1986 and Huang, 1991).

Control of root rot disease pathogens through amended soil by organic materials or agricultural wastes alone or in combined with bio control agents may be attributed to 1-increasing the activity of the indigenous microflora resulting suppression of pathogens population through competition or specific inhibition 2-releasing degradation compounds such carbon dioxide, ammonia, nitrites, saponins or enzymes which are generally toxic to the pathogens 3-inducing plant defense mechanisms 4- cellulase and glucanase are prevalent to high concentration as a result of the breakdown of cellulase and lignin by microorganisms in the soil (Kloepper *et al.*, 1980 ; Tsao and Oster, 1981;; Tumey and Menge, 1994 ; Wang,1999 ;Walker and Morry ,1999 and Liu and Huang 2000)

In field experiments, the soil around stems of naturally infected mandarin trees with Fusarium root rot disease were amended with bioenhanced bagasse, bioenhanced bagasse + Topsin-M or Topsin-M treatments as twice application per season. Such treatments resulted in recovering some of diseased trees and reduced disease severity on the others. The best soil amendment treatments in controlling the disease were bioenhanced bagasse followed by bioenhanced bagasse + Topsin-M. These treatments reduced the number of infected mandarin trees, disease severity on diseased trees and also caused high reduction in population density of *Fusarium spp.* in rhizosphere soil of treated trees compared with control (non treated diseased trees). Moreover, population density of Trichoderma propagules counts were highly increased in rhizosphere soil of trees that treated with the same treatments. These results are relatively in accordance with results recorded by (Fang *et al.,* 1995; Sawand *et al.,* 1995; Prasad *et al.,* 1999 and Tewari and Mukhadhyay, 2001) found that Phytopthora root rot of mandarin trees was significantly reduced when the soil of the trees were drenched with Ridoml and amended with *Trichoderma* spp. grown on coffee waste.. In addition, these treatments cause an increasing in fruit weight, fruit yield per tree and also total yield per feddan. Increasing fruit yield of mandarin trees may be due to the high number of recovered diseased trees, enhanced in the growth of treated trees due to inhibition of pathogen (Fusarium) activity and increasing population density of bio agent (Trichoderma) in rhizosphere soil. Amended soil with agricultural wastes inoculated by different bio control agents were recoded to improve plant growth and increased their yield (Tumey and Meng, 1994; Mitra and Nandi, 1994 ;Nemec *et al.*, 1996 and Prasad *et al.*, 2000)

It could be suggest that supplemented agricultural wastes with bio control agents such *Trichoderma spp.*, then added to the soil as soil amendments, or biofertilizer for controlling soil born pathogens can be successfully used under field conditions replacing traditional fungicides treatments and avoid environmental pollution.

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مقاومة مرض عفن الجذور الفيوزاريومى علـى اليوسفي باضافة الفطر *Trichoderma harzianum* النامي على مخلفات قصب السكر (مصاص القصب) إلى التربة .

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تحميل بعض الكائنات الحيوية على المخلفات الزراعية ثم إضافتها إلى التربة كسماد عضوي حيوي لتحسين صفات التربة الزراعية أو مقاومة مسببات أمراض اعفان الجذور هو الهدف من هذه الدراسات .

في هذه الدراسة تم دراسة تأثير إضافة مصاصة القصب بمفرده أو النامي عليها الفطر Trichoderma harzianum أوالمصاصة النامي عليها الفطر + التوبسين-م (٥و٠جم /لتر) أو إضافة التوبسين بمفردة (١جم /لتر) إلى التربة المحقونة بالمسبب المرضى في الصوبة أو المصابة طبيعيا تحت ظروف الحقل على حدوث مرض عفن الجذور الفيوزاريومى على شتلات واشجار اليوسفي . وكذلك على المحصول وأعداد كل من المسبب المرض والكائن الحيوى حول جذور النباتات المعاملة .

وجد انه عند إضافة مصاصة القصب إلى بيئة Dcx medium بمعدلات مختلفة ٢٥و١ ،٥ و٢ ، ٥ % سببت زيادة في القدرة التضادية وكذلك النشاط الأنزيمي (الشيتينيز والسليوليز) للفطر Trichoderma harzianum .

في تجارب الصوبة وجد أن أضافه كل من المعاملات السبق ذكرها الى التربة المحقونة بالمسبب المرضى سببت نقص فى أعداد النباتات المصابة وكذلك انخفاض فى شدة المرض على النباتات المصابة ووافق ذلك تناقص فى أعداد المسبب المرضى بينما زادت أعدادالفطر Trichoderma harzianum حول جذور النباتات المعاملة .

وجد ان معاملة التربة للأشجار اليوسفى المصابة بمرض عفن الجذور بمصاصة القصب النامي عليه الفطر Trichoderma harzianum أو النامى عليها الفطر + توبسين ٠م أو اضافة التوبسين بمفردة وذلك بمعدل مرتين خلال الموسم سببت شفاء العديد من الأشجار المريضة وقللت من شدة المرض على بعض النباتات الأخرى المريضة . أيضا سببت تلك المعاملات انخفاضا ملحوظا فى أعداد المسبب المرضى حول جذور النباتات المعاملة بيتما زادت أعداد الفطر Trichoderma harzianum وانعكس ذلك على المحصول حيث زاد محصول أشجار اليوسفي بنسبة٢و١٣٠ ، ٣و١١٦، ٤و٦٧% عند أضافه المعاملات السابقة للتربة

تشير النتائج إلى إمكانية استخدام المخلفات الزراعية مثل مصاصة قصب السكر كمواد حاملة للكائنات الحيوية مثل الفطر Trichoderma harzianum ثم أضافتها للتربة لمقاومة مسببات أمراض أعفان الجذور على الموالح وبذلك نقلل من استخدام المبيدات ويزداد المحافظة على البيئة من التلوث .