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Utilization of bio composted agricultural wastes in management of Fusarium dry root rot disease on lime (*Citrus aurantifolia* L.)

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Abstract Plant diseases caused by soil borne pathogens cause greet losses in yield and products of agricultural crops. Controlling these pathogens manly depends on bromide methyl and chemical fungicides treatments that cause hazards to human health and increase environmental pollution. The modern agricultural systems aimed to reduce and/or eliminate fungicide, through eco-friendly safely controlling systems. In this study manipulation of soil with bio compost (composted of sugarcane bagasse ,rice straw and soybean straw inoculated with spore suspension $5x10^6$ cfu/ml of *T. harzianum* isolate NB10) , *T. harzianum* (spore suspension $5x10^6$ cfu / ml) and Topsin M 70% successfully controlled Fusarium dry root rot disease of lime(*Citrus aurantifolia* L.) . Complete reduction of the *F. solani* linear growth was recorded at 100 ppm of Topsin M and *T. harzianum* . Under Field condition, amended soil around stems of diseased lime trees by bio compost (BCAW) and Topsin-M (1 g/L) treatments as twice applications per season resulted 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 treated trees by bio compost (BCAW). It could be concluded that application of bio composed agricultural wastes has the same effect of fungicide Topsin –M ,so it could be safely used commercially as substitute of bromide methyl and fungicides for controlling soil borne plant pathogens and avoid health hazards and environmental pollution.

Keywords: Citrus - Root rot, *Fusarium solani* , Control, Bio compost.

Introduction

Citrus is the main fruit crop in Egypt, its cultivation expanding in the newly reclaimed land. Citrus trees are cultivated across Egypt. The cultivation is gradually spreading to the North, in Sharkia, Behira, Kalubia, Monofia, Giza, Ismailia, Gharbia, Asuit and Beni swif, including the newly reclaimed desert areas in Noubaria district . Egyptian Lime (*Citrus aurantifolia* L.) accounts for

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10.7% of the total cultivated varieties in Egypt. The most important lime varieties are Balady or Egyptian Rashidy (local), Mexican, Tahiti and Persian (Salem and Sheta, 2002).

Dry root rot disease of citrus caused by *Fusarium solani* (Mart.) is one of the most serious diseases attacked citrus trees especially that cultivated in new reclaimed lands in Egypt , it has been estimated to affect 8.9% of lime trees and caused 39.6% loss in fruit yield (El- Mohamedy, 1998) . Such disease of citrus was reported to attack all citrus varieties (El-Mohamedy, 1998; Catara and Polizzi, 1999).). Control of this disease depends mainly on fungicides application (El-Mohamedy, 1998 and Verma *et al.*, 1999).Meanwhile fungicides always desirable due to 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.

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 (Godwin and Arinze, 2000; Liu and Huany, 2000; Prasad and Ragashwaran, 1999;El-Mohamedy et al., 2006 , 2008;2010 and 2012). 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 *Stroptomyces* spp reduced root rot and Grown rot diseases on tomato, ball pepper, celery and citrus.

The purpose of this research is to evaluate the efficiency of amended soil with bio composed agricultural wastes colonized by *T. harzianum* on control of Fusarium dry root rot disease on mandarin as well as 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 ,rice straw and soybean straw into a bio manure for land application using *T. harzianum* in clean agricultural system.

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, Egypt.

Effect of T. harzianum and Topsin M 70% on F. solani in vitro

Antagonistic ability of *T. harzianum* against *F. solani* was carried out on PDA medium using duel culture technique (Ferreira *et al*., 1991) ,four each treatment Five Petri dishes were used. All plates were incubated at 25 ^{o}C for 5 and 10 days. Antagonistic ability was expressed as percent of reduction in linear growth of *F.solani* over the Reduction in linear growth of *F. solani* was calculated. Serial quantities of Topsin- M 70% were added to conical flasks containing melted PDA medium to obtain final concentrations of 0.0, 25, 50, 100, 200, and 400 ppm and mixed gently and then dispensed in sterilized Petri dishes (10 cm diameter). Plates were individually inoculated at the centre with equal disks (5 mm diameter) of 10 day old culture of *F. solani* and incubated at 25℃. The overage linear growth of fungi was calculated after 7 days. Each treatment was represented by 5 replicates. The spores of *Fusarium solani* obtained from the 10-days-old cultures on PDA medium were collected, suspended in distilled sterile water, and mixed with appropriate aliquots of stock aqueous suspension/solutions of Topsin M 70% to obtain a density of 5 x 10^5 spores/ml, and the concentrations of Topsin -M . Three drops (about 50µl) of each treatment were then placed on microscopic slide , kept at 20℃ in moisten filter paper placed in Petri dishes for 28 h. .The germination percentages of conidia (50 conidia for each treatment) were measured using an Olympus Cx40 microscope. The conidium's was considered germinated when the germ-tube length was at least equal to the conidial diameter. The experiment was replicated four times.

Preparation of bio composted agricultural wastes (BCAW)

Agricultural wastes such as Sugarcane bagasse ,rice straw and soybean straw were ground to powder ,250 g of each waste powder was mixed with sand soil (4 : 1) in polyethylene bags ,then 2.0 g ammonium sulphate ,5.0 g super phosphate , 5.0 g potassium sulphate and 400 ml water per 1000 g powder were added to each bag .All bags were sterilized for 1 hr in autoclave at 121C and left at room temperature for two weeks for complete composted.

The compost in a half of these bags were inoculated by spore suspension of *T. harzianum* 3 x 10^6 spore / mL (this mixture = bio composed) and the other left as cheek treatment control (this mixture = compost). All bags were incubated at 25^{o}C for 14 days at room temperature, then used as bio composted agricultural wastes (BCAW) (composed agricltrural wastes colonized by *T. harzianum*) or composted agricultural wastes (CAW) for direct delivery into the soil.

Field experiments

The experiments were carried out at Nobaria province (Behiera Governorate) during 2011 and 2012 seasons in two Privet orchards of lime (8-year old Baladi cv), Grafted on sour orange rootstock) with the history of root rot disease. The following treatments were evaluated.

Bio composted agricultural wastes (BCAW) colonized by *T. harzianum*) at the rate of 10 % (w/w) of soil.

Composted agricultural wastes (CAW) at the rate 10 % (w/w) of soil.

T. harzianum at the rate of 5% (v/w) of soil.

Topsin .M 70% at the rate of 20 g / tree.

Control (non-treated diseased trees).

One hundred and twenty diseased lime trees showing typical symptoms of Fusarium dry root rot disease were selected. 10 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 treatments were applied to soil around main stem of tree twice per season, first, in spring (March) and the second in summer (July). After 60 and 90 days from the adding second application a number of recovered trees and the development of disease severity on diseased trees were recorded. The development of disease symptoms (severity) on lime trees 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 60 and 90 days from treatments application were recorded and calculated.

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 lime trees respectively. Five plates were used as replicated for each treatment. Ttotal count of each fungus were expressed as cell forming units (cfu) per gram dry soil.

Statistical analysis

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

Results

Effect of T. harzianum and Topsin M on F. solani in vitro

The inhibitory effect of different concentrations of Topsin -M and *T.harzainum* against linear growth and spore germination of *F. solani* were tested . Results in Table (1) show that increasing fungicide concentration caused strongly decrease on both linear growth and spore germination of *F. solani* . The linear growth and spore germination of *F. solani* were completely inhibited at 200 ppm of Topsin-M .*T. harzainum* was highly antagonistic to *F. solani*, as complete reduction (100%) of the growth of *F .solani* recorded after eight days of incubation.

Table 1. Percentage reduction (%) of linear growth and spore germination of *F. solani* as affected by Topsin -M 70% and *T .harzianum* on PDA medium

Treatment		**Linear growth reduction %**	**Spore germination reduction %**
Tosin -M 70%	25 ppm	0.0 a	0.0
(fungicide)	50 ppm	25.0 b	30
	100 ppm	77.0 c	84
	200 ppm	100 d	100
	400 ppm	100 d	100
T .harzianum		100 d	100
Control		0.0 a	0.0

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

Field experiments

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

Effect on Fusarium rot root disease incidence

Data in Table (2 and 3) show that all soil treatments reduced the number of infected Egyptian lime trees as well as the percentages of disease severity on these trees. But there are no significant differences between all tested treatments and control (untreated diseased trees). The highly records of recovering trees and the highly percentages of reduction in disease development (disease severity) were recorded after 90days from soil treatments .The highly effective soil treatments were BCAW, Topsin-M and *T.harzianum* .These treatments cause reduction in Fusarium rot root infection and severity reach to 66.6, 33.3 , 25.0% and 66.8 , 40.0, 20.0% respectively during first season 2011 and 64.0,48.0 , 20.0% and 64.0, 48.0 , 20.0% during the second season 2012 after 90 days from treatments. Treated soil of diseased lime with *Trichoderma harzienum* cause considerable effect in reduce both disease infection and severity superior to fungicide soil treatment (Topsin –M) . Meanwhile CAW soil treatment case least effect in diseases management during two seasons.

Table 2. Fusarium dry root rot on Egyptian lime trees as affected by different soil treatments under field conditions during 2011 and 2012 seasons

Soil treatment	**Fusarium dry root rot incidence**					
	No. of diseased trees after application			Fusarium dry root rot Reduction after treatments		
	0 day	60 days	90 days	0 day	60 days	90 days
			Season 2011			
BCAW (10% w/w)[1]	12 a[3]	8 b	4 c	0.0	33.0	66.6
CAW (10% w/w) [2]	12 a	10 b	9 c	0.0	16.6	25.0
T.harzianum	12 a	10 a	8 a	0.0	16.6	33.3
Topsin –M 70% (1 g/L)	12 a	9 a	7b	0.0	25.0	41.6
Control	12 a	10 a	10 a	0.0	16.6	16.6
			Season 2012			
BCAW (10% w/w)	12 a	6.0 b	4c	0.0	50.0	66.6
CAW (10% w/w)	12 a	9 b	9 c	0.0	25.0	25.0
T.harzianum	12 a	9 a	9a	0.0	25.0	25.0
Topsin –M 70% (1 g/L)	12 a	8 a	8 b	0.0	33.3	33.3
Control	12 a	12 a	12 a	0.0	0.0	0.0

1- BCAW: bio composed agricultural wastes 2- CAW : composed agricultural wastes
3- Figures with the same letters are not significant different (P =0.05)

Table 3. Development of Fusarium dry root rot on Egyptian lime trees as affected by different soil treatments under field conditions during 2011 and 2012seasons

Soil treatment	Fusarium dry root rot disease severity					
	Disease severity (%) after application			Disease severity Reduction after application		
	0 Day	60 day	90day	0 Day	60 day	90 day
Season2011						
BCAW (10% w/w)(1)	50 a(3)	40.0 c	16.6 d	0.0	20.0	66.8
CAW (10% w/w) (2)	50 a	46.2 b	42.0 b	0.0	8.0	16.0
T.harzianum	50a	42.0	40.0	0.0	16.0	20.0
Topsin –M 70% (1 g/L)	50 a	33.6 c	30.5 c	0.0	34.0	40.0
Control	50 a	63.4 a	74.8 a	0.0	0.0	0.0
Season2012						
BCAW (10% w/w)	50 a	30.0 c	18.0 c	0.0	40.0	64.0
CAW (10% w/w)	50 a	42.0 b	26.0 b	0.0	16.0	48.0
T.harzianum	50a	40.0	40.0	0.0	20.0	20.0
Topsin –M 70% (1 g/L)	50 a	35.0 c	26.0 b	0.0	30.0	48.0
Control	50 a	65.0 a	75 a	0.0	0.0	0.0

1-BCAW : bio composed agricultural wastes 2- CAW : composed agricultural wastes
3-Figures with the same letters are not significant different (P =0.05)

Effect on Population density of Fusarium and Trichaderma propagules in soil

Data in Tables (4) indicate that all soil treatments were significantly reduced the number of Fusarium propagules counts in rhizosphere soil of treated trees, while it increased in untreated diseased trees (control). BCAW , Topsin-M and *T. harzianum* treatments show highly effective in inhibition of Fusarium activity (density) around roots of trees followed by CAW treatment. These treatments reduced the counts of Fusarium propagules counts by 53.8, 52.5, 50.0% and 60.0, 57.0, 30.0% during 2011 and 2.012 seasons respectively. Moreover, these treatments cause increasing in population density of *Trichoderma* spp around roots of treated trees Table (5) . The total propagules count of *Trichoderm* spp in rhizosphere soil of treated trees with BCAW ,Topsin-M and *T. harzianum* increased by 140.0,30.0,66.6% and by 136.0,20.0,62.5% during the fist and second seasons 2011 and 2012 . The highly effective treatments in decreasing Fusarium density and increasing the density of bio agent *T. harzianum* in rhizosphere soil of treated lime trees were BCAW and Topsin-M followed by T. *harzianum* respectively.

Table 4. Fusarium propagules counts in rhizosphere soil of infested lime trees affected by different soil treatments under field conditions during 2011 and 2012seasons

Soil treatment	**Fusarium propagules count (cfu x 10^3) / g dry soil**			**Reduction(%) of Fusarium propagules count**		
	0 day	60 day	90 day	0 day	60 day	90 day
Season2011						
BCAW (10% w/w)(1)	6.5 a(3)	3.8 c	3.0 c	0.0	41.5	53.8
CAW (10% w/w) (2)	6.2 a	5.0 b	4.2 b	0.0	19.3	32.2
Trichoderma harzianum	6.0 a	3.8 c	3.0 d	0.0	36.0	50.0
Topsin –M 70% (1 g/L)	5.5 a	3.8 c	3.1 d	0.0	30.9	52.5
Control (diseased tree)	6.5 a	9.6 a	11.5 a	0.0	0.0	0.0
Season2012						
BCAW (10% w/w)	5.5 c	3.3 c	2.2 c	0.0	40.0	60.0
CAW (10% w/w)	5.0 b	4.2 b	4.2 b	0.0	16.0	16.0
Trichoderma harzianum	6.0 b	4.0 b	4.2 b	0.0	33.3	30.0
Topsin –M 70% (1 g/L)	5.5 b	3.4 b	2.3	0.0	37.0	57.0
Control (diseased tree)	7.5 a	10.0 a	12.0 a	0.0	0.0	0.0

1-BCAW : bio composed agricultural wastes 2- CAW : composed agri0.0cultural wastes
3-Figures with the same letters are not significant different (P =0.05)0.0

Table 5. Trichoderm propagules counts in rhizosphere soil of infested lime trees affected by different soil treatments under field conditions during 2011 and 2012seasons

Soil treatment	**Trichoderma propagules count (cfu x 10^3) / g dry soil**			**Increasing(%)Trichoderma propagules count**		
	0 day	60 day	90 day	0 day	60 day	90 day
Season2011						
BCAW (10% w/w)(1)	2.7 ab(3)	6.2 a	8.8 a	0.0	94.2	140.0
CAW (10% w/w) (2)	2.5 b	2.2 c	23.4 c	0.0	18.2	30.2
Trichoderma harzianum	3.0b	4.8 e	5.0e	0.0	60.0	66.6
Topsin –M 70% (1 g/L)	3.0 b	3.6 b	3. 8 b	0.0	20.0	30.0
Control (diseased tree)	1.8 ab	2.0 c	2.2 c	0.0	11.1	22.2
Season2012						
BCAW (10% w/w)	2.8 b	9.0 a	8.5 a	0.0	96.0	136.0
CAW (10% w/w)	2.4 a	2.5 b	3.0 b	0.0	14.3	25.0
Trichoderma harzianum	3.2b	5.0a	5.2a	0.0	56.2	62.5
Topsin –M 70% (1 g/L)	2.5 b	2.2 b	3.0 b	0.0	12.0	20.0
Control (diseased tree)	2.0 a	2.4 b	2.8 b	0.0	20.0	40.0

1-BCAW : bio composed agricultural wastes CAW : composed agri0.0cultural wastes
2- Figures with the same letters are not significant different (P =0.05)0.0

Discussion

Using fungicides alternatives in management of plant pathogens is one of the most methods to keep environment clean and green. Utilization of agricultural wastes especially rice straw and bio control agents as substitutes of bromide methyl and fungicides that cause many hazards to human and environment is powerful approach in management of soil borne plant pathogens . Fusarium root rot disease caused by *F. 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. The linear growth and spore germination of *F. solani* were completely inhibited at 200 ppm of Topsin M . *T. harzainum* was highly antagonistic to *F. solani* ,as complete reduction (100%) of the growth of *F .solani* recorded after eight days of incubation . The same results are in agreement with El-Mohamedy 1998 and 2004 ; El-Mohamedy *et al*., 2008 and 2010. as they indicated that *T.harzainum* alone or in combination with fungicides have been used in chemical-biological integrated control programs against several soil borne plant pathogens .Elad *et.al*.,1980 found that the mechanisms of the antagonism of *T. harzainum* against different pathogens may be due to mycoparasitism , competition and antibiosis .

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 ; Godwin and Arinze, 2000).

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*., 1981 ; Tsao and Oster, 1981; Tumey and Menge, 1994; Wang,1999; Walker and Morry, 1999 ;Liu and Huang, 2000)

In field experiments, the soil around stems of naturally infected trees with Fusarium dry root rot disease were amended with BCAW, CAW 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 treatments in controlling the disease were BCAW followed by 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 and Tsao, 1995; Sawand *et al.*, 1995; Tewari and Mukhadhyay, 2001). 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 Tables (4 and 5) . Moreover the antagonistic ability of the bio agent against the pathogen and its enzymatic activity (chitinase and cellulase) were highly increased due to growing the bio agent on sugar can bagasse (El-Mohamedy, 2004). *Trichoderma* spp. enhanced the growth and induced systemic resistance of plants (Klopper *et al.*, 1981; Windham *et al.*, 1986). Sawant *et al*., 1995 fount 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, restricted the development of feeder roots providing a healthy and vigorous trees capable of producing maximum yield .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)

Conculusion

In the recent years alternative control methods are strongly desired for sustainable agricultural, where organic amendments play an important role in clean and green environment, as well as sustainable alternatives of bromide methyl and fungicides to protect plants against soil borne pathogens. Utilization of organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases. In this study we can suggest that supplemented agricultural wastes with bio control agents such *Trichoderma* spp, then added to the soil as soil amendments or bio fertilizer for controlling soil born pathogens can be successfully used under field conditions replacing traditional fungicides treatments and avoid environmental pollution.

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