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Suppression of Damping-off and Charcoal-Rot of Sunflower with Composted and Non-composted Agricultural Wastes

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Pythium sp., *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Macrophomina phaseolina* were found to be associated with damping-off and charcoal-rot of sunflower in Etay Elbaroud region, Behera governorate, Egypt. However, *Pythium* sp. was not able to incite any disease to sunflower in the pathogenicity test while the other fungi incited pre- and post- emergence damping-off in different degrees. Charcoal-rot was incited by *Macrophomina phaseolina* only. Field amendments with different straw-based composts, produced with cow manure, poultry manure, or urea, inoculated or not with *Trichoderma harzianum*, as well as the noncomposted farmyard manure, mostly produced less damping-off and charcoal-rot in naturally infested fields. The highest disease suppression (61.6%) was obtained with rice straw composted with cow manure and inoculated with *Trichoderma harzianum*. Amendment of compost with *Trichoderma harzianum* accelerated composting and improved its suppressive effect. The other compost amendments, *i.e.* rice straw composted with poultry or cow manure were equally effective in checking sunflower diseases where total disease suppressions were 51.3% and 47.6% with these composts, respectively. The noncompposted farmyard manure also suppressed damping-off and charcoal-rot of sunflower and composts became more suppressive by increasing compost age. The rice straw-urea compost, however, showed the lowest suppressive effect in checking sunflower diseases. The suppressive effect was accompanied, mostly, by significant reduction in pathogen population of *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Macrophomina phaseolina* in sunflower rhizosphere. Also, a strong correlation was recorded between disease suppression and both of the total microbial population (bacteria and actinomycetes) and activity in the sunflower rhizosphere. Total bacteria and actinomycetes were considerably increased (134.7%- 375.3%) in plant rhizosphere. However, rate of increase of the microbial activity was higher (259.2%- 657.1%) and its correlation coefficients with the disease suppression were even higher being 0.783, 0.978, and 0.950 compared to 0.749, 0.736, and 0.531 with the microbial population for the pre-emergence damping-off, post-emergence damping-off, and charcoal-rot, respectively. This indicated that compost disease suppressiveness effect was more correlated with the microbial activity than the microbial population in sunflower plant rhizosphere.

Keywords: Charcoal-rot, compost, damping-off and sunflower.

Soil-borne diseases are still a major threat to sunflower cultivation in Egypt and all over the world because of the wide host range of the pathogens and their strong survival ability in the soil (Mousa *et al.*, 2006 and Bokor, 2007). Chemical control was massively applied, however, for the increasing public concern over the fungicide usage, alternative control methods are strongly desired for sustainable agriculture where organic amendments play an important role (Workneh and van Bruggen, 1994 and Lazarouits, 2001). The use of the organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of the agricultural residues and could provide a powerful tool for management of plant diseases (Boulter *et al*, 2000).

It has been reported that several composts, used as soil amendments, protected plants from soil-borne plant pathogens (Scheuerellss *et al.*, 2005). It was shown that such composts reduced the pathogen propagule density of *R. solani*, *M. phaseolina*, *Fusarium* spp., *Virticillium* spp, *Pythium* spp., *Streptomyces scabies*, *Ralstonia solanecearum*, and plant parasitic nematodes (Aryantha *et al.*, 2000; Sato and Toyota, 2004; Scheuerellss *et al.*, 2005 and Mousa *et al.*, 2006). It was also reported that composts increased microbial activity and consequently suppressed root rot and damping-off pathogens (Scheuerellss *et al.*, 2005). However, little is still known regarding the suppression mechanisms (Sato and Toyota, 2004).

Agricultural residues such as rice straw are abundant in farms in Egypt and field burning of rice stubble constitutes an annual environmental problem (El-Mashad *et al.* 2003). Composting of rice straw and soil amendments of such agricultural residues along with farm wastes could be of great benefit for soil fertility and management of the occurred soil-borne diseases (Osunlaja, 1990, Lazarouits, 2001; Scheuerellss *et al.*, 2005, Asari *et al.*, 2007 and Hameeda *et al.*, 2007). Amendment of compost with *Trichoderma harzianum* was reported (Hoitink and Boehm, 1999) to accelerate agricultural residues composting and improved its disease-suppressive effect. The present study, therefore, was conducted to investigate potential of different kinds of rice-straw composts to suppress damping-off and charcoal-rot of sunflower and to analyze the possible involved mechanism.

Materials and Methods

Fungi associated with damping-off and charcoal-rot of sunflower:

During 2005 growing season, three fields with recent history of severe damping-off and charcoal-rot were monitored at Etay Elbaroud region. Diseased sunflower plants were collected 15, 45, and 90 days after sowing. Isolation of the causal fungi was conducted on PDA and identification was carried out according to Gilman (1971), Booth (1977) and Barnett and Hunter (1987).

Pathogenicity test:

During 2006 summer season pathogenicity tests were conducted in a pot experiment according to El-Zarka (1976) in Etay Elbaroud agricultural station. Inocula of the recovered isolates were prepared on autoclaved barley grains medium under aseptic conditions.

Seeds of sunflower cv. Giza 102 were obtained from Seed Dept., Ministry of Agric., Giza. Egypt. Seeds were surface disinfested with 2% sodium hypochlorite, rinsed in sterile distilled water, and sown in 25-cm plastic pots filled with autoclaved clay and sand (1:1, v/v). Twelve replicated pots for each tested isolate were prepared; each was sown with two seeds of sunflower. Plants were watered as needed and treated according to the normal agricultural practices. Re-isolation was conducted to insure the association of the tested isolates with the developed disease.

Field studies:

Synchronized with the laboratory work, two field experiments were conducted at Etay Elbaroud region during the summer season of 2005 and 2006. This was to investigate the disease suppressive effect of various rice of straw-based composts in fields naturally infested with damping-off and charcoal-rot pathogens of sunflower. The experimental plot was 21 m^2 with five ridges. Sunflower seeds (cv. Giza 102) were surface disinfested with 2% sodium hypochlorite, rinsed in sterile distilled water and sown as 200 seeds/ plot, after field had been amended with different types of composts. Experiment was planned in a complete randomized block design with four replicate plots.

Composts:

Agricultural residues, *i.e.* rice straw (*Oryza sativa*), which is widely abundant in the Egyptian farms was used as a raw material for compost production according to the rapid composting methods described by Cuevas (1993).

Three rice straw-based composts were prepared using fresh cow manure, poultry manure (at 3:1 w/w of rice straw to animal manure) or urea as 2%, on dry weight basis. Animal manures and urea were applied as a nitrogen source to speed up decomposition and to get good compost quality. Also, *Trichoderma harzianum* ($30x10^6$ spore/ml, El-Nasr Company) was applied (500 ml/ 100 L water/ 1000 kg dry rice substrate) to rice straw mixed with cow manure (as 10:1 rice straw to cow manure, on dry weight basis) to produce another type of compost (Hoitink and Boehm. 1999). Completion of composting was determined by the pleasant odors, reduction of the produced heat, the change into dark brown colour and the crumbling texture type of the mixture (Cuevas, 1993). Also, fresh farmyard manure (FYM) which was rice straw, cow dung mixture was obtained from the surrounding farms at Etay Elbaroud region and used as noncomposted agricultural manure. Characteristics of composts as well as the FYM are shown in Table (1) as analyzed according to Page *et al.* (1982) Compost pH was determined by suspending 5.0 g of compost in 10 ml deionized water, then measuring the pH of the supernatant after 1h. All composts were sun dried until 10-20% moisture and applied at the rate of $20m^3$/fed (El-Gizawy. 2005). Composts were applied to field plots during field preparation one week before sowing in the first week of May. No fungicides were applied and plants were treated according the normal agricultural practices for sunflower cultivation.

Table 1. Characteristics of composts produced and used in the present study

Compost	%C	%N	%P	%K	%Ca	pH	Moisture
RS+Urea	44.7*	1.9	0.1	1.1	0.17	8.1	26
RS+CM	41.3	1.3	0.8	1.3	0.19	9.0	41
RS+PM	39.4	2.7	1.9	1.9	0.35	7.6	29
RS+CM+*Tr.*	40.8	0.8	0.4	1.5	0.16	7.9	23
FYM (Non-composted)	6.6	0.9	1.2	1.4	0.21	8.3	42

* Values are averages of components of the prepared composts used in the two growing seasons, relative to the oven dried composts (105°C for 24h.). RS = Rice straw, CM = Cow manure, PM= Poultry manure, *Tr.* = *Trichoderma harzianum*, FYM = Farmyard manure.

Compost disease-suppressive effect:

This was assessed in terms of the following parameters:

1- Incidence of damping-off and charcoal-rot:

Incidence of the pre-emergence damping-off, post-emergence damping-off, and the charcoal-rot disease was determined 15, 45, and 90 days after sowing, respectively, as percentage of the infected plants out of the total number of seeds cultivated (200) in each plot for the different compost amendments.

2- Survival of the fungal pathogens in plant rhizosphere:

Soil samples were collected from each plot at 15, 45, and 75 days after sowing. Samples were taken from plant rhizosphere 10-15 cm of the soil surface where four soil extracts were prepared for each plot. Serial dilution of 1.0g soil/ 9.0 ml of sterile distilled water were made to obtain 10^{-3} dilution and 0.1ml of each dilution was spread plated on appropriate selective media (Nash and Synder, 1962, Ko and Hora, 1971, Cloud and Rupe, 1991, and Steadman *et al.*, 1994) for enumeration of the most expected pathogenic fungi. Inoculated plates were incubated at 25°C in darkness and developed colonies were enumerated five days after inoculation.

3- Microbial population:

Soil extracts were prepared and 10^{-3} dilutions were made as above mentioned. A 0.1ml of each dilution was spread plated on each of Lingappa and Lockwood (1962), and Collins and Lyne (1985) media for the total counting of actinomycetes and bacteria, respectively. Plates were incubated at 30°C in darkness for three days before counting bacterial colonies, and for five days for counting actinomycetes. Microbial population was estimated on the dry weight basis of the soil (105°C/ 24h.).

4- Microbial activity:

Total microbial activity was estimated by determining the rate of microbial hydrolysis of fluorescein diacetate according to Schnurer and Rosswall (1982). Soil samples (5.0g), taken from plant rhizosphere 10-15 cm of the soil surface, were mixed with 20 ml of phosphate buffer (60 mM, pH 7.6), to which 400µg of fluorescein diacetate (FDA, Sigma) was added.

The mixture was shaken for 20 min at 90 rpm for 24°C before addition of 20 ml acetone. The supernatant was filtered through Whatman No.1 filter paper and the absorbance was assessed using spectrophotometer (Analytic Jana 40) at 490 nm. A standard curve was prepared by boiling a range of FDA concentrations from 0 to 1,000 µg / ml in 5 ml of buffer to complete hydrolysis and measuring the absorbance of the resulting supernatant at 490 nm. Microbial activity was expressed as micrograms of hydrolyzed FDA per gram of dry soil (oven dried at 105 °C for 24 days) per minute.

Statistic analysis:

The obtained data were statistically analyzed using the American SAS/STAT software, version 6. Combined analysis was conducted for the two growing seasons and means were compared by the least significant difference test (LSD). Correlation analysis was conducted for mean increase in microbial (bacteria and actinomycetes) population and activity with the disease suppression using the Microsoft Office Excel, 2003.

Results

Fungi associated with damping-off and charcoal-rot:

Sampling of diseased sunflower seedlings, 15 days after sowing, revealed the occurrence of *Rhizoctonia solani* and *Sclerotium rolfsii* in frequencies of 59.4% and 34.3%, respectively, in seedlings affected with pre-emergence damping-off. Also, *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Pythium* sp. also occurred but at lower frequencies of 21.6%, 13.2%, and 9.3%, respectively. Sampling for the post-emergence damping-off at 45 days after sowing, revealed an increase in the frequency of *S. rolfsii* (47.1%) and *M. phaseolina* (21.5%) but a decrease in the frequency of *R. solani* and *F. oxysporum* while the *Pythium* sp. disappeared. At 90 days after sowing, sampling for the charcoal-rot revealed a 100% occurrence of *M. phaseolina* while *R. solani* and *S. rolfsii* occurred in 17.3% and 19.5%, respectively, no *Fusarium oxysporum*, or *Pythium* sp. were recorded (Table 2).

Pathogenicity test:

Pathogenicity of the recovered fungi revealed that *R. solani*, *S. rolfsii* and *F. oxysporum* were pathogenic to sunflower at the pre-emergence damping-off stage assessed 15 days after sowing. These fungi incited 29.1%, 25.0%, and 16.5% pre-emergence damping-off, respectively. However, *M. phaseolina* incited only 4.1% while *Pythium* sp. was not able to incite any disease (Table 3). At the post-emergence damping-off stage 45 days after sowing, *R. solani*, *S. rolfsii* and *F. oxysporum* fungi incited more disease being 37.5%, 41.6%, and 20.8%, respectively. Moreover, *M. phaseolina* incited 16.5 % damping-off while *Pythium* sp. did not incite any disease. However, at 90 days after sowing, *M. phaseolina* incited 70.8% as charcoal-rot as evident by the presence of sclerotia in plant base while none of the other tested fungi incited any disease in this stage (Table 3).

Table 2. Frequency of fungi recovered from sunflower plants, showed damping-off and charcoal-rot symptoms, collected from different fields in Etay Elbaroud region 15, 45, and 90 days after sowing, during 2005 growing season

Fungus	Frequency (%) of fungi in;		
	Pre-emergence damping-off (15 days)	Post-emergence damping-off (45 days)	Charcoal-rot (90 days)
Pythium sp.	9.3 *	0.0	0.0
Fusarium oxysporum	21.6	18.2	0.0
Rhizoctonia solani	59.4	43.6	17.3
Sclerotium rolfsii	34.3	47.1	19.5
Macrophomina phaseolina	13.2	21.5	100

* Values are percentages of recovery from 100 samples plated on PDA. for each sampling date.

Table 3. Pathogenicity of the recovered fungi in a pot experiment conducted during the 2006 growing season and monitored for damping-off and charcoal-rot symptoms on sunflower (cv. Giza 102), 15, 45, and 90 days after sowing

Fungus	Monitored diseases & days after sowing		
	Pre-emergence (15 days)	Post-emergence (45 days)	Charcoal-rot (90 days)
Pythium sp.	0.0 *	0.0	0.0
Fusarium oxysporum	16.5	20.8	0.0
Rhizoctonia solani	29.1	37.5	0.0
Sclerotium rolfsii	25.0	41.6	0.0
Macrophomina phaseolina	4.1	16.5	70.8
Control	0.0	0.0	0.0

* Values are percentage of disease incidence in 12 replicate pots, each was sown with two seeds, for each tested fungus.

Field experiment:

1. Compost disease-suppressive effect:

1.1. Effect on disease incidence:

Field amendments with the tested rice-straw based composts significantly suppressed pre-emergence damping-off with most compost amendments. This was in the range of 17.5%-51.2% with most pronounced effect was for the rice straw + cow manure compost inoculated with *Trichoderma harzianum* (RS+CM+ *Tr.*). The fresh farmyard manure (FYM) amendment or the rice straw composted with urea (RS+Urea), however, did not pose a significant effect in this stage. At the post emergence damping-off stage, a suppressive disease effect was obtained with all the tested composts. This ranged between 33.3% and 62.1% with most pronounced effect was obtained with (RS+CM+ *Tr.*) compost followed by the rice straw+ poultry manure (RS+PM) and the rice straw+ cow manure (RS+CM) composts. while the rice straw+ urea (RS+Urea) compost exhibited the lowest effect. Incidence of charcoal-rot was also suppressed with the soil amendments with the tested composts. The highest suppression (69.1%) was also obtained with RS+CM+*Tr.* followed by FYM. RS+PM. and RS+CM composts where suppressions of 66.5%. 59.4, and 57.8%, were recorded, respectively. The RS+Urea, however, exhibited the lowest suppressive effect of 28.2%. Concerning the total disease suppression, the RS+CM+*Tr* posed the highest disease suppression (61.6%) followed by RS+PM (51.3%), RS+CM (47.6%), and FYM (39.9%), respectively. The RS+Urea, however, showed the least disease suppression effect of 26.2% (Table 4).

Table 4. Effect of straw-based compost amendments on incidence of damping-off and charcoal-rot of sunflower in fields naturally infested with the causal fungi

Compost	Disease incidence (%)							
	Pre-emergence		Post-emergence		Charcoal-rot		Total	
	Value	Supr.(%)	Value	Supr.(%)	Value	Supr.(%)	Value	Supr.(%)
RS+Urea	19.3 *	17.5	13.2	33.3	22.3	28.2	54.8	26.2
RS+CM	16.1	31.1	9.7	51.0	13.1	57.8	38.9	47.6
RS+PM	13.9	40.5	8.3	58.0	12.6	59.4	34.8	51.3
RS+CM+*Tr.*	11.4	51.2	7.5	62.1	9.6	69.1	28.5	61.6
FYM	21.1	9.8	13.1	33.8	10.4	66.5	44.6	39.9
Unamended (control)	23.4	0.0	19.8	0.0	31.1	0.0	74.3	0.0
LSD 5%	4.3	-	3.1	-	2.2	-	9.2	-

* Values are averages of two growing seasons (2005 and 2006). statistically analyzed according to the combined analysis. Disease incidence was calculated as mean percentage of infection of total 200 plants /plot. four replicate plots for each treatment. Pre-emergence. post-emergence. and charcoal-rot were assessed 15. 45. and 90 days after sowing. % Supr. = percentage of disease suppression. RS = Rice straw. CM = Cow manure. PM = Poultry manure. *Tr.* = *Trichoderma harzianum*. FYM = Farmyard manure (noncomposted).

1.2. Effect on the fungal pathogens population:

Sampling of soil rhizosphere in the pre-emergence damping-off stage showed that rice straw-based compost amendments decreased populations of the associated fungal pathogens, *i.e. F. oxysporum, R. solani, S. rolfsii* and *M. phaseolina*. This effect was most pronounced with RS+CM+*Tr.* for all fungal pathogens followed by the RS+PM, the RS+CM, and the RS+Urea composts. However, the fresh FYM did not exhibit a significant effect in this stage. At the post emergence damping-off stage, a similar but more pronounced trend was revealed. The highest effect was also recorded with the RS+CM+*Tr.*, and RS+PM composts while rest of the composts were equally effective. At the charcoal-rot stage, assessed 75 days after sowing, also a similar trend was obtained with the different compost amendments but populations were the lowest compared to the other two stages (Table 5).

Table 5. Effect of straw-based compost amendments on damping-off and charcoal-rot fungal populations in sunflower rhizosphere, 15, 45, and 75 days after sowing

Monitored disease & Days after sowing	Compost	Fungus population (colonies x10^3 /g soil) *				
		F. oxysporum	*R. solani*	*S. rolfsii*	*M. phaseolina*	Total
Pre-emergency Damping-off (15 days)	RS+Urea	3.39	1.83	1.67	1.93	8.82
	RS+CM	2.61	2.02	1.56	1.77	7.96
	RS+PM	2.52	1.71	1.40	1.69	7.32
	RS+CM+*Tr.*	1.94	1.51	1.33	1.37	6.15
	FYM	3.93	1.85	1.96	2.19	9.93
	Control	4.16	2.11	1.97	2.21	10.45
Post- emergency Damping-off (45 days)	RS+Urea	2.93	1.09	0.93	1.36	6.31
	RS+CM	2.72	0.67	0.95	1.21	5.55
	RS+PM	2.19	0.48	0.60	1.23	4.50
	RS+CM+*Tr.*	1.37	0.37	0.56	1.13	3.43
	FYM	1.41	1.95	1.31	1.76	6.43
	Control	3.87	2.09	1.65	2.61	10.22
Charcoal-rot (75 days)	RS+Urea	1.91	0.95	0.53	0.43	3.82
	RS+CM	1.83	0.66	0.57	0.46	3.52
	RS+PM	1.70	0.52	0.48	0.37	3.07
	RS+CM+*Tr.*	1.72	0.32	0.35	0.34	2.73
	FYM	1.84	0.60	0.61	1.26	4.31
	Control	1.93	1.41	1.19	2.38	6.91
LSD at 5%		0.23	0.29	0.19	0.24	0.89

* Values are averages of two growing seasons (2005 and 2006). statistically analyzed according to the combined analysis. RS= Rice straw, CM= Cow manure, PM= Poultry manure, *Tr.*= *Trichoderma harzianum*, FYM= Farmyard manure (noncomposted).

2. Factors affecting compost disease-suppressive effect:

2.1. Microbial population:

Soil amendments with the tested composts significantly increased microbial population (total bacteria and actinomycetes) in sunflower rhizosphere in the three stages of assessment.

At the pre-emergence damping-off stage, microbial population increased to the range of 210.5%- 375.3% compared to the unamended control with highest increase was recorded with RS+CM compost followed by RS+PM and RS+CM+*Tr* in this stage.

The RS+Urea compost enhanced microbial population to 237.3% compared to the control while the fresh FYM compost amendment exhibited the lowest effect. However, at the post-emergence damping-off stage, microbial populations were 202.6%- 335.0% compared to the control with the tested compost amendments, and FYM exhibited an even higher increase of 315.7%. A similar trend was revealed 75 days after sowing. However, rate of increase was lower (134.7%-224.6%) with all composts except that the FYM where an increase of 346.3% was recorded which was higher than the other two assessments (Table 6).

Table 6. Effect of straw-based compost amendments on total microbial population (bacteria and actinomycetes) in rhizosphere of sunflower grown in field naturally infested with damping-off and charcoal-rot fungi, 15, 45, and 75 days after sowing

Compost	Microbial population (colonies x 10^6/g soil) *					
	Pre-emergence		Post-emergence		Charcoal-rot	
	value	% **	value	%	value	%
RS+Urea	33.7	237.3	25.6	224.5	9.3	134.7
RS+CM	53.3	375.3	38.2	335.0	15.5	224.6
RS+PM	41.9	295.0	33.3	292.1	11.4	165.2
RS+CM+*Tr.*	39.8	280.2	23.1	202.6	13.4	194.2
FYM	29.9	210.5	36.0	315.7	23.9	346.3
Control	14.2	100	11.4	100	6.9	100
LSD at 5%	5.3	-	6.1	-	3.4	-

* As described in footnote of Table (5).
** %= percentage relative to the unamended control.

2.2. Microbial activity:

Soil amendment with the tested rice-straw composts increased total microbial activity in sunflower rhizosphere in the three intervals of assessment compared to the unamended control. In the pre-emergence damping-off stage, assessed15 days after sowing, the RS+PM, RS+CM, and RS+CM+*Tr* amendments were the most pronounced treatments with the highest increase (543.2%) was for RS+PM compost. The noncomposted FYM also increased microbial activity but at less level (283.9%) while the RS+Urea did not exhibit a significant effect in this stage. In the post emergence damping-off stage, assessed 45 days after sowing, there was more pronounced increase (442.8%-657.1%) in microbial activity with the different compost amendments and the RS+CM+*Tr* compost showed the highest microbial activity (657.1%). In the charcoal-rot stage assessed 75 days after sowing, microbial activity was relatively lower (263.8%-472.2%) than of the other two stages. However, microbial activity was still significantly higher for all compost amendments with the most pronounced effect was for the RS+CM+*Tr* (Table 7).

2.3. Correlation between disease suppression with compost and microbial population & activity in plant rhizosphere:

A considerable positive correlation (r= 0.531- 0.749) was revealed between total microbial population (bacteria and actinomycetes) in sunflower plant rhizosphere, as influenced by compost amendments, and the disease suppression in the three stages tested.

In the pre-emergence damping-off stage and the post-emergence damping-off stage, high correlation coefficients of 0.749 and 0.736 were obtained, respectively, while a less correlation coefficient of 0.531 was recorded for the charcoal-rot stage (Fig. 1).

Table 7. Effect of straw-based compost amendments on total microbial activity in rhizosphere of sunflower grown in soil naturally infested with damping-off and charcoal-rot fungi

Compost	Microbial activity as FDA hydrolyzed (µg /min/g soil) *					
	Pre-emergence		Post-emergence		Charcoal-rot	
	value	% **	value	%	value	%
RS+Urea	2.1	259.2	3.1	442.8	1.9	263.8
RS+CM	3.7	456.7	3.9	557.1	3.1	430.5
RS+PM	4.4	543.2	4.3	614.2	2.7	375.0
RS+CM+*Tr.*	3.2	359.0	4.6	657.1	3.4	472.2
FYM	2.3	283.9	3.1	442.8	2.1	291.6
Control	0.81	100	0.70	100	0.72	100
LSD	1.4	-	1.3	-	1.1	-

* Values are averages of two growing seasons (2005 and 2006), statistically analyzed according to the combined analysis. FDA= fluorescein diacetate. Oven dried soil (105°C for 24 days).

** %= percentage relative to the control (unamended). RS= Rice straw. CM= Cow manure. PM= Poultry manure. *Tr.= Trichoderma harzianum*, FYM= Farmyard manure (noncomposted).

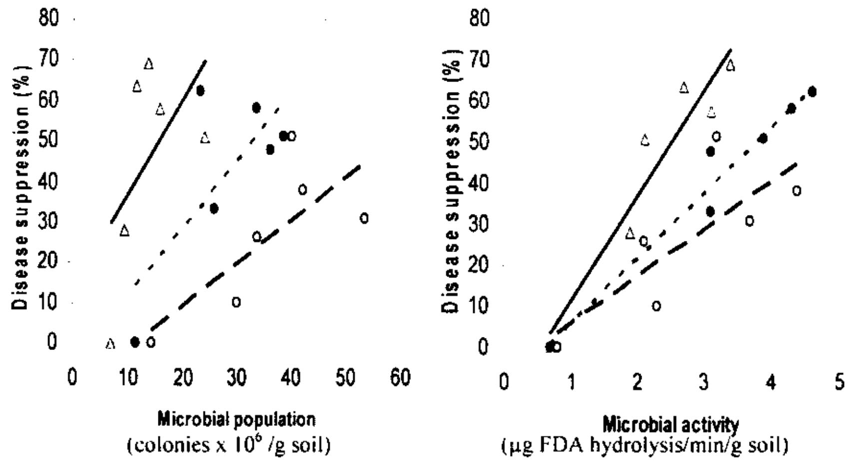

Fig.1. Correlation between disease suppression on sunflower and total microbial population (bacteria and actinomycetes) and activity in plant rhizosphere in fields naturally affected with damping-off and charcoal-rot diseases and amended with straw-based composts. (_ _ _) pre-emergence damping-off, (-----) post-emergence damping-off, and (———) charcoal-rot.

On the other hand, a stronger correlation (r= 0.783- 0.978) was found between total microbial activity in sunflower rhizosphere and rate of disease suppression by compost amendments. The highest correlation (r= 0.978), however, was recorded for the post-emergence damping-off, followed by r= 0.950 at the charcoal-rot stage, while the lowest correlation (r= 0.792) was linked to the pre-emergence damping-off stage (Fig. 1).

Discussion

Several fungi were found to be associated with damping-off and charcoal-rot of sunflower in Etay Elbaroud locality, Behera governorate. These were *Pythium* sp. *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Macrophomina phaseolina*. However, *Pythium* sp. was not able to incite any disease to sunflower in the pathogenicity tests while the other fungi incited pre- and post- emergence damping-off at different degrees. Meantime, charcoal-rot disease, as evident by the presence of black sclerotia in plant stem base, was only developed with *Macrophomina phaseolina*. These findings are in agreement with several reports in Egypt and other parts of the world (El-Zarka, 1976; Ibrahim, 2006 and Bokor, 2007).

All straw-based compost amendments tested showed a disease suppressive effect and produced less damping-off and charcoal-rot in field plots naturally infested with the causal fungi. The suppressive trend of composts was almost similar on pre- and post-emergence damping-off and the charcoal-rot with the highest total suppressive effect (61.6%) obtained with the rice straw composted with cow manure and inoculated with *Trichoderma harzianum* (RS+CM+*Tr*).

Amendment of compost with *Trichoderma harzianum* was found to accelerate rice-straw composting and produce a highly disease suppressive compost which is supported by Hointink and Boehm (1999)' view as *Trichoderma harzianum* fortified the compost suppressive effect in checking the soil-borne diseases. Effect of *T. harzianum* in this respect was widely documented (Aryantha *et al.*, 2000 and Scheuerells *et al.*, 2005) and was interpreted by the high ability of *T. harzianum* conidia to germinate, colonize plant rhizosphere, and to parasitize the pathogen hyphae. Also, enzymes and inhibitory substances might be involved (Inbar *et al.*, 1996). The other composts, *i.e.* RS+PM, RS+CM and FYM, were almost equally effective in checking the disease where total disease suppressions of 51.3%, 47.6%, and 39.9% were recorded with these composts, respectively. However, it seems that composts became more suppressive to sunflower diseases by increasing compost age as the soil amendment with the fresh noncomposted farmyard manure (FYM) did not exhibit a significant suppressive effect at 15 days after sowing while it was significantly disease suppressive at 45 and 75 days after sowing.

The RS+Urea compost, however, showed the lowest suppressive effect over the three intervals of assessment but still significantly effective in checking the total developed disease on sunflower. These findings are in agreement with several reports on the disease suppressive effect of such rice based composts (Osunlaja, 1990; Voland and Epstein, 1994; Aryantha *et al.*, 2000; Lodha *et al.*, 2002; Scheuerellss *et al.*, 2005; Yogev *et al.*, 2006 and Hameeda, 2007). Such disease suppressive effect was explained in view of effect of such organic mulching to improve vigour of plants, increase nutrient uptake, facilitate drainage, and to provide rich substrate to soil antagonistic microbes (Hoitink *et al.*, 1993; Aryantha *et al.*, 2000 and Mousa, *et al.*, 2006). It was also suggested (Workneh and van Bruggen, 1994; Aryantha *et al.*, 2000; El-Gizawy, 2005 and Scheuerellss *et al.*, 2005) that compost disease suppressiveness could be attributed to the direct effect of such organic materials to suppress (or to kill) the pathogen population, or to the indirect effect to enhance both the microbial population and activity in rhizosphere of the affected plants. In the present study, a significant reduction was recorded, with most composts, in pathogen populations of *F. oxysporum*, *R. solani*, *S. rolfsii*, and *M. phaseolina* assessed in plant rhizosphere. This was most pronounced with increasing compost age. Meantime, total antagonistic and saprophytic bacteria and actinomycetes were considerably increased (134.7%- 375.3%) in plant rhizosphere with compost amendments at the three stages assessed. Positive correlation coefficients of 0.749, 0.736, and 0.531 were revealed between microbial population and the suppression of pre-emergence damping-off, post-emergence damping-off, and charcoal-rot, respectively. However, it was recorded that microbial activity rather than microbial population was most associated with compost suppressiveness to fungal diseases (Scheuerellss *et al.*, 2005). In the present study a high increase (259.2% - 657.1%) in microbial activity in plant rhizosphere was obtained with compost amendments.

Also, the revealed correlation coefficients for microbial activity with the disease suppression were even higher than that of the microbial population being 0.783, 0.978, and 0.950 for pre-emergence damping-off, post-emergence damping-off, and charcoal-rot, respectively. This indicated that disease suppression in sunflower was more correlated with microbial activity rather than microbial population. Such biological effect could comprise competition for nutrients with pathogens, parasitism, predation, production of antibiotics, lytic or other extracellular enzymes, induction of host resistance in plants and other reactions which affect disease development. These findings are in harmony with Chen *et al.* (1988), Hoitink *et al.* (1993), Workneh and van Bruggen (1994), Hoitink and Boehm (1999), Boulter, *et al.* (2000), Lodha *et al.* (2002), El-Gizawy (2005), Scheuerellss *et al.* (2005), and Asari *et al.* (2007). Hence, the present study revealed that composting of the agricultural wastes could turn the problem into a solution. Farmers are strongly encouraged to adapt such environmentally and economically sound measures for management of crop diseases. Although the use of compost may not control diseases to a level which may replace the use of fungicides, their integration into current disease management practices will reduce the fungicide use and the associated problems.

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تأثير أنواع مختلفة من السماد العضوى المجهزمن المخلفات الزراعية على تثبيط أمراض موت البادرات والعفن الفحمي في عباد الشمس

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في دراسة بمنطقة إيتاى البارود بمحافظة البحيرة للموسم الزراعى ٢٠٠٥ - ٢٠٠٦ عزلت فطريات *Pythium* sp., *Rhizoctonia solani* , *Fusarium oxysporum*, *Macrophomina phaseolina*, *Sclerotium rolfsii* من النباتات المصابة بأمراض موت البادرات والعفن الفحمي لعباد الشمس حيث وجد ان الفطر *Pythium* sp. لم يكن قادرا على إحداث أى إصابة لنباتات عباد الشمس فى إختبارات القدرة المرضية بينما أحدثت الفطريات الأخرى درجات مختلفة من الذبول قبل وبعد ظهور البادرات ، وكانت أعراض العفن الفحمى على النباتات المعداه قاصرة فقط على فطر الماكروفومينا فاسيولينا .أجريت دراسة حقلية لمدة عامين فى الموسم الصيفى لعامى ٢٠٠٥ - ٢٠٠٦ فى حقل مصا ب طبيعيا بفطريات موت البادرات والعفن الفحمى بمنطقة إيتاى البارود وفيها أضيفت أنواع مختلفة من الكمبوست المجهز من قش الأرز مع كل من روث الماشية ، زرق الدواجن ، سماد اليوريا، ملقحا بفطر *Trichoderma* أو غير ملقح به، وكذا أستخدمت المخلفات الغير مكمورة لمزارع الماشيةللمقارنه، أدى إستخدام الكمبوست الى إنخفاض معنوى فى شدة مرض الذبول والعفن الفحمى لعباد الشمس و كان الكمبوست الملقح بفطر *Trichoderma* الأكثر تثبيطا لإجمالى المرض (٦١,٦ %) بينما أحدثت أنواع الكمبوست الأخرى المنتجة مع زرق الدواجن أو روث الماشية دون فطر *Trichoderma* تثبيطا بنسبة ٥١,٣% ، ٤٧,٦% على التوالى، وأحدثت المخلفات الغير مكمورة لمزارع الماشية تثبيطا بنسبة ٣٩,٩% وزاد التثبيط مع إزدياد عمرهذة المخلفات الغير مكمورة. و أظهر الكمبوست المنتج مع اليوريا أقل تثبيط (٢٦,٢%) للمرض المتكون على عباد الشمس. هذا وقد وجد أن التثبيط للمرض كان مصحوبا بإنخفاض أعداد المسببات المرضية بمنطقة المجال الجذرى لنباتات عباد الشمس نتيجة اضافات انواع الكمبوست، وكذلك كان التثبيط مصحوبا بزيادة حجم العشيرة الميكروبية من البكتيرات والاكتينو ميسيتات الموجوده بمنطقة المجال الجذرى بمعدل تراوح بين ١٣٤,٧% و ٣٧٥,٣%، كما وجد إرتباط موجب بين شدة التثبيط المرضى والزيادة فى أعداد البكتيرات والأكتينو ميسيتات المصاحبة بمنطقة المجال الجذرى وكان معامل الإرتباط ٠,٧٤٩ ، ٠,٧٣٦ ، ٠,٥٣١ للذبول قبل ظهور البادرات ، الذبول بعد ظهور البادرات والعفن الفحمى، على التوالى، هذا كما أدت أنواع الكمبوست المضافة الى زيادة فى النشاط الميكروبى الكلى بمنطقة المجال الجذرى بمعدل كبير (٢٥٩,٢% - ٦٥٧,١%) و كان معامل الإرتباط ٠,٧٨٣ ، ٠,٩٧٨ ، ٠,٩٥٠ للامراض الثلاثة على التوالى وهو أعلى منه مع العشيرة الميكروبية مما يشير الى أن النشاط الميكروبى الكلى ربما هو الأكثر تأثيرا فى التثبيط المرضى للأمراض المحمولة بالتربة لنباتات عباد الشمس عن حجم العشيرة الميكروبية ذاتها.