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Integrated Suppressive Effect(s) of Micronutrients and Compost against Chickpea Fusarium Wilt in Relation to Microbial Activity

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Two micronutrients, *i.e.* manganese and zinc sulphate(s), used as seed treatment along with compost used as soil amendment were evaluated for their control efficiency against Fusarium wilt disease of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f.sp. *ciceris*. *In vitro* evaluation revealed that out of 21 isolates of bacteria recovered from compost extract, five isolates, belonged to genus *Bacillus* reduced linear growth of *F. oxysporum* on PDA medium. Enrichment the medium with zinc or manganese sulphate(s) increased antagonistic activity of the tested bacteria. Application of micronutrients, alone or mixed with compost, as soil amendment, effectively reduced wilt disease incidence under greenhouse and field conditions in two growing seasons (2010/11 and 2011/12). Mixed applications recorded the highest disease decrements which dramatically associated with a rapid accumulation of salicylic acid content in roots compared to untreated-unamended control. Higher salicylic acid content caused lower disease incidence. Also, microbial activity in the rhizosphere of chickpea plants, as shown by greater dehydrogenase activity, markedly increased as a result of application of micronutrients, alone or mixed with compost. The statistical analysis revealed a negative correlation between disease incidence and microbial activity. Furthermore, application of micronutrients and compost increased crop parameters, nodulation of chickpea plants as well as seed protein content compared to untreated-unamended control.

Keywords: Manganese sulphate, microbial activity, rhizosphere, salicylic acid, seed protein, wilt and zinc sulphate.

Chickpea (*Cicer arietinum* L.) is an important food legume crop with high protein content. It is considered as a good nitrogen fixer. The major constraints to chickpea production are susceptibility to diseases (Pande *et al*., 2005). Fusarium wilt disease caused by *Fusarium oxysporum* Schlecht. Fr. f.sp. *ciceris* (Padwick) Matuo & K. Sato, is a widespread disease that occurs in most chickpea growing areas, causing considerable yield losses (Badawi *et al*., 2007 and Sarwar *et al*., 2010).

Biological and nutrient management of soil borne diseases are increasingly gaining stature as possible practical and safe approach to control fungal diseases (Adhilakshmi *et al*., 2008). The use of composts to suppress soil borne diseases, especially Fusarium wilt disease has been extensively reviewed (Escuadra and Amemiya, 2008; Yogev *et al.*, 2011 and Singh *et al*., 2012).

The suppressive effect of compost is due to a combination of biotic and abiotic factors (Kavroulakis *et al.,* 2010 and Pane *et al*., 2012). Suarez-Estrerella *et al*. (2012) isolated several microorganisms from composts which have been identified as biocontrol agents. Also, diversification of different organic materials in the compost increase microbial activity in the rhizosphere, thereby enhancing disease suppressiveness (Yogev *et al*., 2011 and Xiao *et al*., 2012).

Compost-amended soil increased phenolic content of the growing plants, suggesting that induced resistance could be an additional mechanism involved in soil borne disease suppression by compost (Wang and Millner, 2009 and Abdel-Fattah and Al-Amri, 2012).

On the other hand, micronutrients actively participate in the control of soil borne diseases (Ahmed, 2007; Das, 2007 and Adhilakshmi *et al*., 2008), through increasing plant's resistance and induction of defence related compounds (Hill *et al*., 1999 and Ahmed, 2007), in addition to the increase of population and activity of the potential microorganisms in the rhizosphere (Hameeda *et al*., 2006 and Das, 2007).

This investigation was designed to; a) *In vitro* evaluate the efficiency of some isolates of bacteria isolated from compost on mycelial growth of *F. oxysporum* f.sp. *ciceris*; b) Study the effect of micronutrients as seed treatment, alone or mixed with compost amended soil, against chickpea Fusarium wilt under greenhouse and field conditions; c) To monitor the involvement of salicylic acid content and d) Study the effect of micronutrients on microbial activity (as dehydrogenase), plant growth parameters and nodulation as well as seed protein content.

Materials and Methods

Compost:

Compost (rice straw and cow dung) obtained from Soil, Water and Environ. Res. Inst. (SWERI), ARC, was used in this investigation. The main properties of the compost are shown in Table (1).

Isolation of bacteria from compost extract:

Two grams of the tested compost was transferred to flask (100ml) containing 20ml sterilized distilled water, and shaked for 1h using an electric shaker, then the extract was serially diluted to (10^{-6}). Plates containing peptone dextrose agar medium were inoculated with 1ml of the diluted compost extract, then incubated at 28ºC for 5 days. Apparently different isolates of bacteria were selected, purified and examined for antagonistic effect against *Fusarium oxysporum* f.sp. *ciceris.*

Effect of different isolates of bacteria isolated from compost on mycelial growth of F. oxysporum f.sp. *ciceris:*

Plates containing potato dextrose agar (PDA) medium, PDA supplemented with manganese sulphate (2mM) or zinc sulphate (2mM), were inoculated at one side of the dish with a disc (5-mm-diam.) of *F. oxysporum* f.sp. *ciceris* taken from the periphery of 8-day-old culture. Each isolate of tested bacteria was streaked on the opposite side. Control plates of PDA medium only, or PDA supplemented with manganese sulphate ($MnSO_4$) or zinc sulphate ($ZnSO_4$), were inoculated with a disc of the pathogenic fungus only.

Table 1. Characteristics of compost used in this investigation

Character	Value
pH	8.12
E.C (ds/m)	1.57
Organic Matter (%)	33.79
C/N ratio	18.90
Total-N (%)	1.46
NH_4-N (ppm)	200.00
NO_3-N (ppm)	270.00
Total-P (%)	0.63
Total-K (%)	1.14

Three plates were used as replicates for each treatment. All plates were incubated at 25ºC. When the mycelial growth covered the whole surfaces in control of each treatment, the plates were then examined and linear growth of *F. oxysporum* f.sp. *ciceris* was determined. Isolates of bacteria which showed antifungal activity were identified according to (Vos *et al.,* 2009) in Microbial Dept. at SWERI, ARC.

Greenhouse experiment:

Seed treatment:

Surface sterilized chickpea seeds (cv. Giza 3) were soaked in 2mM solution of $MnSO_4$ or $ZnSO_4$ for 6 h then allowed to air dry.

Preparation of fungal inoculum:

Glass bottles (500ml) containing corn meal-sand medium (3:1 w/w) were autoclaved at 121ºC for 30 min, then inoculated with discs (5-mm-diam.) taken from 8-day-old culture of *F. oxysporum* f.sp. *ciceris* and incubated at 25ºC for 15 days.

Soil infestation was carried out by mixing fungal inoculum with sterilized potted-soil at the rate of 4% (w/w). The infested soil was watered for 5 days to enhance fungal growth and to ensure even distribution of the inoculum.

Chickpea seeds, either untreated or pre-treated with $ZnSO_4$ or $MnSO_4$, were individually sown in pots (30-cm-diam) containing, either infested soil + compost at the rate of 5% (w/w) or infested soil only. The treatments were prepared as follows:

1. Untreated seeds sown in pots containing infested soil (Control treatment).
2. Seed treated with $ZnSO_4$ and sown in pots containing infested soil.
3. Seed treated with $MnSO_4$ and sown in pots containing infested soil.
4. Untreated seeds sown in pots containing infested soil + compost 5% (w/w).
5. Seed treated with $ZnSO_4$ and sown in pots containing infested soil + compost 5% (w/w).
6. Seed treated with $MnSO_4$ and sown in pots containing infested soil + compost 5% (w/w).

Three pots were used as replicates for each treatment, and seven seeds were sown in each pot.

Disease assessment:

Growing chickpea plants were periodically examined and disease incidence was recorded 65 days after sowing, according to (Sarwar *et al.*, 2010).

Salicylic acid content:

Estimation of salicylic acid content was carried out in Micro Analytic Centre, Cairo Univ. Root samples of the growing chickpea plants of each of the aforementioned treatments were collected 10, 15 and 20 days after sowing.

One gram of each sample tissues was extracted and homogenized with acetonitrile : 0.1% phosphoric acid (15:85 v/v). The extract was filtered through Whatman (No.1) filter paper and micro filter (0.45µm), and then stored in vials. HPLC analysis was carried out to determine salicylic acid in the extracts. The analysis was performed on a model "HP1050" HPLC equipped with UV detector. Separations and determinations were performed on RP 18 (ODS) Column (4.6×250mm). The mobile phase was the same one which used in the extraction. UV detector was 254 nm and flow rate was 1.5ml/min according to (Gertz, 1990).

Field experiments:

These experiments were carried out at Etay El-Baroud Agric. Res. Station in Behera Governorate during two successive seasons of 2010/11 and 2011/12, in a field naturally infested with the wilt disease causal organism, divided into $2\times3m^2$ plots. Tested compost was applied, at the rate of 5 ton/fed, to the prepared field plots before sowing.

Chickpea seeds treated, or untreated, with $ZnSO_4$ or $MnSO_4$ (as mentioned before) were sown (2 seeds/hill), with approximately 20cm space distance between hills, in plots amended, or not, with compost. The experiment was arranged in split plot design. Three replicated plots were used for each treatment. Seeds of each treatment were treated before sowing with gamma irradiated vermiculite based inoculum (rhizobial inoculum) at the rate of 300g/40kg seed.

Growing plants were periodically examined and wilt disease incidence was recorded 65 days after sowing (Sarwar *et al.*, 2010). Number and dry weight of nodules per plant was recorded 55 days after sowing and other crop parameters were recorded at harvest time.

Microbial activity:

Microbial activity in the rhizosphere of chickpea plants (pre-treated with micronutrients and grown in soil amended, or not, with compost) grown under field condition was estimated as dehydrogenase activity.

Soil samples from the rhizosphere of each treatment were collected 45 and 75 days after sowing, air dried and mixed with $CaCO_3$ 0.1g/10g soil. Determination of dehydrogenase activity was carried out according to the method of (Dick and Tabatabai, 1993) using spectrophotometer (UV2600) at 485 nm. Triphenyel Tetrazolium Chloride (TTC) used as artificial electron acceptor to show the microbial activity in the rhizosphere through microbial respiration.

Seed protein content:

Determination of seed protein content was carried out in Central Lab. Soil, Water and Environ. Res. Inst., ARC, Giza. Chickpea seed samples of each treatment were collected at harvest time, and total protein content was determined according to the method described by (Chapman and Pratt, 1961).

Statistical analysis:

Analysis of variance (ANOVA) of the obtained data was carried out according to (Gomez and Gomez, 1984). Correlation and regression were performed with SPSS software package. Least significant difference (LSD) was used to compare means.

Results

Effect of some isolates of bacteria on mycelial growth of the pathogen:

Results in Table (2) show that out of 21 isolates of bacteria recovered from compost, only five isolates belonged to genus *Bacillus* significantly decreased mycelial growth of *Fusarium oxysporum* f.sp. *ciceris* grown on PDA medium. Supplying PDA medium with $ZnSO_4$ or $MnSO_4$ significantly increased the reduction in mycelial growth. Isolates number B-3 and B-4 recorded the highest mean decrease being 3.6 and 3.7, respectively.

Table 2. Effect of different isolates of bacteria isolated from compost on mycelial growth of *F. oxysporum* f. sp. *ciceris* grown on PDA medium supplemented with $ZnSO_4$ or $MnSO_4$

Bacterial isolate	Linear growth (cm)			
	PDA	(PDA+Zn)	(PDA+Mn)	Mean
B-1	5.6	3.8	4.7	4.7
B-2	6.1	5.4	5.5	5.7
B-3	4.0	3.1	3.6	3.6
B-4	4.4	3.7	3.8	3.7
B-5	5.1	4.6	4.4	4.7
Control	9.0	9.0	9.0	9.0
Mean	5.7	4.9	5.2	-
LSD at 0.05 for: Bacteria (B)= 0.39	Medium (M)= 0.27	(B)×(M)= N.S		

Effect of micronutrients and compost on disease incidence under greenhouse conditions:

Seed treatment with either $ZnSO_4$ or $MnSO_4$ significantly decreased wilt disease incidence compared to untreated control (Table 3). Seed treatment with $ZnSO_4$ was more effective than $MnSO_4$. The decrease in disease incidence was significant and much greater in the presence of compost. Compost mixed with micronutrients recorded a maximum decrease being 12.5% followed by treatments of $ZnSO_4$, compost and $MnSO_4$ being 16.67%, 20.83% and 29.17%, respectively.

Effect of micronutrients and compost on salicylic acid content in chickpea plants:

Salicylic acid content in chickpea plants increased as a result of application of compost and micronutrients upon infection with the pathogen, compared to untreated control. Maximum increase was recorded 10 days after sowing (Fig.1). Application of micronutrients alone was more effective than compost alone. However mixed application of micronutrients and compost (which caused the lowest disease incidence) recorded maximum increases in salicylic acid content 10 day after sowing. Nevertheless, salicylic acid content showed sharp decrease 15 and 20 days after sowing.

Table 3. Effect of seed treatment with micronutrients either alone or mixed with compost soil amendment on chickpea wilt disease incidence under greenhouse conditions

Treatment	Disease incidence (%)		
	(-) Compost	(+) Compost	Mean
$ZnSO_4$	16.67	12.50	14.58
$MnSO_4$	29.17	12.50	20.83
Control	58.33	20.83	39.58
Mean	34.72	15.28	
LSD at 0.05 for: Micronutrient (M)= 10.45	Compost (C)= 8.53	(M)×(C)= 14.78	

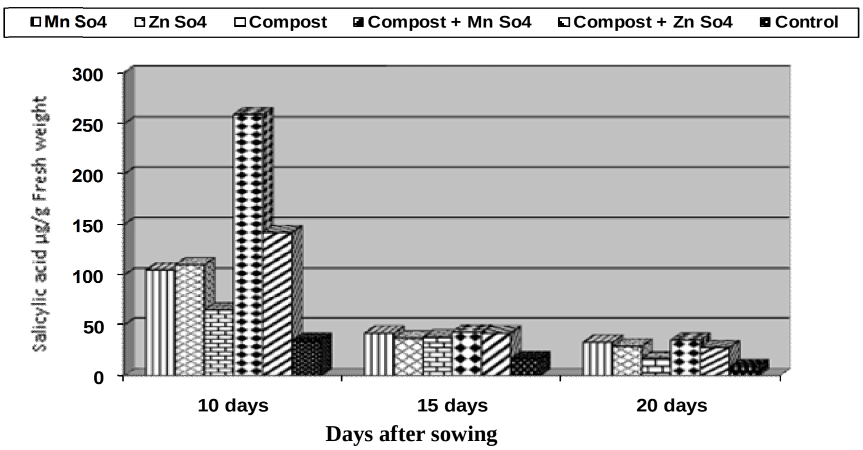


Fig. 1. Effect of seed treatment with micronutrient alone or mixed with compost soil amendment on salicylic acid content in chickpea plants, 10, 15 and 20 days after sowing.

Effect of micronutrients and compost on wilt disease incidence under field conditions:

Results in Table (4) show that in the two season's application of $MnSO_4$ or $ZnSO_4$ as seed treatment alone significantly decreased the wilt incidence compared to the untreated control. Addition of compost significantly decreased the wilt incidence. Mixed application of compost and micronutrients was more effective than compost alone. Compost mixed with $ZnSO_4$ recorded the lowest disease incidence being 1.67 and 2.77%, followed by compost mixed with $MnSO_4$ being 2.78 and 4.44%, in the two tested seasons, respectively.

Effect of micronutrients and compost on microbial activity (as shown by dehydrogenase):

Results in Table (5) show that single treatment of seed either with $ZnSO_4$ or $MnSO_4$ significantly increased dehydrogenase activity, in the two seasons treatments compared with untreated control.

Table 4. Effect of seed treatment with micronutrients either alone or mixed with compost soil amended on chickpea wilt disease under field conditions, in season 2010/11 and 2011/12 at Behera Governorate

Treatment	Disease incidence (%)					
	Season 2010/11			Season 2011/12		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
$ZnSO_4$	6.11	1.67	3.89	6.67	2.77	4.72
$MnSO_4$	5.56	2.78	4.17	7.22	4.44	5.83
Control	13.33	6.67	10.00	16.11	5.0	10.56
Mean	8.33	3.71		10.00	4.07	
L.S.D at 0.05 for:	Micronutrient (M)=		2.67			2.46
	Compost (C) =		2.18			2.01
	(M)×(C) =		N.S			3.49

Table 5. Effect of seed treatment with micronutrients either alone or mixed with compost soil amended on microbial activity (as dehydrogenase) in the rhizosphere of chickpea plants grown under field conditions in seasons 2010/11 and 2011/12 at Behera Governorate

Treatment	Dehydrogenase activity (µg TPF*/g soil/24h.)					
	Season 2010/11					
	45 days			75 days		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
$ZnSO_4$	109.8	178.6	144.2	125.0	210.7	167.8
$MnSO_4$	115.0	149.8	132.4	122.8	173.5	148.2
Control	97.3	133.0	115.2	112.3	154.3	133.3
Mean	107.4	153.8		120.0	179.5	
L.S.D at 0.05 for:	Micronutrient (M)=		21.05			22.09
	Compost (C) =		17.19			18.04
	(M)×(C) =		N.S			N.S
	Season 2011/12					
$ZnSO_4$	121.6	142.3	131.9	120.5	227.8	174.2
$MnSO_4$	101.9	131.2	116.6	141.4	181.9	161.7
Control	78.0	128.5	103.3	103.6	170.8	137.2
Mean	100.5	134.00		121.8	195.5	
L.S.D at 0.05 for:	Micronutrient (M)=		6.38			14.00
	Compost (C) =		5.21			11.43
	(M)×(C) =		9.02			19.80

*TPF= Triphenyl-Formazan.

Maximum increase was recorded after 75 days from sowing. However, the activity was much higher and significantly greater in the presence of compost. Mixed application of compost and micronutrient was more effective than compost alone. Compost combined with $ZnSO_4$ recorded the highest increase being 210.7 and 227.8 (µg TPF/g soil/24h) after 75 days from sowing in the two seasons, respectively.

Results in Table (6) and Fig. (2) show that the correlation between disease incidence and dehydrogenase activity in the seasons 2010/11 and 2011/12 was negatively and highly significant during examination periods, except 75 day in the first season 2010/11 the correlation was non-significant (r = -0.381).

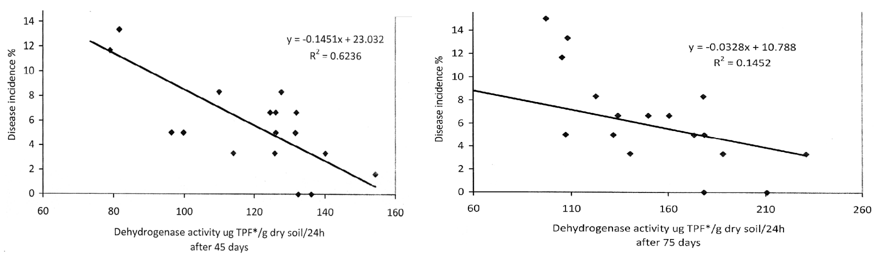
Table 6. Correlation between chickpea wilt incidence and microbial activity (based on dehydrogenase activity) in the rhizosphere of chickpea plants grown at Behera Governorate in seasons 2010/11 and 2011/12

	Dehydrogenase activity µg TPF*/g soil/24h. after	
	45 days	75 days
Wilt disease incidence	(r)[a]	(r)
Season 2010/11	-0.790**	-0.381
Season 2011/12	-0.654**	-0.691**

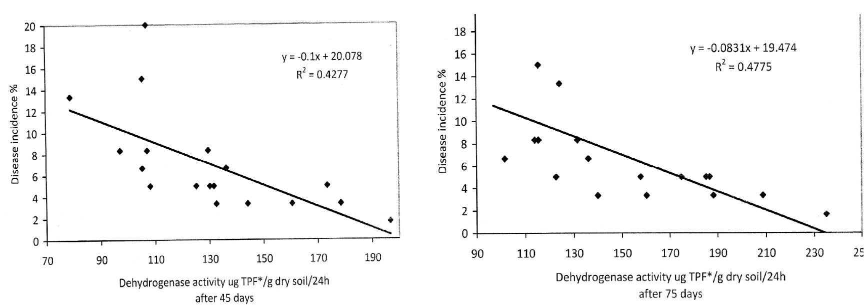
* TPF= Triphenyl-Formazan.

** Significant at p ≤ 0.01

[a] = Correlation coefficient.



Season 2010/11



Season 2011/12

Fig. 2. Regression equation that describes the relationship between dehydrogenase activity and chickpea wilt disease incidence 45 and 75 days after sowing in season 2010/11 and 2011/12

Effect of micronutrients and compost on nodulation status:

In the two seasons, single seed treatment, either with $ZnSO_4$ or $MnSO_4$, significantly increased number of nodules per plant compared to untreated control (Table 7). Seed treatment with $ZnSO_4$ was more effective than $MnSO_4$ treatment. The increase was much higher and significantly greater in the presence of compost. Moreover, mixed application of compost and micronutrients was more effective than compost alone. Mixed application of compost and $ZnSO_4$ recorded the maximum number of nodules being 49.67 in the first season. Whereas mixed application of compost and $MnSO_4$ recorded maximum number of nodules in the second season being 42.

Also, application of $MnSO_4$ or $ZnSO_4$ significantly increased dry weight of nodules in the presence or absence of compost in the two seasons (Table 7). The increase was more pronounced in the presence of compost. Mixed application of compost and micronutrients was more effective than compost alone. Compost mixed with $ZnSO_4$ recorded the highest dry weight being 593 mg in the first season. Whereas compost mixed with $MnSO_4$ recorded the highest dry weight being 453 mg in the second season.

Effect of micronutrients and compost on crop parameters:

Crop parameters of chickpea plants increased in both seasons as a result of application of compost and micronutrients (Tables 8a & 8b). Seeds treated with micronutrients increased the number of branches per plant in season 2010/11. This increase was significantly greater and much higher in the presence of compost. Mixed application of compost and $ZnSO_4$ was superior. Regardless of compost amendment in season 2011/12, seed treated with micronutrients significantly increased number of branches per plant compared to untreated control.

Number of pods/plant significantly increased when the seeds were treated with either $ZnSO_4$ or $MnSO_4$ in both seasons (Table 8a). Addition of compost significantly increased the number of pods/plant. Mixed application of compost and micronutrients was more effective than compost alone. Mixed application of $MnSO_4$ and compost recorded the highest number of pods being 114.00 and 103.00 pods/plant in the two seasons, respectively.

Application of micronutrients either alone or mixed with compost increased the weight of 100 seed in the two seasons (Table 8b). The increase was significant in season 2011/12. However, the increase was more pronounced in mixed application of compost and micronutrients than micronutrients alone. In season 2010/11, seed treated with micronutrients significantly increased seed yield per plant compared to untreated control. Whereas, in season 2011/12 addition of compost significantly increased seed yield per plants in mixed application treatment of compost and micronutrients compared to single ones. Mixed application of compost and $MnSO_4$ recorded the highest seed yield/plant being 22.4 and 18.9 g/plant in the two seasons, respectively.

Table 7. Effect of seed treatment with micronutrients either alone or mixed with compost soil amended on nodulation status of chickpea plants grown under field conditions in 2010/11 and 2011/12 seasons

Treatment	No. of nodules/plant			Dry weight of nodules/plants (mg)		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
Season 2010/11						
$ZnSO_4$	41.67	49.67	45.67	360.0	593.0	476.5
$MnSO_4$	32.00	43.33	37.67	203.0	566.0	384.5
Control	17.33	38.67	28.00	30.0	348.0	219.0
Mean	30.33	43.89		217.67	502.33	
L.S.D at 0.05 for: Micronutrient (M)=			5.00			63.89
Compost (C) =			4.09			52.17
(M)×(C) =			7.08			N.S
Season 2011/12						
$ZnSO_4$	36.00	37.67	36.84	328.0	372.0	350.0
$MnSO_4$	27.33	42.00	34.67	219.0	453.0	336.0
Control	14.67	31.67	23.17	76.0	270.0	173.0
Mean	26.00	37.11		207.66	365.0	
L.S.D at 0.05 for: Micronutrient (M)=			6.57			59.70
Compost (C) =			5.36			48.75
(M)×(C) =			7.08			84.43

Table 8a. Effect of seed treatment with micronutrients either alone or mixed with compost soil amendment on some crop parameters of chickpea plants grown under field conditions 2010/11 and 2011/12 growing seasons at Behera Governorate

Treatment	No. of branches/plant			No. of pods/plant		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
Season 2010/11						
$ZnSO_4$	6.7	8.0	7.3	89.3	107.0	98.2
$MnSO_4$	6.7	7.0	6.8	85.7	114.0	99.8
Control	6.3	7.7	7.0	75.7	82.3	79.0
Mean	6.6	7.6		83.6	101.1	
L.S.D at 0.05 for: Micronutrient (M)=			N.S			10.97
Compost (C) =			4.09			8.96
(M)×(C) =			7.08			N.S
Season 2011/12						
$ZnSO_4$	6.67	7.33	7.0	85.33	97.67	91.50
$MnSO_4$	7.67	7.67	7.67	85.00	103.00	94.00
Control	6.0	7.00	6.50	71.67	88.33	80.00
Mean	6.78	7.33		80.67	96.33	
L.S.D at 0.05 for: Micronutrient (M)=			0.79			7.08
Compost (C) =			N.S			5.78
(M)×(C) =			N.S			N.S

Table 8b. Effect of seed treatment with micronutrients either alone or mixed with compost soil amendment on some crop parameters of chickpea plants grown under field conditions 2010/11 and 2011/12 growing seasons at Behera Governorate

Treatment	Weight of 100 seed (g)			Seed yield/plant (g)		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
Season 2010/11						
$ZnSO_4$	21.5	22.8	22.2	18.7	19.7	19.2
$MnSO_4$	18.8	21.0	19.9	18.0	22.4	20.2
Control	15.7	19.2	17.4	14.4	16.5	15.5
Mean	18.7	21.0		17.0	19.5	
L.S.D at 0.05 for: Micronutrient (M)=			N.S			3.09
Compost (C) =			N.S			N.S
(M)×(C) =			N.S			N.S
Season 2011/12						
$ZnSO_4$	19.3	20.3	19.8	15.7	18.7	17.2
$MnSO_4$	19.2	21.1	20.1	14.8	18.9	16.9
Control	14.7	18.2	16.5	13.5	15.5	14.5
Mean	17.7	19.9		14.68	17.68	
L.S.D at 0.05 for: Micronutrient M)=			2.54			N.S
Compost (C) =			2.07			2.11
(M)×(C) =			N.S			N.S

Effect of micronutrients and compost on seed protein content:

Results in Table (9) show that in the two seasons, single application of $ZnSO_4$ or $MnSO_4$ significantly increased seed protein content compared to untreated control. However, $ZnSO_4$ was more effective than $MnSO_4$. Moreover, the increase was much higher in the presence of compost (except $ZnSO_4$ treatment in the first season). Mixed application of compost and $ZnSO_4$ or $MnSO_4$ was more effective than compost alone.

Table 9. Effect of seed treatment with micronutrients either alone or mixed with compost soil amendment on seed protein content of chickpea plants grown under field conditions in season 2010/11 and 2011/12

Treatment	Protein (%)					
	Season 2010/11			Season 2011/12		
	(-) Compost	(+) Compost	Mean	(-) Compost	(+) Compost	Mean
$ZnSO_4$	22.5	22.0	22.2	20.1	21.2	20.7
$MnSO_4$	20.9	22.2	21.6	20.0	22.3	21.2
Control	19.5	21.2	20.3	18.7	19.3	19.0
Mean	20.96	21.83		19.61	20.96	
L.S.D at 0.05 for: Micronutrient (M)=			0.78			1.60
Compost (C) =			0.64			1.31
(M)×(C) =			1.10			N.S

Results in Table (10) show a significant (P≤0.05) or highly significant (P≤0.01) negative correlation between chickpea wilt disease and all crop parameters in concern both seasons. Number of branches was a no Table exception because it was not correlated with wilt disease incidence in 2011/12 season.

Table 10. Correlation between chickpea wilt incidence and some crop parameters in seasons 2010/11 and 2011/12

Crop parameter	(2010/11) first season (r)[a]	(2011/12) second season (r)
Number of nodules/plant	-0.764**	-0.818**
Dry weight of nodules (mg)	-0.826**	-0.774**
Number of pods/plant	-0.658**	-0.787**
Weight of 100 seed	-0.594**	-0.621**
Number of branches/plant	-0.516*	-0.380
Weight of seed yield/plant	-0.748**	-0.629**
Seed protein content	-0.728**	-0.541**

[a]= Correlation coefficient
** = Significant at p≤0.01
* = significant at P≤0.05

Discussion

Among 21 isolates of bacteria, taken from compost extract, only five isolates, affiliated to the genus *Bacillus*, decreased the *in vitro* linear growth of *Fusarium oxysporum* f.sp. *ciceris* on PDA medium. This decrease may be due to the production of antifungal materials secreted by the tested bacteria (Fen *et al.*, 2004). Furthermore, supplying the medium with manganese or zinc sulphate increased the inhibitory effects of the tested bacteria. In this regard, Hameeda *et al.* (2006) tested different isolates of Bacillus, taken from composts, against *F. oxysporum*, *F. solani* and *Macrophomina phaseolina*. They found that PDA medium amended with zinc sulphate greatly improved antifungal activity of the tested bacteria. Obtained results are in harmony with those reported by Kavroulakis *et al.* (2010) and Pane *et al.* (2012) who found that some of isolated bacteria from composts showed antagonistic potential against *F. oxysporum* f.sp. *lycopersici*, *F. solani*, *Phytophthora cinnamomi*, *Rhizoctonia solani* and *Sclerotinia minor*.

Obtained results herein show that application of micronutrients as seed treatment and compost as soil amendment, alone or in combination, effectively provided a good protection against Fusarium wilt disease of chickpea under greenhouse and field conditions. Moreover, mixed application recorded maximum decrease in disease incidence.

The suppressive effect of compost is due to combination of biotic and abiotic factors. The biotic factor including the inhabiting microbes (bioagents) is might be partly responsible for the efficacy of compost in decreasing soil borne diseases (Naware, 2008). The abiotic factor is probably related to fungistatic compounds occurring in the compost (Carlile and Coules, 2009). Moreover, application of micronutrients led to increase the plant resistance to diseases through utilization of these elements in increasing synthesis of phenolic and ligneous compounds. These compounds may serve as chemical barriers in plants against pathogen invasion (Hill *et al.,* 1999). Also, zinc sulphate has been reported to increase the activity of bioagents and depresses the production of toxin by the pathogen (Duffy and Defego, 1997). This may explain the high decrease in disease incidence in mixed treatments.

The present results are similar to those reported by Dasgupta *et al.* (2000) who found that seeds treated with zinc or manganese sulphate before sowing in soil amended with compost effectively decreased collar rot disease of groundnut caused by *Aspergillus niger*. Recently, several investigators used many types of composts to control Fusarium wilt disease on different plant species (Omer and Mohamed, 2012; Singh *et al.,* 2012 and Xiao *et al.,* 2012). They indicated that the tested composts significantly reduced wilt disease incidence compared to untreated control. Moreover, Ahmed (2007) and Adhilakshmi *et al.* (2008) found that application of manganese or zinc sulphate effectively reduced Fusarium wilt disease on tomato and alfalfa plants, respectively.

Salicylic acid is one of numerous phenolic compounds found in plants, which have been reported as a constitutive defence compound (Klessing and Malamy, 1994). HPLC analysis revealed that time-course of salicylic acid showed marked increase in salicylic acid content in chickpea plants as a result of application of compost or micronutrients. However, mixed application resulted in marvellous increase in salicylic acid content 10 days after sowing. This increase reached up to 4 and 7 folds than untreated-unamended control, in combined treatment of zinc sulphate with compost and manganese sulphate with compost, respectively.

The increase in salicylic acid content may be due to the presence of microorganisms inhabiting compost, which have been previously reported to increase salicylic acid content in plants against pathogen invasion (Zhang *et al.,* 2002 and Singh *et al.,* 2003). Also, applications of micronutrients, especially zinc and manganese, increased synthesis of phenolic compounds (Hill *et al.,* 1999 and Ahmed, 2007).

Increased salicylic acid in plant may inhibit activity of catalase and ascorbic peroxidase, which then leads to increased levels of H_2O_2. The elevated H_2O_2 levels activate PR gene expression and increased the rate of polymerization of phenolic compounds into lignin like substances and making the plants more resistant to pathogen attack (Zhang *et al.,* 2002). So, the rapid accumulation of salicylic acid will restrict pathogen invasion. Obtained results were in agreement with those reported by Erhart *et al.* (1999) and Wang and Millner (2009). They indicated that application of composts to control soil borne diseases was associated with pronounced increase in flavonoids and different phenolic acids. Also, Kalim *et al.* (2003) and Ahmed (2007) found marked increase in total phenols and O-dihydroxy phenol contents in plants pre-treated with manganese or zinc sulphate and sown in soil infested with *Rhizoctonia solani, Sclerotium rolfesii* and *F. oxysporum* and correlated it with resistance to pathogens infection.

Microbial activity has been reported to be a key factor in suppression of Fusarium wilt disease (Serra-Wittling *et al.,* 1996). In the present investigation, obtained results revealed that seed treated with micronutrients before sowing increased microbial activity as expressed by greater dehydrogenase in the rhizosphere of chickpea roots compared to untreated control. However, combined application of micronutrients and compost resulted in additional increase in microbial activity.

The previous work of Lumsdan *et al.* (1986) attributed suppression of soil borne diseases to the enhanced soil microbial activity that resulting from compost amendment. Diversity in the organic materials in the compost promotes higher microbial activity and population in the soil, actively taking up nutrients creating a nutrients sink, which eventually increased disease suppressiveness (Escuadra and Amemiya, 2008). In addition, the presence of micronutrients in the rhizosphere increases the activity of microorganisms (Duffy and Defego, 1997 and Hameeda *et al.,* 2006). Recently, several researchers used many types of composts to control Fusarium wilt disease on different plant species (Yogev *et al.,* 2011; Salem *et al.*; 2012 and Xiao *et al.*, 2012). They found that disease suppressiveness was positively correlated with microbial activity.

Under field conditions, application of micronutrients and compost also increased crop parameters over the two tested seasons. Mixed application of micronutrients and compost recorded maximum increase in number and dry weight of nodules per plant compared with individual application. These increments might be due to the production of phytohormones by microorganisms inhabiting compost. These phytohormones are implicated in nodule formation (Hirsch *et al.,* 1997), as they stimulate root growth, providing further sites for infection and nodulation (Cameco *et al.,* 2001 and Zhang *et al.,* 2004).

Obtained results are confirmed by the observation recorded by Singh *et al.* (2008) and Singh *et al.* (2012) who found a great increase in number and dry weight of nodules of chickpea and cowpea plants as a result of application of compost to control some soil borne diseases. Meanwhile, Kalbhor *et al.* (1988) and Kostin *et al.* (2004) indicated that application of manganese or zinc sulphate increased number and dry weight of nodules of chickpea and pea plants.

Also, other crop parameters, *i.e.* number of branches, number of pods, weight of 100 seed as well as seed yield per plant, markedly increased as a result of application of compost and micronutrients in two successive seasons. These increments are not only attributed to the decrease in disease incidence but also due to the positive effects of micronutrients and compost. The increase in crop parameters of groundnut and chickpea plants as a result of application of compost was previously reported by many researchers (Rao and Shaktawar, 2002; Abdel-Wahab *et al.*, 2006 and Pandey *et al.*, 2011). They attributed such increases with the additions of organic manures, which supply the nutrients to the plants through mineralization and improvement in physico-chemical properties of the soil. Whereas, Kostin *et al.* (2004) and Bondruzzaman *et al.* (2005) reported that seed treatment with manganese or zinc sulphate pre-sowing, increased seed yield of rice and wheat relative to untreated control.

Chemical analysis of chickpea seed revealed that appreciable increase in seed protein content as a result of application of compost and micronutrients in the two tested seasons. Obtained results are in harmony with those reported earlier by Abdel-Wahab *et al.* (2006) and Singh *et al.* (2008) who indicated that soil amended with compost before sowing increased seed protein content of groundnut and chickpea plants. Moreover, Mohamed *et al.* (2003) and Abd-El-Rahman and El-Khatib (2010) observed an increase in seed protein content of soybean and guar as a result of application of micronutrients.

Strong negative correlation was found between wilt disease incidence and most crop parameters in the two tested seasons. These results indicated the positive effects of micronutrients and compost on chickpea productivity. Therefore, seed treatment with micronutrients along with soil amendment with compost can be recommended to control Fusarium wilt disease as they are safe and eco-friendly.

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التأثير المثبط المشترك للعناصر الصغرى والكمبوست ضد مرض ذبول الفيوزاريوم في الحمص وعلاقته بالنشاط الميكروبي

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إستخدمت كل من كبريتات الزنك وكبريتات المنجنيز كمعاملة للبذرة وكذلك الكمبوست كمعاملة للتربة لدراسة تأثيرهما ضد مرض الذبول الفيوزاريومي في الحمص المتسبب عن الفطر فيوزاريوم اوكسسبوريوم. تحت ظروف المعمل ومن بين ٢١ عزلة بكتريا معزولة من مستخلص الكمبوست وجدت ٥ عزلات بكتيرية تابعة للجنس (*Bacillus*) لها القدرة على تثبيط النمو الميسليومي للفطر، كما لوحظ أن إضافة كل من كبريتات الزنك أو المنجنيز إلي البيئة النامي عليها الفطر يزيد من كفاءة البكتريا في تثبيط النمو الفطري.

أدت معاملة البذور بكبريتات المنجنيز أو الزنك مفردة أو مصحوبة بمعاملة التربة بالكمبوست إلى خفض نسبة الإصابة بالمرض (مقارنة بالكنترول غير المعامل) سواء تحت ظروف الصوبة أو تحت ظروف الحقل خلال موسمي ٢٠١١/٢٠١٠ و ٢٠١٢/٢٠١١، وحققت المعاملات المشتركة للعناصر الصغرى والكمبوست أقصى انخفاض في نسبة الإصابة مقارنة بالمعاملة بالعناصر مفردة أو الكنترول.

وجد أن المعاملات السابقة أدت إلى تراكم سريع لحامض السليسلك في جذور نباتات الحمص مقارنة بالكنترول غير المعامل وحققت المعاملات المشتركة من العناصر الصغرى والكمبوست أقصى زيادة في حامض السليسليك.

كان هناك زيادة ملحوظة للنشاط الميكروبي (مقدراً كإنزيم الديهيدروجينيز) في منطقة الريزوسفير نتيجة استخدام العناصر الصغرى والكمبوست وحققت المعاملات المشتركة أعلى نشاط ميكروبي. أظهر التحليل الإحصائي وجود علاقة إرتباط سالبة بين نسبة حدوث الإصابة بالمرض والنشاط الميكروبي خلال الموسمين – كما أدت المعاملات السابقة إلى زيادة في القياسات المحصولية لنباتات الحمص، والعقد البكتيرية وكذلك محتوى البذور من البروتين مقارنة بالكنترول غير المعامل.