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Control of Maize Late Wilt and Enhancing Plant Growth Parameters using Rhizobacteria and Organic Compounds

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Rhizobacterial strains and organic compounds were tested for their capability to decrease maize late wilt disease caused by *Cephalosporium maydis* and promote plant growth under greenhouse and field conditions. *In vitro*, three *Bacillus subtilis* out of seven isolates and one *Pseudomonas fluorescens* out of eight isolates were found to show antifungal activity against the highly virulent *C. maydis* isolates. Also, application of organic compounds, *i.e*. compost-tea, olive mill wastewater (OMW) and humic acid at different concentrations caused significant reduction to the fungal growth rate. Meanwhile, humic acid was the most efficient one. Under greenhouse and/or field conditions, significant reduction in disease incidence was recorded when seed treated with rhizobacterial strains and/or organic compounds, either individually or in combination. Seed coating was the best method for treating seeds with rhizobacteria, while the best one for treating with organic compounds was seed soaking. However, the most significant reductions in disease incidence were recorded in case of treatments of seed coating with *B. subtilis*-1 and *P. fluorescens*, as well as seed soaking with compost-tea and the combination of each of the tested biological agents with compost-tea. Moreover, these treatments caused increments in seed germination percentages and significantly stimulated maize vegetative growth characters, *i.e*. plant height and dry weight, either in infested or uninfested soil with *C. maydis* under greenhouse conditions. Likewise, significant increments in grain yield (100 grain weight and grain yield/fed), as well as ear parameters (ear length, ear diameter, rows number/ear, kernels number/row and kernels number/ear) were recorded, when seeds were treated with rhizobacterial strains and/or organic compounds, either alone or in combinations, under field conditions.

Keywords: *Bacillus subtilis, Cephalosporium maydis,* compost-tea, humic acid, maize, olive mill wastewater and *Pseudomonas fluorescens.*

Maize (Z*ea mays* L.) is considered as one of the most important cereal crops in Egypt. The late wilt disease of maize, caused by *Cephalosporium maydis* Samra, Sabet and Hingorani, is the most economical disease of maize in Egypt (Ali, 2000). In naturally infested fields with *C. maydis*, infection reached up to 80% in susceptible cultivars, and the yield losses reached up to 40% (El-Shafey and Claflin, 1999).

Since *C. maydis* is a poor saprophytic competitor (Zeller *et al.,* 2000), various attempts of biological control by inoculating maize seeds with competitive or antagonistic organisms, *i.e. Trichurus spiralis*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Verticillium tricorpus*, have been evaluated (El-Assiuty *et al.,* 1991 and El-Mehalowy *et al.,* 2004). Sellam *et al*. (1978) reported that suspensions of *B. subtilis,* or its culture filtrate, reduced maize infection with *C. maydis* when added to the infested soil in pots, either at or after the time of sowing. Under field conditions, certain biocontrol formulations of *B. subtilis*, *P. fluorescens* and *Epicoccum nigrum* were reported as the most effective treatments against late wilt disease compared with bentocide, zinc oxide nanoparticles and nanosilica (Hamza *et al.,* 2013).

Gholami *et al.* (2009) observed that seed inoculation with *P. fluorescens, P. putida,* and *B. subtilis* significantly enhanced seed germination and seedling vigour of maize. Moreover, maize seed priming with *Azospirillum lipoferum, B. subtilis* and *P. fluorescens* significantly increased plant height, number of kernels/ear, grains rows number, grains number/row, 100 seed weight and grain yield (Noumavo *et al.*, 2013).

Compost-tea has been used for control of a variety of plant pathogens and as a source of soluble nutrients, which can be used as a liquid fertilizer (Al-Mughrabi, 2006). Furthermore, compost application significantly increased the vegetative and yield parameters of maize (plant height, dry matter yield, leaf area and grain yield), and performed better than inorganic fertilizer (Osoro *et al.,* 2014). Also, humic acid has been early recorded to have appositive effect against plant pathogens and consider suitable candidates for use as liquid biofertilizers (Ayuso *et al*., 1996). Moreover, humic substances were found to reduce the radial growth of *Fusarium culmorum, F. oxysporum* f.sp. *lycopersici*, *Pythium ultimum* and *Alternaria alternata* on PDA medium (Loffredo *et al.,* 2007). Additionally, significant increases in maize vegetative growth characters; ear characters and grain yield were recorded when soil application and seed soaking with humic acid was evaluated by several investigators (Bakry *et al.,* 2009 and Daur and Bakhashwain, 2013). Furthermore, several studies have shown that olive mill wastewater (OMW) can be used as fertilizer, weed suppressor and show antifungal and antibacterial properties due to the high content of phenolics and other compounds (Boz *et al*., 2003, and Vagelas *et al.,* 2009). Moreover, germination index and plant growth characters of maize were efficiently increased by seed and/or soil treatments with olive mill wastewater (Hanifi and El-Hadrami, 2007 and Mekki *et al.,* 2013).

This study was designed to evaluate the efficiency of some rhizobacterial strains and/or organic compounds against late wilt disease caused by *C. maydis* under laboratory, greenhouse and field conditions. Also, the effect of the tested control agents on some crop parameters of maize was taken in consideration.

Materials and Methods

1. Source of tested Cephalosporium maydis and biocontrol agents:

Three highly virulent *C. maydis* isolates, previously isolated from infected maize plants during 2011 growing season, were used in the present study. However, Different rhizobacterial strains (*Pseudomonas fluorescens* and *Bacillus subtilis*), isolated previously from the rhizosphere of maize plant fields at Gharbiya Governorate were used. Isolation of these strains was done according to the method described by Meera and Balabaskar (2012). To identify the bacterial isolates, certain biochemical tests were conducted according to Breed *et al*. (1957). Moreover, three organic compounds, *i.e.* compost–tea, humic acid and olive mill wastewater, obtained from Dept. of Microbiol., Soil, Water and Environ. Res. Inst., as well as from Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza, were tested in this study.

2. In vitro experiments:

2.1. The antagonism of rhizobacteria:

The antifungal activity of rhizobacterial isolates was evaluated against three highly virulent *C. maydis* isolates on PDA + 0.2% yeast extract (PDAY) medium by using the dual culture technique described by Dennis and Webster (1971). Bacterial isolates were streaked at one side of Petri dishes 9-cm-diam. containing 15-20 ml of PDAY medium. Discs (6-mm-diam.), taken from 7-day-old *C. maydis* culture were placed at the opposite side of Petri dishes perpendicular to the bacterial streak and incubated at 27±2 ºC for 7 days. Five replicate plates were prepared for each isolate. Petri dishes inoculated with fungal discs alone served as control. The inhibition zone was recorded as the distance between the fungal colony margin and the area of antagonist growth after 7 days. Inhibition zones or cessation of the fungal growth were used as criteria for the positive effect as follows: (-): no inhibition zone, (+) = ≤ 5 mm inhibition zone; (++) = 5 to 10 mm inhibition zone; (+++) = ≥ 10mm inhibition zone and (++++): no fungal growth and bacteria fill the plate.

2.2. Effect of organic compounds on radial growth of C. maydis:

The inhibitory effect of compost-tea, humic acid and olive mill wastewater was evaluated against the linear growth of highly virulent *C. maydis* isolate using the culture technique described by Abdel-Kader *et al*. (2012) and modified as follows: substances were micro-filtered (0.2 µm) and separately incorporated into sterilized PDAY medium before solidification to obtain the proposed concentrations of 10, 25, 40 and 60% (v:v) and mixed gently then poured into 9-cm-diam. sterilized Petri dishes and left to solidify. Discs (6-mm-diam.), taken from 7-day-old *C. maydis* culture, were placed in the centre of each plate. Five plates were used as replicates for each concentration of each substance. Dishes were incubated at 27± 2ºC. The average of linear growth diameter of colonies was measured when the fungal growth covered the plate in the control treatment and growth inhibition percentage was determined according to the following equation:

$$\text{Reduction (\%)} = [(C - T) / C] \times 100$$

Whereas: C= Radial growth in control and T= Radial growth in treatment.

3. Greenhouse experiments:

These experiments were carried out in potted soils under greenhouse conditions at the Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza, following the soil infestation technique as described by Samra *et al*. (1966), during summer of 2012 and 2013 growing seasons. Autoclaved clay loam soil was used.

Maize seeds (cv. Boushy), susceptible to *C. maydis*, obtained from Maize and Sugar Crops Dis. Dept., Plant Pathol. Res. Inst., ARC, Giza, was used in this study.

3.1. Fungal inoculum preparation:

Fungal inoculum was prepared by growing the highly virulent *C. maydis* isolate on autoclaved sorghum grains in 500 ml glass bottles and incubation at 27 ± 2ºC for 2 weeks until sufficient growth of the fungus was obtained (El-Shafey *et al.*, 1979). Contents of the bottles were poured out and mixed to get homogenized inoculum, and then inoculum was used for soil infestation at the rate of 30 g /kg soil.

3.2. Rhizobacterial inoculum preparation:

Two loopfulls of *B. subtilis* and/or *P. fluorescens* isolates from 3-day-old cultures grown on nutrient agar and/or King's B media, respectively, were transferred aseptically into conical flasks (250 ml) containing 100 ml of broth medium and incubated at 27± 2ºC for 48h on a rotary shaker (150 rev min^{-1}). Bacterial cells were removed by centrifugation at 10000 rpm for 10 min. at 4ºC and washed in sterile distilled water. The pellet was re-suspended in small quantity of sterile distilled water. Serial decimal dilutions were done to obtain 10^8 cfu/ml for each isolate homogenate (Cavaglieri *et al.*, 2005).

3.3. Seed and soil treatment methods:

3.3.1. Seed soaking technique:

Maize seeds were soaked in the prepared bacterial suspension and organic compounds at the rate of 100ml/100seed in 250 ml Erlenmeyer flasks. Control seeds were soaked in sterile distilled water only. Flasks were incubated at 25ºC on a rotator shaker at 70 rpm for 6 h. After incubation, excess inoculum was removed and seeds were left to air dry for 30 min. at room temperature and then immediately planted in the infested-potted soil (Cavaglieri *et al.,* 2005).

3.3.2. Seed coating technique:

Seed coating was done using the method described by Bardin *et al.* (2004). Seeds were soaked for 15min. in 1% methyl cellulose (MC) solution at the rate of 3ml per 100 seeds. Thereafter, seeds were removed and placed in plastic bags then coated with bacterial suspension and organic compounds at the rate of 5 ml per 100 seeds. Bags were inflated with air and shaken vigorously. After that, seeds were directly planted in the potted soil. Seed coated with sterile distilled water acted as control.

3.3.3. Soil drench technique:

Soil drenching was carried out according to Jenana *et al.* (2009) and modified as follows: Potted soil was thoroughly drenched at sowing time with 100 ml of prepared solution from bacterial suspension and/or organic compounds. Soil drenching was weekly repeated for two times starting from the planting time. Control soil was drenched by sterile distilled water only.

3.4. Experimental design:

Three isolates of *B. subtilis* and one of *P. fluorescens* as well as three organic compounds, *i.e.* compost-tea, humic acid and OMW, were applied by three methods, seed soaking, seed coating and soil drench, during 2012 growing season. Meanwhile, combined treatments among rhizobacterial strains and organic compounds were investigated during 2013 growing season. The combination between seed coating with two isolates of *B. subtilis* or one of *P. fluorescens* and seed soaking in compost-tea or humic acid were studied.

Generally, 8 treated seeds were sown in each pot (25-cm-diam.). Uninfested-potted soil sown with treated seeds was served as check treatment. Untreated seeds were used as control in both infested and/or uninfested soil. Three pots were used as replicates for each treatment. Pots were labelled and distributed in greenhouse under ambient conditions of growing.

Disease incidence and vegetative growth were recorded 95 days after sowing. Meanwhile, Seed germination percentage was recorded 10 days after sowing.

4. Field experiment:

Combined treatments among rhizobacterial strains and organic compounds, as mentioned in greenhouse experiment, were studied under field conditions in disease nursery at Gemmiza Res. Station, Plant Pathol. Res. Inst., ARC, Gharbiya, Egypt, during summer of 2013 growing season. Untreated seeds were used as check treatment. Three replicate plots, each of 20 plants were used for each treatment.

Disease incidence as infection percentage was recorded 95 days after sowing. Maize grain yield parameters were evaluated during harvest period.

5. Statistical analyses:

Most of data were subjected to statistical analysis, whenever needed, according to the method described by Steel and Torrie (1960), whereas the differences between treatments were tested by calculating L.S.D. at 5% level.

Results

1. Laboratory studies:

1.1. Antagonistic effect of rhizobacterial strains on the radial growth of Cephalosporium maydis:

Data presented in Table (1) show that 3 out of the 7 isolates of *Bacillus subtilis* and one isolate of *Pseudomonas fluorescens* showed significant antifungal activity against the highly virulent *C. maydis* isolates. It was observed that *B. subtilis*-1 filled the plate during 48h of incubation and recorded as (++++), meanwhile, *B. subtilis*-4 and *B. subtilis*-5 as well as *P. fluorescens*-3 recorded as (+++). On the other hand, some isolates were unable to challenge the fungal growth causing no inhibition zone.

1.2. Effect of organic compounds on the radial growth of C. maydis:

Data presented in Table (2) show that all concentrations of the tested compounds resulted in a significant reduction in *C. maydis* radial growth, compared with the check treatment. In general, growth inhibition (%) was increased with the increasing of the concentrations of all tested substances. The 70% efficiency in inhibiting the fungal growth was considered as a reasonable level to consider the material as a potent inhibitor. However, the highest effect was recorded at 60% concentration of any tested material. On the average, humic acid was obviously the most effective to reduce the radial growth of the tested fungus (being 62.2% reduction). Meanwhile, OMW recorded the lowest (40.0%) reduction.

Table 1. *In vitro* antagonistic effect of *B. subtilis* and *P. fluorescens* against *C. maydis* on PDAY medium

Isolate of bacteria	*C. maydis* I*	*C. maydis* II	*C. maydis* III
B. subtilis-1	++++**	++++	++++
B. subtilis-2	-	-	-
B. subtilis-3	-	-	-
B. subtilis-4	+++	+++	+++
B. subtilis-5	+++	+++	+++
B. subtilis-6	-	-	+
B. subtilis-7	-	-	-
P. fluorescens-1	+	-	-
P. fluorescens-2	-	-	-
P. fluorescens-3	+++	+++	+++
P. fluorescens-4	-	-	-
P. fluorescens-5	-	+	-
P. fluorescens-6	-	-	-
P. fluorescens-7	+	-	-
P. fluorescens-8	-	-	+
Control	-	-	-

* *C. maydis* I, II and III, were the highly virulent isolates.
** (-)= No inhibition zone, (+)= ≤ 5 mm inhibition zone; (++)= 5 to 10 mm inhibition zone and (+++)= ≥ 10 mm inhibition zone.

Table 2. *In vitro* effect of compost-tea, humic acid and olive mill wastewater on radial growth of *C. maydis* on PDAY medium

<table>
<tr><th>Treatment</th><th>Concentration (%)</th><th>Radial growth (cm)</th><th>Reduction (%)</th></tr>
<tr><td rowspan="4">Compost-tea</td><td>10</td><td>7.9 b</td><td>12.2</td></tr>
<tr><td>25</td><td>6.9 c</td><td>23.3</td></tr>
<tr><td>40</td><td>4.3 e</td><td>52.2</td></tr>
<tr><td>60</td><td>1.8 h</td><td>80.0</td></tr>
<tr><td colspan="2">Mean</td><td>5.2</td><td>42.2</td></tr>
<tr><td rowspan="4">Humic acid</td><td>10</td><td>6.3 d</td><td>30.0</td></tr>
<tr><td>25</td><td>4.1 f</td><td>54.4</td></tr>
<tr><td>40</td><td>2.7 g</td><td>70.0</td></tr>
<tr><td>60</td><td>0.4 i</td><td>95.6</td></tr>
<tr><td colspan="2">Mean</td><td>3.4</td><td>62.2</td></tr>
<tr><td rowspan="4">Olive mill wastewater</td><td>10</td><td>8.0 b</td><td>11.1</td></tr>
<tr><td>25</td><td>7.0 c</td><td>22.2</td></tr>
<tr><td>40</td><td>4.5 e</td><td>50.0</td></tr>
<tr><td>60</td><td>1.9 h</td><td>78.9</td></tr>
<tr><td colspan="2">Mean</td><td>5.4</td><td>40.0</td></tr>
<tr><td colspan="2">Control</td><td>9.0 a</td><td>----</td></tr>
</table>

2. Greenhouse studies:

2.1. Effect of treatments with some rhizobacterial strains or organic compounds on maize late wilt and plant growth parameters under greenhouse conditions:

2.1.1. Effect on disease incidence:

Data presented in Table (3) show that seed treated with either rhizobacterial strains or organic compounds significantly reduced the infection percentage with late wilt compared to check treatment (88.9% infection) under artificial infestation. No infection percentage was recorded in case of uninfested soil in all treatments. Among of all rhizobacterial strains, seed treatment with *B. subtilis* (No. 1) and *P. fluorescens* were the best treatments in reducing infection percentage, being 64.6 and 62.5% reduction in disease incidence, respectively. On the other hand, treatment with compost-tea gave the highest effect in reducing infection percentages compared with other organic compounds, being 43.8% reduction than 33.4 and 20.9% reduction in case of humic acid and olive mill wastewater treatments, respectively.

Regarding to treatment methods, it was observed that the best method for treating seeds with rhizobacteria was seed coating. Meanwhile, seed soaking was the best one for treating with organic compounds. Soil drench was the lowest effective method for treatment with rhizobacterial strains and/or organic compounds to control late wilt disease (Table 3).

Generally, data in presented in Table (3) reveal that seeds coated with any of *B. subtilis*-1 and *P. fluorescens* caused the highest reduction in disease incidence, which recorded 75.0% reduction. Meanwhile, soil drenched with the same isolates gave 50.1 and 43.8% reduction, respectively. On the other hand, seeds soaked in compost-tea led to 56.2% reduction in infection percentage followed by 43.8% and 31.3% reduction when seeds were soaked in any of humic acid and olive mill wastewater, respectively. Conversely, soil drenched with these compounds resulted in 25.0, 18.8 and 12.5% reduction, respectively.

2.1.2. Effect on maize plant growth characters:

Data presented in Table (4) show that seed treatments with rhizobacterial strains and/or organic compounds significantly promoted plant growth compared to check treatment either in infested or uninfested soil. No significant differences among treatments and/or treatment methods in their effect on seed germination percentages, whether in infested or uninfested soil. Generally, the percentage of seed germination was higher in uninfested soil compared with that in infested ones.

Regarding to plant height, it was observed that all treatments significantly increased plant height in comparable to check plants either in infested or uninfested soil, being 66.6 and 76.5 cm, respectively. Whether in infested or uninfested soil, the highest plant height was recorded when the seeds were coated with any of *P. fluorescens* and *B. subtilis*-1 as well as seeds soaked in compost-tea.

On the other hand, data in Table (4) reveal that significant increment in dry weight of treated plants was recorded compared with check plants, either in infested or uninfested soil, being 14.1 and 22.9 g, respectively. Generally, plant dry weight was higher in uninfested soil than that in infested soil. Among rhizobacterial strains, seeds coated with any of *P. fluorescens* and *B. subtilis* (No. 3) were the best

Table 3. Effect of some rhizobacterial strains and organic compounds on maize late wilt under greenhouse conditions during 2012 growing season

Treatment	Infection (%)*				Reduction (%)			
	Seed soaking	Seed coating	Soil drench	Mean	Seed soaking	Seed coating	Soil drench	Mean
B. subtilis-1	27.8	22.2	44.4	31.5	68.7	75.0	50.1	64.6
B. subtilis-2	38.9	27.8	55.6	40.7	56.2	68.7	37.5	54.2
B. subtilis-3	33.3	27.8	50.0	37.0	62.5	68.7	43.8	58.3
P. fluorescens	27.8	22.2	50.0	33.3	68.7	75.0	43.8	62.5
Compost-t	38.9	44.4	66.7	50.0	56.2	50.1	25.0	43.8
Humic acid	50.0	55.6	72.2	59.3	43.8	37.5	18.8	33.4
OMW	61.1	72.2	77.8	70.4	31.3	18.8	12.5	20.9
Mean	39.7	38.9	59.5	46.0	55.3	56.3	33.1	48.2
Control	88.9	88.9	88.9	88.9				
LSD** $_{(0.05)}$ for: Treatment (T) 0.2								
Treatment method (M) 0.1								
T × M 0.2								

* Infection percentage of both treated and non-treated plants in non-infested soil was 0.0%.

** Percentage data were transformed in to arc sine angles before carrying out the analysis of variance.

treatments in increasing plant dry weight, whether in infested (35.4 and 35.1 g, respectively) or uninfested soil (38.7 and 37.6 g, respectively). Meanwhile, seeds soaked in compost-tea gave the highest dry weight increment in both infested and uninfested soil (31.4 and 36.2 g, respectively) compared with other compounds.

2.2. Effect of combined treatments with some rhizobacterial strains and organic compounds on maize late wilt and plant growth under greenhouse conditions:

2.2.1. Effect on disease incidence:

Data presented in Table (5) indicate that, under artificial infestation, seed treatment with rhizobacteria and/or organic compounds, either alone or in combination, significantly reduced maize infection by late wilt compared with check plants under greenhouse conditions. Meanwhile, no infection was recorded in uninfested soil. Moreover, it was observed that combination of rhizobacterial strains with some organic compounds caused extremely reduction in maize late wilt compared with individual treatments of rhizobacteria and/or organic compounds. However, the combined treatment of compost-tea with *P. fluorescens* and/or *B. subtilis*-1 gave the highest reduction in late wilt (78.5%) in comparable to treatment with *P. fluorescens*, *B. subtilis*-1 or compost-tea alone, being 71.5, 64.3 and 50.0% reduction, respectively.

No significant variations among treatments were recorded when these treatments were applied under greenhouse conditions.

Table 4. Effect of some rhizobacterial strains and organic compounds on maize plant growth characters under greenhouse conditions in infested and uninfested soil during 2012 growing season

Soil	Treatment	Germination (%)				Plant height (cm)				Dry weight (g)			
		S.S[a].	S.C.	S.D.	Mean	S.S.	S.C.	S.D.	Mean	S.S.	S.C.	S.D.	Mean
Infested soil [b]	*B. subtilis*-1	83.3	87.5	83.3	84.7	82.5	89.9	78.6	83.7	31.3	35.0	20.8	29.0
	B. subtilis-2	91.7	91.7	87.5	90.3	83.1	88.2	72.9	81.4	27.7	29.2	22.0	26.3
	B. subtilis-3	87.5	95.8	87.5	90.3	83.8	87.0	74.3	81.7	34.0	35.1	21.7	30.3
	P. fluorescens	91.7	95.8	87.5	91.7	85.5	93.6	73.8	84.3	30.9	35.4	21.4	29.2
	Compost-t	87.5	87.5	87.5	87.5	87.1	75.2	71.7	78.0	31.4	25.3	16.1	24.3
	Humic acid	79.2	79.2	79.2	79.2	78.9	72.5	69.4	73.6	25.9	21.6	15.4	21.0
	OMW	79.2	83.3	79.2	80.6	69.5	67.9	66.7	68.0	20.8	14.6	14.1	16.5
	Mean	85.7	88.7	84.5	86.3	81.5	82.0	72.5	78.7	28.9	28.0	18.8	25.2
	Control	79.2	79.2	79.2	79.2	66.6	66.6	66.6	66.6	14.1	14.1	14.1	14.1
	LSD[c] $_{(0.05)}$ for:												
	Treatment (T)	n.s.[d]				1.3				0.8			
	Treatment method (M)					0.7				0.6			
	T × M					1.9				1.6			
Uninfested soil	*B. subtilis*-1	87.5	91.7	87.5	88.9	87.6	95.1	77.4	86.7	33.6	36.9	22.9	31.1
	B. subtilis-2	95.8	91.7	91.7	93.1	86.4	90.5	77.8	84.9	33.4	36.7	23.0	31.1
	B. subtilis-3	91.7	91.7	87.5	90.3	87.8	91.2	77.4	85.5	35.1	37.6	23.9	32.2
	P. fluorescens	95.8	95.8	91.7	94.4	90.3	99.2	77.6	89.1	36.6	38.7	24.7	33.3
	Compost-t	91.7	87.5	87.5	88.9	92.9	79.5	79.2	83.9	36.2	28.9	23.8	29.7
	Humic acid	87.5	87.5	91.7	88.9	81.1	77.0	76.0	78.0	30.3	28.8	22.9	27.3
	OMW	91.7	87.5	87.5	88.9	78.0	76.8	76.7	77.2	27.0	22.9	23.1	24.3
	Mean	91.7	90.5	89.3	90.5	86.3	87.0	77.4	83.6	33.2	32.9	23.5	29.9
	Control	87.5	87.5	87.5	87.5	76.5	76.5	76.5	76.5	22.9	22.9	22.9	22.9
	LSD[**] $_{(0.05)}$ for:												
	Treatment (T)	n.s.				0.7				1.0			
	Treatment method (M)					0.6				0.5			
	T × M					1.6				1.4			

a: S.S: Seed soaking, S.C: Seed coating, S.D: Soil drenching.

b: Soil infested with *C. maydis.*

c: Percentage data were transformed into arc sine angles before carrying out the analysis of variance.

d: n.s.: Insignificant differences among germination percentages.

Table 5. Effect of combined treatment with some rhizobacterial strains and organic compounds on maize late wilt under greenhouse conditions during 2013 growing season

Treatment	Infection (%)	Reduction (%)
B. subtilis-1	27.8	64.3
B. subtilis-3	33.3	57.2
P. fluorescens	22.2	71.5
Compost-t	38.9	50.0
Humic acid	44.4	42.9
B. subtilis-1 + Compost-t	16.7	78.5
B. subtilis-1 + Humic acid	22.2	71.5
B. subtilis-3 + Compost-t	22.2	71.5
B. subtilis-3+ Humic acid	27.8	64.3
P. fluorescens + Compost-t	16.7	78.5
P. fluorescens + Humic acid	22.2	71.5
Control	77.8	-
LSD** $_{(0.05)}$	0.5	

* Infection percentage of both treated and un-treated plants in uninfested soil was 0.0%.

** Percentage data were transformed in to arc sine angles before carrying out the analysis of variance.

2.2.2. Effect on growth parameters of maize:

Data presented in Table (6) show that seed treatments with rhizobacterial strains and/or organic compounds, either alone or in combination, significantly promoted plant growth compared to check treatment whether in infested or uninfested soil. Moreover, combination of rhizobacterial strains with organic compounds caused significant increment in maize growth parameters compared with treatment of rhizobacteria and organic compounds alone except seed germination (%), where statistical analysis referred that no significant differences among treatments were recorded whether in infested or uninfested soil.

Regarding to plant height, data in Table (6) indicate that all treatments significantly increased plant height in comparable to check plants either in infested or uninfested soil, being 67.7 and 77.4 cm, respectively. Furthermore, combined treatments between rhizobacteria and organic compounds lead to significant increment in plant height compared with individual treatments. However, the combined treatment of *P. fluorescens* with compost-tea and/or humic acid caused the highest plant height comparable with individual treatments. Seed treatment with humic acid alone gave the lowest effect on plant height either in infested soil, being 79.9 cm, or in uninfested soil (81.5 cm).

Table 6. Combined effect of some rhizobacterial strains and organic compounds on maize plant growth parameters under greenhouse conditions during 2013 growing season

Treatment	Effect of treatment on growth characters					
	Infested soil[a]			Un-infested soil		
	G[b] (%)	P.H (cm)	D.W (g)	G (%)	P.H (cm)	D.W (g)
B. subtilis-1	87.5	92.3	34.7	91.7	97.3	37.4
B. subtilis-3	87.5	88.4	33.9	91.7	94.4	35.4
P. fluorescens	91.7	97.0	36.7	95.8	102.7	39.0
Compost-t	91.7	89.4	32.3	91.7	93.9	35.2
Humic acid	83.3	79.9	25.3	87.5	81.5	31.3
B. subtilis-1 + Compost-t	91.7	101.3	41.1	95.8	116.0	43.5
B. subtilis-1 + Humic acid	87.5	99.3	36.6	91.7	107.3	39.5
B. subtilis-3 + Compost-t	91.7	99.0	36.3	91.7	106.7	39.1
B. subtilis-3 + Humic acid	87.5	98.3	35.5	87.5	102.7	38.4
P. fluorescens + Compost-t	95.8	106.3	43.3	95.8	122.3	46.5
P. fluorescens + Humic	91.7	101.7	40.8	91.7	116.7	41.7
Control	79.2	67.7	16.4	83.3	77.4	24.1
$LSD^{c}_{(0.05)}$	n.s.	2.9	1.9	n.s.	3.5	1.3

a: Soil was infected by *Cephalosporium maydis.*

b: G: seed germination percentage, P.H: plant height (cm), D.W: dry weight of plant (g).

c: n.s.: no significant differences between treatments.

3. Field studies:

3.1. Effect of combined treatments with some rhizobacterial strains and organic compounds on maize late wilt and yield parameters under field conditions:

3.1.1. Effect on disease incidence:

Data presented in Table (7) reveal that seed treatment with rhizobacteria and/or organic compounds, either individually or in combination, significantly reduced maize infection with late wilt compared with check plants (52.4% infection). Moreover, the combination treatments of rhizobacterial strains with organic compounds caused more reduction in maize late wilt compared with individual treatments of rhizobacteria or organic compounds.

However, the combined treatment of compost-tea with *P. fluorescens* and/or *B. subtilis*-1 gave the highest reduction in late wilt, being 76.1 and 71.9%, respectively comparable to treatment with *P. fluorescens*, *B. subtilis*-1 or compost-tea alone, being 66.2, 61.3 and 45.1% reduction, respectively. The lowest reduction in disease incidence was recorded when seeds were soaked in humic acid only (38.0% reduction). Meanwhile, the combination between humic acid and any of *P. fluorescens*, *B. subtilis*-1 and *B. subtilis*-3 lead to 68.7, 66.0 and 63.4% reduction in infection percentage, respectively.

Table 7. Combined effect of some rhizobacterial strains and organic compounds on maize late wilt under field conditions at Gemmiza disease nursery during 2013 growing season

Treatment	Infection (%)	Reduction (%)
B. subtilis-1	20.3	61.3
B. subtilis-3	22.6	56.9
P. fluorescens	17.7	66.2
Compost-t	28.8	45.1
Humic acid	32.5	38.0
B. subtilis-1 + compost-t	14.7	71.9
B. subtilis-1 + humic acid	17.8	66.0
B. subtilis-3 + compost-t	18.3	65.1
B. subtilis-3 + humic acid	19.2	63.4
P. fluorescens + compost-t	12.5	76.1
P. fluorescens + humic acid	16.4	68.7
Control	52.4	----
$LSD^{*}_{(0.05)}$	0.1	----

* Data were transformed into arc sine angles before carrying out the analysis of variance.

3.1.2. Effect on maize yield characters:

Data presented in Table (8) show that seed treatments with rhizobacterial strains and/or organic compounds either alone or in combination significantly improved crop production compared to check plants. Moreover, combination of rhizobacterial strains with organic compounds caused markedly an increment in maize yield parameters compared with individual treatment.

Concerning the yield, it was observed that all treatments significantly increased yield of treated plants in comparable to check ones (14.2 ard/fed). Seed treatment with compost-tea in combination with *P. fluorescens* and/or *B. subtilis*-1 caused the highest produced yield, being 30.2 and 30.0 ard/fed, respectively compared with the other treatments. In contrast, plants treated with humic acid alone produced the lowest yield (17.4 ard/fed) compared with 27.1, 25.2 and 24.7 ard/fed when combined with any of *P. fluorescens, B. subtilis*-1 and *B. subtilis*-3, respectively.

The same trend was observed in regarding to 100 kernels weight. The highest weight was recorded in case of combination treatments between compost-tea and any of *P. fluorescens* and *B. subtilis*-1, being 33.5 and 33.0 g, respectively compared with check treatments (25.3 g). Meanwhile, the lowest 100 grains weight was observed when the seeds were soaked in humic acid alone (29.6 g).

Data (Table 8) also indicate that no significant differences among treatments were observed concerning to ear parameters, *i.e.* ear length, ear diameter, No. of rows per ear and No. of kernels per ear, except of slightly variations in their effect on No. of kernels per row. However, all treatments were increased ear parameters compared to check plants. Combined treatments of compost-tea with any of *P. fluorescens* and *B. subtilis*-1 led to produce the highest number of kernels per ear (515.1 and 510.6, respectively). Meanwhile, individual treatment of humic acid gave the lowest number (427.2) of grains per ear.

Table 8. Combined effect of some rhizobacterial strains and organic compounds on maize crop production under field conditions at Gemmiza disease nursery during 2013 growing season

Treatment	Yield* (ard/fed)	Ear length (cm)	Ear diam. (cm)	No. rows/ ear	No. kernels/ row	No. kernels/ ear	100 kernels weight (g)
B. subtilis-1	25.0	21.2	4.4	11.7	41.3	481.2	32.0
B. subtilis-3	23.3	21.0	4.3	12.0	39.1	466.7	30.3
P. fluorescens	26.1	21.4	4.4	11.7	42.5	495.4	31.6
Compost-t	22.1	20.5	4.3	11.0	40.4	444.1	30.0
Humic acid	17.4	20.7	4.3	11.2	38.3	427.2	29.6
B. subtilis-1 + Compost-t	30.0	21.9	4.3	12.0	42.5	510.6	33.0
B. subtilis-1 + Humic acid	25.2	21.2	4.4	11.7	41.4	481.9	30.8
B. subtilis-3 + Compost-t	26.3	22.4	4.4	11.7	42.0	490.3	31.0
B. subtilis-3 + Humic acid	24.7	21.3	4.3	11.4	41.3	470.1	30.8
P. fluorescens + Compost-t	30.2	22.4	4.3	12.2	42.3	515.1	33.5
P. fluorescens + Humic acid	27.1	21.7	4.4	12.0	42.5	510.0	32.8
Control	14.2	20.0	3.8	10.9	33.8	366.8	25.3
$LSD_{(0.05)}$	4.6	n.s.**	n.s.	n.s.	3.7	n.s.	3.6

* Grain yield per feddan, in ardab by adjusting grain yield / plot to grain yield per feddan (adjusted at 15.5% grain moisture).
** Ns: no significant differences between treatments.

Discussion

In current study, various attempts of biological control by treating maize seeds with rhizobacterial strains and/or organic compounds have been evaluated *in vitro* and *in vivo* to manage maize late wilt caused by *Cephalosporium maydis*. Three treatment techniques were used, *i.e.*, seed soaking, seed coating and soil drench.

Under laboratory conditions, dual culture experiments revealed that 3 out of the 7 isolates of *Bacillus subtilis* and one *Pseudomonas fluorescens* out of 8 isolates showed antifungal activity against the highly virulent *C. maydis* isolates. Also, it was observed that *B. subtilis*-1 filled the plate and completely inhibited the growth of *C. maydis*. This finding suggests that *B. subtilis*-1grew rapidly and colonized the surface of the medium so suppressed fungal growth. Meanwhile, the effect of other isolates may be due to secretion antibiosis in the medium. These results are in accordance with the findings of Cavaglieri *et al.* (2005) and Kim *et al.* (2008). Likewise, Nalisha *et al.* (2006) reported that *B. subtilis* produces antifungal compounds and suppressed radial growth of *S. rolfsii*. Similarly, *P. fluorescens,* which isolated from the rhizosphere of rice and sugar beet fields inhibited the mycelial growth of *Sarocladium oryzae* and *R. solani*, respectively (Ebrahim, 2010 and Meera and Balabaskar, 2012). In fact, antibiosis and competition on space and nutrients are generally the mode of antagonism observed with *Bacillus* species (Edwards *et al.,* 1994). Most *Bacillus* spp. produces many kinds of antibiotics such as bacillomycin, fengycin, mycosubtilin, and zwittermicin, which are effective on suppressing growth of target pathogens *in vitro* and/or *in situ* (Kim *et al.,* 2008). On the other hand, *P. fluorescens* antagonism is related to mechanisms more diverse than those found for *Bacillus.* The traits found included siderophores, volatile compounds, antibiotics (*e.g.* pyrrolnitrin, tensin, and pyoluterin), cell-wall degrading molecules, extracellular chitinase, and protease enzymes as well as competition and induced systemic resistance (Mavrodi *et al.*, 2001).

Moreover, obtained results indicated that all tested organic compounds in the present study had an inhibitory effect on the *in vitro* radial growth of *C. maydis* at any of the used concentrations. Growth inhibition (%) was increased with the increasing of concentrations, in all substances. Humic acid caused the highest inhibitory effect on the fungal growth. These results are in harmony with the findings of several investigators (Charest *et al*., 2005 and El-Mohamedy and Ahmed, 2009). Loffredo *et al.* (2007) found that the addition of humic acids to the growth medium completely inhibited the radial growth of *F. solani, F. culmorum, F. oxysporum* f.sp. *lycopersici*, *P. ultimum* and *A. alternata* on PDA medium. Yigit and Dikilitas, (2008) reported that humic acid has antimicrobial activity against several plant pathogens. Furthermore, the effect of compost-tea on radial growth of fungi was investigated by many investigators (Hsiang and Tian, 2007 and Jenana *et al.,* 2009). Similarly, i*n vitro* assays carried out by Koné *et al.* (2010) showed that compost-tea provided significant inhibition of mycelial growth of *A. solani*, *B. cinerea*, and *P. infestans*. The mechanism by which compost-tea may cause inhibition to fungal growth is its microbial content whereas the microorganisms may act as pathogen antagonists through their ability to compete for space and nutrients (Al-Mughrabi 2006), or to produce antimicrobial compounds (Zhang *et al.,* 1998). Moreover, the antifungal activity of olive mill wastewater (OMW) was reported by several authors (Vagelas *et al.,* 2009 and Özdemir, 2009). As it was found in the present study, Vagelas *et al.* (2009) found that filter sterilized olive OMW significantly reduced the radial growth of important soil borne plant pathogens such as *Fusarium oxysporum, Pythium* spp., *Sclerotinia sclerotiorum* and *Verticillium dahliae*. Ramos-Cormenzana *et al.* (1995) reported that olive mill wastewater contains a number of biologically active substances capable of inhibiting the growth of microorganisms. Many low molecular-weight phenolic compounds, high molecular-mass polyphenols, free fatty acids and aromatic compounds as well as oligosaccharides and glycoproteins have been detected in olive oil mill residues associated with phytotoxic and antimicrobial properties of these residues (Capasso *et al.,* 2002).

Greenhouse and field experimental results indicated that significant reduction in the disease was recorded by seed treatments with rhizobacterial strains and/or organic compounds, either individually or in combination. Moreover, it was observed that seed coating was the best method for treating seeds with rhizobacteria, whereas the best one for treating with organic compounds was seed soaking. However, seed coating with *B. subtilis*-1 and *P. fluorescens,* in addition to seed soaking with compost-tea and their combination caused the most significant reduction in disease incidence under greenhouse and field conditions. These results are in harmony with those reported by many researchers (El-Assiuty *et al.*, 1991 and El-Mehalowy *et al.,* 2004). Similarly, Hamza *et al.* (2013) reported that the formulations of *B. subtilis*, *B. pumilus*, *P. fluorescens* and *Epicoccum nigrum* caused noticeably reduction to maize late wilt under field conditions. Ebrahim (2010) found that the most effective antibiotics (DAPG, PLT, PCA and PLN) and several enzymes (lipase, protease) as well as siderophores (pyoverdine and/or pyochelin) are produced by *P. fluorescens,* which showed antagonistic activity toward *R. solani* and significantly inhibited root infection when coated onto sugar beet seeds. Also, Bacillus-based biological control agents have modes of action that include antibiosis, parasitism, colonization, competition and induced systemic resistance (Jacobsen *et al.,* 2004).

In agreement with previous results, the severity of various diseases caused by *Fusarium* spp. and *Rhizoctonia* spp. has been reduced by using compost amendments (Diab *et al.,* 2003). Also, Scheuerell and Mahaffee (2004) indicated that drench application of compost-tea can suppress *P. ultimum* damping-off of cucumber in soilless container media. The most reported factor influencing the efficacy of compost-tea in inhibiting the development of plant disease is its content of microorganisms, which have the ability to destroy pathogens by parasitism or to induce systemic resistance in plants (Zhang *et al.,* 1998). Other works hypothesized that the physicochemical properties of the compost-tea, namely nutrients and organic molecules such as humic or phenolic compounds may protect the plant against diseases through improved nutritional status, direct toxicity toward the pathogens or induced systemic resistance (Siddiqui *et al.,* 2008). On the other hand, Varanini and Pinton (2001) reported that humic acid can affect directly on the number of physiological and biochemical processes occurring in plants and other soil-borne organisms, especially in the rhizosphere compartment. Generally, as found in the present study, Abdel-Monaim *et al.* (2011) reported that seed soaking of soybean seeds with humic acid was able to protect soybean plants against damping-off and wilt diseases caused by *F. oxysporum.* In contrast, Yigit and Dikilitas (2008) found that the application of humic acid caused an increment in root fresh weight and increase the susceptibility of tomato plants to the root rot disease. Furthermore, it was mentioned that OMW can be safely employed as a seed treatment with no concern for phytotoxicity or negative impact on germination (Boz *et al*., 2003 and Özdemir, 2009). Similarly, Özdemir (2009) reported that OMW significantly reduced tomato infection by *Clavibacter michiganensis* subsp. *michiganesis* and *P. syringae* pv. *tomato*.

Also, obtained results in the current investigation revealed that seed treatment with rhizobacterial strains and/or organic compounds significantly promoted growth of maize plants, either under greenhouse or field conditions. However, under greenhouse conditions, it was observed that all treatments, either alone or in combination, resulted in better germination and significantly increased plant height and dry weight in comparable to check plants whether in infested or un-infested soil.

On the other hand, under field conditions, seed treatments with rhizobacterial strains and/or organic compounds either alone or in combination were significantly improved maize ear and yield characters. The enhancing effect of seed inoculation with rhizobacteria on shoot dry weight and yield of maize were reported by many researchers (Pandy *et al.*, 1998 and Shaharoona, *et al.*, 2006). Results of this investigation are similar to that obtained by Vikram (2007) and Gholami *et al.* (2009) who stated that auxins produced by rhizobacteria can influence plants growth, including seed germination and root development which improve uptake of essential nutrients thus increasing plant growth. Gholami *et al.* (2009) suggested that the increased synthesis of hormones like gibberellins trigger the activity of specific enzymes that promote early germination, such as α-amylase, which have brought an increase in availability of starch assimilation.

Also, Results of this study are in harmony with those of Hanifi and El-Hadrami (2007) and Bakry *et al.* (2009). Similarly, Osoro *et al.* (2014) reported that the application of composts caused significant increments on the vegetative and yield parameters of maize. The effect of compost on plant growth may due to the enrichment content of minerals (Siddiqui *et al.*, 2008). Likewise, humic acid preparations were reported to increase the uptake of mineral elements, promoted the root length and increase the fresh and dry weight of crop plants (Yigit and Dikilitas, 2008). Daur and Bakhashwain (2013) reported that humic acid amendments increased maize vegetative growth characters, ear characters and grain yield. They demonstrated that the increase in the growth parameters of maize in the humic acid-amended treatments most probably due to the improvement of soil condition of the root zone. On the other hand, OMW contain an enormous supply of organic matter very rich in phenolic compounds, and can be used to fertilize the soil (Mekki *et al.*, 2006). Obtained results are similarly to that of Hanifi and El-Hadrami (2007) who reported that olive mill wastewater-fertilised plots showed a net improvement in maize plant growth characters.

In conclusion, usage of fungicides to minimize soil-borne disease seriously affects human and animal health and pollutes the environment. Thus, it is recommended to follow some safe methods, responsive with the environment, to control soil-borne diseases such as late wilt of maize. Current investigation suggesting the usage of some rhizobacterial strains (*e.g. B. subtilis* and *P. fluorescens*) and organic compounds, such as compost and humic substances, as seed treatment, to manage maize infection with late wilt disease.

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مكافحة مرض الذبول المتأخر في الذرة الشامية وتحسين خصائص النمو باستخدام الرايزوبكتيريا والمركبات العضوية

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تم اختبار فعالية بعض عزلات الرايزوبكتيريا والمواد العضوية في خفض الإصابة بمرض الذبول المتأخر في الذرة الشامية المتسبب عن الفطر *C. maydis* وتحسين نمو النبات تحت ظروف الصوبة والحقل. أظهرت ثلاث عزلات من *B. subtilis* وعزلة واحدة من *P. fluorescens* القدرة على تثبيط نمو عزلات الفطر *C. maydis* الشديدة المرضية في المعمل. وجد أيضاً أن المعاملة بتركيزات مختلفة من المواد العضوية (شاي الكومبوست ، المياه المستعملة لمعاصر الزيتون ، حمض الهيوميك) قدسببت انخفاضاً معنوياً في معدل نمو الفطر وكان حمض الهيوميك هو الأكثر تأثيراً في المعمل. أما تحت ظروف الصوبة والحقل ، فقد أدت معاملة الحبوب بالرايزوبكتيريا و/أو المواد العضوية - سواء بمفردها أو بالدمج فيما بينهم - إلى انخفاض معنوي في حدوث المرض ، وكانت طريقة تغليف الحبوب هي الأفضل للمعاملة بالرايزوبكتيريا ، في حين كانت أفضل طريقة للمعاملة بالمواد العضوية هي نقع الحبوب. وقد سبب تغليف الحبوب بـ *B. subtilis*-1 و *P. fluorescens* ونقع الحبوب بشاي الكومبوست وخلط هذه العزلات مع شاي الكومبوست إلى حدوث انخفاضاً كبيراً في ظهور المرض. ومن ناحية أخرى فقد أدت هذه المعاملات إلى زيادة نسبة الإنبات وتحسين خصائص النمو الخضري لنبات الذرة الشامية (ارتفاع النبات و الوزن الجاف) في كل من التربة الملوثة وغير الملوثة بالفطر *C. maydis* تحت ظروف الصوبة. كذلك وجد أن معاملة الحبوب بالرايزوبكتيريا و/أو المواد العضوية , سواء بمفردها أو بالخلط فيما بينهم , قد أدت الى زيادة كبيرة في المحصول (وزن ١٠٠ حبة ومحصول الفدان) ومواصفات الكوز (طول الكوز وقطر الكوز وعدد الصفوف بالكوز وعدد الحبوب في الصف وعدد الحبوب في الكوز) وذلك تحت الظروف الحقلية.

وتشير نتائج الدراسة إلى إمكانية استخدام عزلات الرايزوبكتيريا والكومبوست وحمض الهيومك كمعاملة لحبوب الذرة الشامية قبل الزراعة في مكافحة مرض الذبول المتأخر تحت الظروف الحقلية.