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Bacterial Bioagents and Compost as Two Tools for Management of Eggplant Fusarium Wilt

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Abstract: Isolation trials from the roots of wilted eggplant plants grown at five governorates yielded many fungal isolates. The isolates of the fungus *F. oxysporum* were selected to test their pathogenicity and Kalubia isolate was the most virulent one. The fungus was virulent to eggplant and no apparent infection was found in case of the other seven tested plants. Therefore, the fungus *F. oxysporum* named *Fusarium oxysporum* Schlecht. f. sp. *melongenae* Fomg. All the tested bioagents and compost tea caused significant reduction to the linear growth of *F.o.*f.sp. *melongenae* and the germinated conidiospores compared with control treatment. Adding the two tested bioagents, *i.e. B. subtilis* and *P. fluorescens* each alone or in combination to the infested soil with the causal pathogen resulted in significant reduction to eggplant wilt with significant increase to plant height as well as the number of fruits and their weight / plant compared with control treatment. Moreover, amending the soil with compost increased the efficiency of the two bioagents in reducing the disease and increasing the produced fruit yield compared with the clay soil only. In addition, the fungicide Topsin M-70 was the superior treatment in reducing the severity of the disease and increasing plant height and the produced fruit yield followed by the combination of *B. subtilis* and *P. fluorescens*. The total phenol compounds were greatly increased in the bacterial treated plants and compost as compared to the control (untreated plants with the bioagents) and those infested with the pathogen only.

Keywords: Bacterial Bioagents, Compost, Eggplant, Fusarium Wilt, Disease Management, Fruit Yield, Total Phenol Compounds

1. Introduction

Eggplant (*Solanum melongenae* L.) is considered one of the most important vegetable crops in Egypt for local consumption and exportation. It is a preferable food for poor and rich people. Therefore, improving the bioproduction of this crop is one of the objectives in agriculture in many countries.

The total cultivated area with eggplant in Egypt is unknown exactly, which estimated by about 74.125 feddan during 2016 growing season with a total production of about 466723 ton at the rate of 6.3 ton /feddan.

Eggplant is liable to be attack by many bacterial, fungal, viral and nematode diseases as well as physiological disorder. However, Fusarium wilt is considered the major devastative and destructive disease affecting crop production of eggplant [5], [6], [22], [26].

Due to the cultivated area in Egypt is limited, therefore crop rotation is not applied and this caused great difficulties, especially in case of specific soil-borne pathogens such as eggplant Fusarium wilt. On the other hand, the use of chemical management against such diseases mostly gives good results. However, improper use of fungicides leads mostly to environmental pollution, disasters throughout the world and the phenomena of resistance to the plant pathogens [9]. Therefore, to overcome these difficulties, it is urgent to apply biological control as an alternative safe efficient

method against such diseases, which biological control is considered an important approach of agricultural biotechnology in recent years for controlling many soil-borne plant pathogens [1], [2], [3], [24], [40].

The fungus Fusarium wilt of eggplant (*Fusarium oxysporum* Schlecht. f. sp. *melongena*e Fomg) is one of the major fungal diseases causing economical yield losses in Egypt as well as several other eggplant producing countries. Under optimal infection conditions, such as temperature, high soil moisture level, soil compaction and poor soil drainage, this pathogen can completely destroy the grown plants. Infected plants exhibit leaf chlorosis and slight vein clearing on outer leaflets, followed by yellowing and dropping of leaves, then xylem browning of the stem and finally death of the aboveground parts. The infection and the symptoms are observed when the temperature is about 25° C. Fusarium wilt spreads widely, especially on eggplant plantations in Eastern Mediterranean Region, which causes serious yield losses [5], [22].

Management of plant disease is supposed to be a dynamic and multifactorial process. Hence, it is assumed that plant defense response can be activated by specific recognition of some microorganisms by the plant [18]. There may be whole organisms or products secreted by microorganisms under the influence of which plants initiate defense response [3], [7]

This work was performed to investigate the potential of some *Bacillus* spp., *i.e. Bacillus chitinosporus, B. megaterium, B. pumilus, B. subtilis* and *B. thuringiensis* as well as *Pseudomonas fluorescens* and *P. putida* in addition to compost tea on the growth and germination of the conidiospores of *F.o.*f.sp. *melongenae*. Also, the efficacy of both *B. subtilis* and *P. fluorescens* in combination with compost on management of eggplant Fusarium wilt and the formation of phenol compounds in eggplant plants.

2. Materials and Methods

2.1. Isolation, Purification and Identification of the Associated Fungi to Eggplant Wilt

Eggplant plants (Balady white cv.) showing characteristic symptoms of wilt were collected from Dakahlia, Menofia, Kalubia, Giza and Fayoum governorates. The infected root samples were thoroughly washed in running tap water and cut into small pieces with lesion having half healthy and half diseased tissue. The pieces were surface sterilized with 2 % sodium hypochlorite for two minutes. The tissue pieces were subsequently washed in three changes of sterile water to eliminate excess sodium hypochlorite and then the pieces were transferred onto PDA medium in Petri-dishes. Plates were incubated at 25 ± 2°C and observed periodically for growth of the fungi. The pure cultures of the isolated fungi were obtained by hyphal tip method and/ or single spore technique and maintained on PDA slants throughout the investigation. The emerged fungi were identified on the basis of cultural, morphological characteristics and the description of [10], [13].

2.2. Pathogenicity Test of the Fusarium oxysporum Isolates and Identification of the Forma Specials

The inoculum of *F. oxysporum* was prepared by culturing the fungus on potato dextrose agar (PDA) medium for 7 days in Petri-plates. Conidial suspension was prepared by pouring 20 ml of sterile distilled water in each Petri-plate. The concentration of the conidia was adjusted to $1x10^3$ conidia per milliliter using haemocytometer.

Three transplants (Balady white cv.) of 30 day-old, grown in foam trays contained disinfested soil (peat moss + clay+ sand +vermiculite), were transplanted in each plastic pot (30 cm. in diameter) contained infested clay soil by spore suspension of any of the five isolates of *F. oxysporum* ($1x10^3$ conidia / ml water) at the rate of 100 ml / pot. Also, Three transplants were transplanted in each plastic pot (30 cm. in diameter) contained disinfested clay soil and served as control. Five pots were used for each treatment. The plants were left to grow under greenhouse conditions.

The pots were irrigated when it was necessary and fertilized with recommended doses as recommended by Min. of Agric. and Land Reclamation.

The plants were examined for the severity of infection by the tested fungus two months after transplanting as mentioned under disease assessment and the averages were recorded.

Also, plant height (cm), fresh and dry weight of the foliage growth/ plant of the grown plants were estimated and recorded.

Eight tested plants, i.e. bean (Bronco cv.), cucumber (Admiral cv), eggplant (Balady white cv.), melon (Shahd Edfina cv.), sweet pepper (Balady cv.), strawberry (Camarosa cv.), tomato (GS cv.) and watermelon (Giza 1 cv.) were grown in plastic pots infested or not with *F. oxysporum* isolate of Kalubia governorate and left two grow for two months, then incidence of Fusarium wilt was recorded.

2.3. Isolation, Purification and Identification of the Bacterial Antagonists

Soil samples collected from the rhizospheric soil of healthy eggplant plants grown in a field have severe infection by Fusarium wilt, were used to isolate the antagonists. Serial dilution plate technique was used to isolate native *Bacillus* spp. as well as *Pseudomonas fluorescens* and *P. putida* on nutrient agar medium [21]. The isolated bacteria were purified and identified using the description of [16], [23].

2.4. Effect of the Culture Filtrate of Bacterial Bioagents on the Linear Growth and Germination of Conidiospores of the Causal Pathogen

The effect of the culture filtrate of five isolates of *Bacillus* spp., *i.e. Bacillus chitinosporus, B. megaterium, B. pumilus, B. subtilis* and *B. thuringiensis* as well as *P. fluorescens* and *P. putida* on the linear growth and germination of conidiospores of *F. o.* f.sp. *melongenae* was studied. Hundred ml. of nutrient medium were put in each 250 ml flask and sterilized by steamer for three successive days. The medium

was inoculated with two loops of the culture of any of the tested bioagents (taken from two days-old culture). Inoculated flasks were incubated on a rotary shaker at 200 rpm for 2 days at 30 ± 2°C. The culture filtrate was filtered through Whitman No.1 filter paper and the filtrate was collected in a flask. Bacterial culture filtrate was sterilized using 0.25 µm syringe filter. The culture filtrate of the bioagents was added to PDA medium just before solidify-cation in different proportions (20, 30, 40 and 60 %), shake well and poured into the Petri-dishes (20 ml/plate). After solidification the Petri-plates were inoculated with 5 mm. discs of the test pathogen cut from the five days old culture. PDA plates inoculated with the test pathogen, but not amended with the culture filtrate of the tested bioagents, were maintained as control. Plates were then incubated in an incubator at 30±2°C. Five replications were maintained for each treatment. The linear growth was measured when the plates of the control treatment covered with the fungal growth. Inhibition percentage of the mycelial growth of the tested pathogen was calculated by the following formula:

$$I = (C- T)/C X100$$

Where;

I = Percent of inhibition in growth of the tested pathogen,

C = Linear growth of the pathogen (mm) in control,

T= Linear growth of the pathogen (mm) in treatment.

Also, the prepared concentrations of the tested bioagents were added to the fungal growth in the Petri-dishes to make a conidial spore suspension for the causal fungus. One m1. of conidial suspension was placed on each sterilized slide, borne on two glass rods in a sterilized Petri-dish (two slides in each Petri-dish) containing a piece of wetted cotton by sterilized distilled water to provide high relative humidity. The same was made for a spore suspension put in distilled sterilized water only as control treatment. Preparations were incubated in darkness at 30±2°C for 24 hour. Five Petri dishes for each treatment were used as replicates. The percentages of conidial germination were counted in a total of 100 conidiospore in each glass slide. The germinated conidia were counted and the mean was calculated and recorded for each treatment.

2.5. Effect of Filtrate of Compost Tea on the Linear Growth and Germination of Conidiospores of the Causal Fungus

Compost (500 g.) was soaked in 2 liter tap water over night and filtered through Whitman filter paper No1. Compost tea was sterilized using 0.25 µm syringe filter and added to PDA medium after sterilization at the concentrations of 20, 30, 40 and 60 %, shake well and poured into the Petri-dishes (20 ml/plate). After solidification the Petri-plates were inoculated with 5 mm. discs of the test pathogen cut from the five days old culture. PDA plates inoculated with the test pathogen, but not amended with the compost tea were maintained as control. Plates were then incubated in an incubator at 30±2°C. Five replications were maintained for each treatment. The linear growth was measured when the plates of the control treatment covered with the fungal growth. Inhibition percentage of the mycelial growth of the tested pathogen was calculated as mentioned before.

The prepared concentrations of compost tea were added to the fungal growth in the Petri-dishes to make a conidial spore suspension for the causal fungus. One m1. of conidial suspension was placed on each sterilized slide, borne on two glass rods in a sterilized Petri-dish (two slides in each Petri-dish) containing a piece of wetted cotton by sterilized distilled water to provide high relative humidity. The same was made for a spore suspension put in distilled sterilized water only as control treatment. Preparations were incubated in darkness at 30±2°C for 24 hour. Five Petri-dishes for each treatment were used as replicates. The percentages of conidial germination were counted in a total of 100 conidiospore in each glass slide. The germinated conidia were counted and the mean was calculated and recorded for each treatment.

2.6. Effect of the Combination Among B. subtilis, P. fluorescens and Compost on Wilt Severity and Some Crop Parameters

Plots (1x1 m) containing formalin sterilized clay soil were infested with the conidial spore suspension of the causal pathogen (2 liter of $1x10^6$ spore / liter water). The plots were divided into two groups; the first one contained formalin sterilized clay soil and the second group contained formalin sterilized clay soil amended with compost (2kg./plot). Each group received the following treatments:

1. The plots supplied with the growth suspension of *B. subtilis* at the rate of 2 liter cfu ($1x10^6$ / ml.) / plot, one week before transplanting..
2. The plots supplied with the growth suspension of *P. fluorescens* at the rate of 2 liter cfu ($1x10^6$ / ml.) / plot, one week before transplanting..
3. The plots supplied with compost at the rate of 2 kg. / plot, one week before transplanting.
4. The plots supplied with the growth suspension of *B. subtilis* the rate of 2 liter cfu ($1x10^6$ / ml.) / plot plus compost at the rate of 2 kg. / plot, one week before infestation with *F.o.* f.sp. *melongenae* (FOM) and transplanting.
5. The plots supplied with the growth suspension of *P. fluorescens* at the rate of 2 liter cfu ($1x10^6$ / ml.) / plot plus compost at the rate of 2 kg. / plot, one week before infestation with FOM and transplanting.
6. The plots supplied with one liter growth suspension from both *B. subtilis* and *P. fluorescens*, one week before infestation with FOM and transplanting.
7. The plots were left without any treatment to grow the plants, but received 2 liter water only (control uninfested soil).
8. The plots were left without supplying any additional from the bioagents, but infested with the spore suspension of the causal fungus at the rate of 2 liter

spore suspension / plot, one week before transplanting.

9. The plots were infested with the spore suspension of the causal fungus at the rate of 2 liter / plot, one week before transplanting and 5 liters (one g / liter water) from the fungicide Topsin M-70 (Thiophanate-methyl) were added as soil drench before transplanting.

Six transplants (Balady white cv.) of 30 day-old, grown in foam trays contained disinfested soil (peat moss + clay +sand+ vermiculite soil), were transplanted in each plot. Three plots were used for each treatment, disease severity, plant height (cm), the number and weight of fruits / plant were assessed and recorded.

2.7. Disease Assessment

The severity of Fusarium wilt was assessed five months after transplanting using the devised scale (0 to 5) by [4] on the foliage growth using the following scale:

Where:

0 = No foliar symptoms,

1 = Chlorosis and/or wilt restricted to the first leaf,

2= Chlorosis and/or wilt extending beyond the first leaf,

3 = Moderate to severe foliar symptoms usually with some abscised leaves,

4 = Severe foliar symptoms on the entire plant, and

5 = Dead plant.

Disease severity on foliage growth % = £ (nxv) / 5N X 100

Where:

n = Number of wilted leaves in each category.

v = Numerical values of each category.

N = Total number of the wilted leaves.

The plants were, also, rated for vascular discoloration by the causal fungus using the devised scale (0-5) by [31] using the following formula:

Where:

0 = No discoloration,

1 = Light discoloration evident as spotty areas in the longitudinal-section of the basal stem and upper part of the root,

2 = More continuous discoloration covering an area between one quarter and one half of the basal stem and upper part of the root, but light in color,

3 = Vascular discoloration (moderate in color) evident in a band encircling almost the entire of the basal stem and upper part of the root,

4 = Vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a the basal stem and upper part of the root, and

5 = Plant severely damaged, vascular discoloration evident throughout the basal stem and upper part of the root.

Disease severity on the vascular % = £ (nxv)/5 N X 100

Where:

n = Number of infected vascular in each category.

v = Numerical values of each category.

N = Total number of the infected vascular.

Also, plant height (cm) of the grown plants, the average No. of fruits / plant and weight of fruits (g) / plant were estimated and recorded.

2.8. Estimation of Total Phenol Compounds

One gram of eggplant leaf samples was extracted with 10 ml of 80% methanol at 70 °C for 15 min. Reaction mixture was containing 1 ml of methanol extracts, 5 ml of distilled sterilized water, and 250 µl of Folin–Ciocalteau reagent (1N). This solution was kept at 25±1°C. The absorbance of the developed blue color was measured using a spectrophotometer at 725 nm. Gallic acid was used as the standard. The amount of phenol compounds was expressed as mg gallic acid per g roots material [41]. The assessment was carried out 40 and 45 days after inoculation by the causal fungus(FOM).

2.9. Statistical Analysis

Data were statistically analyzed using the standard procedures for complete randomize block, split and split split designs as mentioned by [28]. The averages were compared at 5% level using least significant differences (L. S. D.) according to [12].

3. Results

3.1. Isolation, Purification and Identification of the Associated Fungi to Eggplant Wilt

Isolation trials from eggplant plants (Balady white cv.), showing characteristic symptoms of wilt disease, collected from Dakahlia, Menofia, Kalubia, Giza and Fauoum governorates yielded many fungal isolates. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *F. oxysporum, F. solani, Macrophomina phaseolina, Pythium* spp., *Rhizontonia solani, Sclerotium rolfsii* and *Verticillium dahliae*.

The isolates of the fungus *F. oxysporum* were selected and tested for their pathogenicity and the most virulent isolate was chosen.

3.2. Pathogenicity Test of F. oxysporum Isolates and Identification of the Forma Specials

The five *F. oxysporum* isolates (Table, 1) reveal that they were pathogenic to eggplant plants and showing typical wilt symptoms on the foliage growth and the xylem vesicles. Moreover, the isolate of Kalubia governorate was the highest virulent one on both the foliage growth (48.2%) and the xylem vesicles (53.0%) and resulted in the lowest values of plant height (70.6 cm), foliage fresh (423.8 g) and dray (52.5g) weight compared with the other isolates. Meanwhile, Fayoum isolate resulted in the lowest figures of the disease, being 37.3 and 43.9% and the lowest effect in reducing plant height and foliage fresh and dry weight, being 81.1 cm, 469.3 and 58.8 g, respectively. The remained three isolates recorded intermediate values. No apparent symptoms of Fusarium wilt were observed on the control plants and showed good growth. Thus, Kalubia isolate was used in the

following experiments.

Testing of bean (Bronco cv.), cucumber (Admiral cv.), eggplant (Balady white cv.), melon (Shahd Edfina cv.), sweet pepper (Balady cv.), strawberry (Camarosa cv.), tomato (GS cv.) and water melon (Giza 1 cv.) to their susceptibility to the infection by *F. oxysporum* indicated that the highest infection by the fungus was occurred only on eggplant and low or no apparent infection were occurred in case of the other plants. Therefore, the fungus *F. oxysporum* named *Fusarium oxysporum* f.sp. *melongenae* (FOM).

Table 1. *Pathogenicity test of five isolates of F. oxysporum using transplants of eggplant (Balady white cv.), greenhouse experiment.*

Isolates	% Disease severity on		Average of		
	Foliage growth	Xylem vesicles	Plant height (cm)	Foliage fresh weight (g)	Foliage dry weight (g)
Dakahlia	38.0	45.4	79.2	460.2	57.2
Menofia	40.2	46.0	76.2	451.4	55.3
Kalubia	48.2	53.0	70.6	423.8	52.5
Giza	44.1	51.4	71.3	435.2	83.2
Fayoum	37.3	43.9	81.1	469.3	58.8
Control	0.0	0.0	98.0	780.5	74.5

3.3. Effect of Culture Filtrate of Five Bacillus Strains as Well as two Pseudomonas Strains on the Linear Growth and the Germinated Conidiospores of the Causal Pathogen

The bacterial bioagents, *i.e. B. chitinosporus, B. megaterium, B.pumilus, B.subtilis, B. thuringiensis, Pseudomonas fluorescens* and *P.putida* were isolated from the rihizosperic soil of the healthy eggplant roots (Table,2).

Data presented in Tables (2 and 3) show that all the tested bacterial bioagents resulted in significant reduction to the linear growth and the germinated conidiospores of the causal pathogen (FOM) compared with control treatment. This reduction was gradually increased by increasing the tested concentration.

Table 2. *Effect of culture filtrate of five Bacillus strains and two Pseudomonas spp. on the linear growth of FOM, six days after incubation at 30±2°C.*

Bioagents	Average linear growth (mm) at concentration of (%)				
	20	30	40	60	Mean
B. chitinosporus	80.4	41.2	23.2	0.0	36.2
B. megaterium	72.8	32.6	0.0	0.0	26.4
B. pumilus	76.4	37.2	22.5	0.0	34.0
B. subtilis	69.4	29.0	0.0	0.0	24.6
B. thuringiensis	71.8	30.2	0.0	0.0	25.5
P. fluorescens	70.0	29.6	0.0	0.0	19.9
P. putida	76.0	35.2	17.6	0.0	32.2
Control	90.0	90.0	90.0	90.0	90.0
Mean	75.9	40.6	19.2	11.3	-----

L. S. D. at 5 % for:
Bioagents (B) = 2.7, Concentration (C)= 3.4, BxC = 4.6.

Table 3. *Effect of culture filtrate of five Bacillus strains and two Pseudomonas strains on the average percentage of the germinated conidiospores of FOM, 24 h. after incubation at 30±2°C.*

Bioagents	Average percentage of the germinated conidiospores (.x10^3) at concentration of (%)				
	20	30	40	60	Mean
B. chitinosporus	72.0	38.2	16.0	0.0	31.6
B. megaterium	64.8	20.8	0.0	0.0	21.4
B. pumilus	74.4	40.2	18.8	0.0	33.4
B. subtilis	58.0	25.4	0.0	0.0	20.9
B. thuringiensis	69.4	26.4	0.0	0.0	24.0
P. fluorescens	56.4	24.0	0.0	0.0	20.1
P. putida	71.8	36.2	15.4	0.0	30.9
Control	95.0	95.0	95.0	95.0	95.0
Mean	60.3	38.3	18.2	11.9	----

L. S. D. at 5 %: Bioagents (B) = 2.8, Concentration (C) = 3.7, BxC = 4.2.

Table (2) reveals that *P. fluorescens* was the most efficient one in reducing the linear growth of the causal pathogen, being 19.9 mm., on the average followed by *B. subtilis*, being 24.6 mm., on the average then *B. pumilus*, being 34.0 mm., on the average and *B. megaterium*, being 26.4 mm., on the average Meanwhile, *B. chitinosporus* was the lowest efficient one being, 36.2 mm, on the average followed by *P. putida,* being 32.6 mm., on the average.

The effect of culture filtrate of the tested *Bacillus* strains as well as *P. fluorescens* and *P. putida* on the germinated conidiospores of the causal pathogen was in the same trend of the effect on the linear growth of the causal pathogen (Table, 3). In this respect, the conidiospores of the causal pathogen failed to geminate at the concentration of 40% of the culture filtrate of *B. megaterium, B. subtilis, B. thuringiensis* and *P. fluorescens* and at the concentration of 60% for *B. chitinosporus*, *B. pumilus* and *P. putida*.

3.4. Effect of Compost Tea Filtrate on the Linear Growth and the Germinated Conidiospores of the Causal Pathogen

Results shown in Table (4) show that the filtrate of the soaked compost (compost tea) caused significant reduction to the linear growth of *F. o.* f. sp. *melongenae,* six days after incubation at 30±°C compared with control treatment This reduction was gradually increased by increasing the used concentration. In addition, the causal fungus failed to grow on the concentration of 60 %. Meanwhile, conidiospores failed to germinate at 40% concentration. However, control treatment recorded 90 mm. linear growth and 92.4 %, on the average conidial germination.

Table 4. *Effect of compost tea on the linear growth and the germinated conidiospores of FOM, 6 and 24 h. after incubation at 30±2°C, respectively.*

Concentration (%)	Linear growth (mm)	% Conidiospores germination (…x10^3)
20	73.0	53.0
30	43.6	35.6
40	24.4	0.0
60	0.0	0.0
Control	90.0	92.4
L. S. D. at 5%	2.7	3.2

3.5. Effect of B. subtilis and P. fluorescens in Combination with Compost on the Severity of Eggplant Wilt as Well as Plant Height and the Produced Fruit Yield

Data presented in Tables (5 and 6) show that the two tested bioagents each alone or in combination resulted in significant reduction to eggplant wilt with significant increase to plant height as well as the number of fruits and their weight/ plant compared with the control treatment (infested soil). In addition, no visual symptoms were observed due to infesting the soil with the tested two bioagents only as well as control plants (un-infested soil). However, soil amended with compost was suppressive to the disease either the two tested bioagents were added to the soil or not. The lowest disease severity was recorded when Topsin M-70 was added to the soil as soil drench, being 2.4 %. on the average. Meanwhile, when any of *B. subtilis* and *P. fluorescens* and both were added to the soil infested with the causal fungus 10.0, 8.1 and 4.1% disease severity, on the average were recorded, respectively. Plants grown in soil infested with the causal fungus only recorded 26.3 % disease severity, on the average. The severity of the disease was, to somewhat, higher on the foliage growth than on the xylem vesicles (vascular), being 6.8 and 5.8%, on the average, respectively. In addition, plants grown in soil infested with any of *B. subtilis* and *P. fluorescens* were of high values of plant height and fruit yield (number and weight / plant) than those grown in the control (un-infested soil). In addition, the efficiency of the two tested bioagents were more efficient in reducing the severity of the disease and increasing values of plant height and fruit yield (number and weight / plant) when the soil was amended with compost compared with un-amended soil with compost.

Table 5. *Effect of B. subtilis, P. fluorescens and compost on the severity of eggplant wilt, greenhouse experiment.*

	% Disease severity on						
	Foliage growth		Xylem vesicles		Mean		General
Treatments	Clay soil	Clay soil + compost	Clay soil only	Clay soil + compost	Clay soil only	Clay soil + compost	Mean
B. subtilis (BS)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P. fluorescens (PF)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
BS + FOM	12.4	9.0	9.9	7.0	11.2	8.8	10.0
PF + FOM	10.6	7.2	8.4	6.2	9.5	6.7	8.1
BS+PF+FOM	5.6	4.2	4.0	2.4	4.8	3.3	4.1
Topsin M-70+FOM	2.8	1.7	3.9	1.0	3.3	1.4	2.4
Control (Un-infested)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control (infested)	32.0	23.5	29.3	20.0	30.7	21.8	26.3
Mean	7.9	5.7	6.9	4.6	7.4	5.3	---
General mean	6.8		5.8		------		-----

LSD at 5 % for: Treatments (T)= 2.5, Site of the disease (SD)=2.3, Soil type (ST)= 1.9, T x SD= 3.0, T x ST=1.9, SD x ST=2.7 and T x SD x ST = 3.8.

Table 6. *Effect of the bioagents B. subtilis and P. fluorescens as well as compost on plant height and the produced fruit yield / plant, greenhouse experiment.*

Treatments	Clay soil only			Clay soil + compost		
	Plant height (cm)	No. of fruits /plant	Weight of fruits (g)/plant	Plant height (cm)	No. of fruits/plant	Weight of fruits (g)/plant
B. *subtilis* (BS)	83.3	21.3	978.0	91.5	25.0	1050.0
P. *fluorescens* (PF)	84.5	22.0	989.8	92.0	25.0	1064.0
BS + FOM	75.0	16.7	745.9	78.0	18.3	890.0
PF + FOM	77.3	16.7	754.4	78.3	18.7	898.0
BS+PF+FOM	80.3	18.0	895.0	86.4	21.7	947.1
Tospin M-70)+FOM	81.7	19.0	920.0	89.0	22.3	972.0
Control (Un-infested	82.9	20.0	963.0	90.0	24.0	1020.0
Control (infested)	57.0	11.3	458.3	66.7	560.0	536.8
L. S. D. at 5%	3.1	2.6	7.8	3.3	7.1	7.1

3.6. Effect of the Two Bacterial Bioagents, Compost and Pathogen Treatment on the Content of Eggplant Roots from Phenol Compounds

Table (7) shows that the tested two bacterial bioagents and compost resulted in considerable increase in the phenol compounds contents in the roots of eggplant plants with low variation in their values compared with the two controls. In addition, compost was the most efficient one in this regard, being 0.65 mg / g plant fresh roots, on the average followed by *B. subtilis* and *P. fluorescens*, being 0.64 and 0.62 mg / g plant fresh roots, on the average, respectively. Meanwhile, the lowest value of the phenol content was found in the roots of the control (infested soil) followed by that found in the roots of the plants grown in un-infested soil by the causal pathogen, being 0.41 and 0.47 mg / g plant fresh roots, on the average, respectively.

Table 7. *Effect of two bacteria bioagents, compost and pathogen treatment on the phenol compounds content of eggplant roots,30 and 45 days after inoculation with the causal pathogen.*

Treatments	Gallic acid in mg / g fresh roots after (days)			Mean
	0.0	30	45	
B. subtilis	0.44	0.68	0.79	0.64
P. fluorescens	0.44	0.65	0.76	0.62
Compost	0.44	0.70	0.81	0.65
Control (infested)	0.44	0.41	0.38	0.41
Control (Uninfested)	0.44	0.46	0.50	0.47

4. Discussion

The fungus *Fusarium oxysporum* f. sp. *melongenae* is the most destructive causal pathogen causing eggplant (*Solanum melongena* l.) wilt. The fungus is a soil-borne pathogen that invades the vascular vesicles, causes severe wilting and dying the above ground parts of the plants by blocking the xylem transport vesicle (s). It is extremely difficult to manage soil-borne fungi by conventional methods. The application of bioagents is important, since they may increase beneficial microbial activity, which extends for a long period of time.

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria found on plant roots and in compost that can induce disease resistance and growth promotion by a wide variety of mechanisms.

Isolation trials from eggplant plants (Balady white cv.), showing characteristic symptoms of wilt disease, collected from Dakahlia, Menofia, Kalubia, Giza and Fayoum governorates yielded many fungal isolates. The isolated fungi were purified and identified as: *Alternaria* spp., *Fusarium* spp., *F. oxysporum, F. solani, Macrophomina phaseolina, Pythium* spp., *Rhizontonia solani, Sclerotium rolfsii* and *Verticillium dahliae*. Pathogenicity test of the five isolate of *F. oxysporum* revealed that Kalubia isolate was the most virulent one. In addition, testing eight plant species, *i.e.* bean (Bronco cv.), cucumber (Admiral cv), egg-plant (Balady white cv.), melon (Shahd Edfina cv.), sweet pepper (Balady cv.), strawberry (Camarosa cv.), tomato (GS cv.) and watermelon (Giza 1 cv.) to their infection by *F. oxysporum* proved that it infected eggplant only causing wilt, therefore named *Fusarium oxysporum* f. sp. *melongenae.*

The bacterial bioagents, *i.e. B. chitinosporus, B. megaterium, B.pumilus, B.subtilis, B. thuringiensis, P. fluorescens* and *P.putida* were isolated from the rhizospheric soil of the healthy eggplant roots.

The tested bacterial bioagents resulted in significant reduction to the linear growth and the germinated conidiospores of the causal pathogen compared with control treatment. This reduction was gradually increased by increasing the tested concentration.

Compost tea caused significant reduction to the linear growth and the germinated conidiospores of the causal fungus. This reduction was, also, gradually increased by increasing the tested concentration. Similar results were obtained by [27].

It has been found that, both linear growth and the germinated conidia of the causal pathogen were completely inhibited by the culture filtrate of *B. megaterium*, *B. subtilis*, *B. thuringiensis* and *P. fluorescens* at the concentration of 40% and by the other bioagents, *i.e. B. chitinosporus, B. pumilus* and *P. putida* at the concentration of 60%. Meanwhile, the linear growth of the causal pathogen was completely inhibited by compost tea at the concentration of 60% and the germinated conidia at the concentration of 40%. In all cases, the conidiospores were more sensitive to the tested bioagents and compost tea than the mycelium of the pathogenic fungus.

The tested bioagents resulted in significant reduction to eggplant wilt with significant increase to the plant height as

well as the number of fruits and their weight / plant when added to soil infested with the causal pathogen compared with infestation with the causal pathogen only. However, Topsin M-70 was the superior treatment in decreasing the severity of the disease and increasing plant height and the produced fruit yield. In addition, the two tested bioagents were more efficient in reducing the severity of the disease and increasing values of plant height and fruit yield number and weight / plant) when the soil was amended with compost compared with un-amended soil with compost. This may be due to compost comprises a substrate medium for the growth and reproduction of the two bioagents, hence increasing mode of action of two bioagents. The obtained are in the harmony with the reported results by [1], [2], [6]. [22]. [26], [30], [39] [40].

Colonization of plant roots by selected strains of nonpathogenic bacteria, such as various species of the genus *Bacillus* [19] can induce a distinct broad-spectrum resistance response in both below- and above-ground parts of the plant. This type of resistance to diseases is named as induced systemic resistance (ISR) [11], [32]. The fungus *Fusarium oxysporum* is one of soil-borne plant pathogens and is widely distributed in various soil types worldwide. Recently, there has been a growing interest in nonpathogenic bacteria due to their efficacy as bioagents in many crops [2], [3], [23], [30], [40]. Application of some *Bacillus* strains to the seedlings has been found to be effective for suppressing soil-borne diseases and has successfully induced systemic resistance in the treated plants [19], [29]. Elicitation of ISR by *Bacillus* strains has been demonstrated in greenhouse or field trials on tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, *Arabidopsis* sp., cucumber, loblolly pine, and two tropical crops [19], [29].

Bacillus spp. grow very fast and occupies the court of infection and preventing pathogen spores to reach susceptible tissues in competition for spaces [36]. This might be due to that treatments with biopreparation induce systemic resistance as the main mechanism of activity on the plant. Also, [39] reported that *B. subtilis* CAS15 has great potential for plant growth promotion and biological control, where reduced the incidence of Fusarium wilt in pepper significantly, by 12.5–56.9 % due to induced systemic resistance. They added that there were significant increases in plant height also enhanced the yield of pepper by shortening the time to 50 percent flowering to 17.26 days, increasing the average fruit weight 36.92%, and increasing the average yield per plant 49.68%. This research showed that *B. subtilis* CAS15 has great potential for plant growth promotion and biological control.

It is supposed that *Bacillus* spp. could be have diverse plant response involved in synthesis and accumulation of antimicrobial phytoalexins [14], [18], induction of hypersensitive response [15], production of defense-related proteins [38] production of activated oxygen species [8] and modification of plant cell wall by deposition of callose [34].

Protection of plants from disease by induction of systemic resistance is a new approach. This is much less harmful to the environment as compared to deadly agrochemicals applied to control plant diseases.

The bioactivity of *P. fluorescens* might be due to produces different types of antibiotics including active 2, 4 diacetyl-phloroglucinole (2, 4 DAPB), which control diseases and/or due to that *P. fluorescens* has several methods to control the disease such as production of antifungal compounds including siderophre production, nutrient competition and the induction of systemic resistance [25]. Also, [20] reported that the reduction in the infection by plant pathogens and the increase in the plant length and fresh weight of the treated plants might be due to *P. fluorescens* produces of indole acetic acid as a growth regulator as well as some antibiotic, *i.e*. pyrrolnitrin, pyoluterin and 2, 4 diacetyl phloroglucino.

Compost is considered one of the different available sustainable approaches that may be used to prevent, mitigate or to control plant diseases. Organic amendments play an important role as environmentally friendly and sustainable alternative approach to protect plants against soil borne pathogens. Soil amendments, using composted agricultural wastes fortified with biocontrol agents could be acceptable approaches in this regard. The use of organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases. It has been reported that several composts and/or composts fortified with bioagent used as soil amendments reduced pathogens propgules density and protected plants from soil-borne plant pathogens [17], [37]. Therefore, although disease control effectiveness by compost can be variable [30], the economic and environmental benefits deriving from its use can win any form of distrust that could hover on operators. In addition, it is supposed that compost may be acts as a nutrient source and shelter for the bioagents that compete with plant pathogens, for those organisms that prey on and parasitize pathogens, and for those beneficial that produce antibiotics.

It has been found that *B. subtilis*, *P. fluorescens* and compost resulted in considerable higher production of phenol compounds without great variation in their values compared with control treatment and soil infested with *F.o.* f.sp. *melongenae*. A great increase was observed in the total phenol compounds of plants treated with compost followed by the two tested bioagents..

Phenol compounds content are compounds whose quantity raised when a plant comes under attack by a pathogen [33], [35]. Systemic induction of phenol compounds under influence of bacterial strains was first reported by [33]. However, this alone is not reliable for indication of disease resistance in plant tissues [35]. [3] reported that a significant increase in total phenol contents was observed in bacterial-treated plants. They added that pathogen alone was able to induce phenol formation in plants but with slightly increased levels. Similar results were obtained [1], [35].

5. Conclusion

Biological control is considered an important approach of

agricultural biotechnology in recent years for controlling many soil-borne plant pathogens, which considers an environmentally friendly and sustainable alternative approach for disease management. The obtained results give a potential of the combination of *B. subtilis* and *P. fluorescens* with compost as efficient protection agents against Fusarium wilt of eggplant.

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