Effect of the combination among compost , bioagents andsoilsolarization on management of strawberry Verticillium wilt

Abada K. A.[1],Eman O.Hassan [2]
1 Plant Pathol. Dept., Fac. Agric., Cairo Univ., Giza, Egypt
2 Plant Pathol. Dept., Fac. Agric. at Moshtohor, Benha Univ., Egypt
Email address: ebadah2013@gmail.com

Abstract---Isolation trials from strawberry plants showing mainly wilt symptoms grown at Behera, Ismailia, Menofia, Kalubia and Giza governorates yielded *Alternaria* spp., *F.oxysporum* , *F.solani, Rhizoctonia solani* and *Verticillium dahliae*. Pathogenicity test of the five isolates of *V.dahliae* revealed that they caused wilt symptoms and Ismailia isolate was the most virulent one. Five isolates of *Bacillus* spp., *i.e. Bacillus coagulans* , *B.pumilus* , *B.megaterium, B.subtilis* and *B. thuringiensis* and two isolates of *Pseudomonas* spp., *i.e. P. fluorescens* and *P.putida* were isolated from the rhizospheric soil of apparently healthy strawberry plants grown in a field have severe infection by Verticillium wilt and screened for their efficacy against *V.dahliae*, *in vitro* and *in vivo* experiments. In general, *P.fluorescens* followed by *Bacillus subtilis* were the most efficient in reducing the linear growth of the pathogenic fungus. Culture filtrate of *B.subtilis* and *P.fluorescens* as well as the aqueous filtrate of compost resulted in significant reduction to the germinated conidiospores of the causal fungus compared with the control . This reduction was gradually increased by increasing the tested concentration. In addition, culture filtrate of the compost was more efficient than the culture filtrate of the tested bioagents. The combination among compost, the bioagents *B.subtilis* and *P.fluorescens* and soil solarization resulted in significant reduction to strawberry Verticillium wilt with significant increase to the produced fruits and their total soluble solids (T.S.S.) , either each of them was used alone or in their different combinations, compared with the control treatment (infested with the causal fungus). On the other hand, soil solarization was the most efficient in this regard compared with the other two items of disease management when each of them was used alone. Moreover, no apparent infection was detected when the bioagents *B.subtilis* and *P.fluorescens* ,compost and soil solarization were used together and the produced fruit yield and it´s T.S.S. were , to somewhat, similar to the control treatment (uninfested soil with any fungus).

Keywords: Bacterial bioagents, compost, fruit yield, management, strawberry, total soluble solids and Verticillium wilt.

1 Introduction

Tr Strawberry (*Fragaria* × *ananassa*) is one of the most important and delicious untraditional crops in Egypt for local consumption and exportation. It is liable to infection by many soil borne fungi. However, Verticillum wilt poses a serious threat to commercial strawberry production worldwide and causes severe economic losses (Attia *et al.,* 1989 ; Berg *et al.*,2000; De Coste *et al.,* 2010 and Perez-Jimenez *et al.*,2012).

Strawberry is enjoyed by millions of people in all kinds of climates including temperate, Mediterranean, subtropical and taiga zones.

During the last two decades many complaints have been received from strawberry growers due to the death of strawberry plants due to the infection by wilt beginning from beginning of March until end of the growing season due to the infection by Verticillium wilt, caused by *Verticillium dahliae*. The fungus is a widespread in the most strawberry-production areas. The pathogen is soil-borne and infects plants through roots (Hanson, 2000 and Subbarao *et al.,* 2007). It invades the plant's vascular system and prevents transport of water and nutrients (Kiraly *et al.,* 1970). The fungus is a polyphage and it can infect about 300 species of host plants, including many fruit plants, vegetables, forest trees, shrubs and flowers, as well as numerous weeds and some field crops. Also, survives in soil as microsclerotia which are produced in the dying tissues of the host plant. These structures can survive over a range of soil moisture and temperature conditions but loss of viability most rapidly in wet, warm soil (Uppal *et al.*, 2008).

Yellowing, chlorosis and necrosis of lower leaves are usually the first symptoms of Verticillium wilt. Premature defoliation, most likely due to the drop in tissue turgor (Boote *et al.,* 1983) and/or to the effect of *V. dahliae* phytotoxic compounds (Meyer *et al*., 1994 and Subbarao *et al.,* 2007) often occur. Younger leaves tend to remain green although stunted. Brownish streaks occur in vascular tissue of crown roots or at the base of the petiole. The losses can reach even 80% of plants under favourable environmental conditions. Control of the disease is very difficult, because of lack of effective fungicides. The fungus grows into the xylem where it colonizes the plant through mycelial growth and conidial production. Fluid movement in the xylem passively transports the conidia. Once in the

xylem, this fungus partially blocks water movement and produces toxins that result in wilt symptoms. Isolates that can attack strawberry have a wide host range, so it is not advisable to plant strawberry after Solanaceous plants (Subbarao *et al.,* 2007).

Soil biology is directly linked to agricultural sustainability as it is the driving force behind decomposition processes that break down complex organic molecules and substances and convert them into plant available forms (Friedel *et al.*, 2001). Large, stable, and active soil microbial communities are important for sustaining the productivity of soils under sustainable and organic farming systems. To develop such systems growers adopt strategies such as crop rotation, cover cropping, and application of organic amendments (manures and composts) that significantly increase soil organic matter and improve soil biology and quality (Buyer *et al.*, 2010).

The demand for organic products is also increasing as people become aware of the benefits of organic produce. This has led to increase the consumer interest in organically grown vegetables including those produced in greenhouses. One of the core philosophies of organic production systems is the development of healthy and productive soil that provides essential nutrients for plant growth, supports diverse and active soil biotic communities and balances the entire farm ecosystem. There is a growing demand for organic products since more and more consumers feel that they are healthier than those conventionally grown (Yiridoe *et al.*, 2007). Globally there are about 37,232,127 ha of organically managed land with Australia accounting for 32.2% (Paull, 2011). It has been reported that overall organic produce contains 5.7% more micronutrients than comparable conventionally grown produce (Hunter *et al.*, 2011).The control of Verticillium wilt is currently accom-plished primarily through the use of soil fumigation by methyl bromide as well as fungicides and, to somewhat, resistant cvs. However the frequent and discriminate use of soil fumigation and fungicides leads to atmosphere pollution and create imbalance in the microbial community, which maybe unfavorable to the activity of beneficial organisms and may lead to development of resistance strains of the pathogen (Martin and Bull, 2002).

In recent years biological control has become a promising safer and ecologically acceptable alternative to chemical control in the management of soil borne diseases (Abada and Ahmed, 2014; Abada *et al.*, 2014 and Ragab *et al.*,2015). Among the bacterial bioagents, genera *Bacillus* and *Pseudomonas* received more attention than many other bacterial groups (Santoyo *et al.*, 2012).

Due to strawberry is consumed mainly as fresh or canned, therefore disease management rather than chemical control must be done. In this regard, biological control has emerged as an alternative and most promising means of the management of plant pathogens. Biocontrol of Verticillium wilt of strawberry can be achieved by either promoting the native antagonists such as that found in compost to reach a density sufficient to suppress pathogen(s) or by introducing alien antagonists. Among the several antagonists tested by various scientists, genera of *Bacillus* and *Pseudomonas* …. etc., have been found effective in inhibiting the causal of many soil borne pathogens (Martín and Bull, 2002; Fang *et al.*,2012 ; Abada and Ahmed, 2014 ; Abada *et al.*,2014 ; Juber *et al.,* 2014 and Ragab *et al.,* 2015). Though introduction of several antagonists against this pathogen seems to hold great promise to suppress the disease and have been found effective in inhibiting the growth of the tested fungus under *in vitro* conditions.

Pinkerton *et al.*(2002) reported that following soil solarization, growth of microflora beneficial to plant growth or antagonistic to pathogens and pests may slow the reinfestation of soil by these organisms for more than one growing season and increase plant growth and yield of annual and perennial field crops. In addition, the availability of increased mineral nutrients following solarization may reduce crop fertilization requirements.

The present investigation aimed to investigate the role of compost in combination with the bioagents and soil solarization in management of strawberry Verticillium wilt.

2. Materials and Methods

2.1.Isolation, purification and identification of the associated fungi to Verticillium wilt :

Strawberry plants showing characteristic wilt symptoms were collected from Behera, Ismailia ,Menofia , Kalubia and Giza governorates. The infected crown 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 distilled water to eliminate excess sodium chlorite 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. Cultures of the isolated fungi was obtained by single spore technique or hyphal tip method and maintained on PDA slants throughout the investigation. The emerged fungi were identified depending on cultural, morphological

characteris-tics and the description of Hawksworth and Talboys (1970),Booth (1971) and Domsch *et al*. (1980).

2.1.Isolation, purification and identification of the antagonistic bacterial :

Soil samples collected from the rhizospheric soil of apparently healthy strawberry plants grown in a field have severe infection by Verticillum wilt, were used to isolate the antagonists. Serial dilution plate technique (Johnson and Curl, 1959) was used to isolate native antagonistic *Bacillus* spp. and *Pseudomonas fluorescens* on nutrient agar medium (Oedjijono and Dragar,1993).The isolated bacteria were then purified and identified depending on the description of Parry *et al.* (1983) and Holt and Krieg (1984). The identification was confirmed by the Biolog System technique (Biological control of faba bean chocolate spot disease project, Plant Pathol. Res. Inst., A.R.C., Giza, Egypt).

2.2.Pathogenicity test of the five isolates of *V.dahliae* :

Formalin disinfested clay soil was infested by 2 % inoculum level of the five isolates of *V.dadliae* each alone and distributed in Plastic post (25 cm in diameter). Oso Grandee strawberry cv. transplants were dipped in 1 % of the fungicide Maxim (Fludioxonil 21% + Metalaxyl-M and S-isomer 8.4%) for 30 minutes then 2 transplants were transplanted in each pot. Transplanted transplants in uninfested soil were used as control. The severity of Verticillium wilt was assessed four months after transplanting. Plant growth vigour (+= poor growth , ++= good growth and +++= excellent growth) was also noticed and recorded.

2.3. Effect of the culture filtrate of some bioagents on the linear growth of the tested pathogen:

The effect of the culture filtrate of the five isolates of *Bacillus* spp. as well as *Pseudomonas fluorescens* and *P. putida* on the growth of the causal pathogen was studied as a method given by Dennis and Webster (1971). One 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 a loop of the bioagent(s) 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. The culture filtrate of the bioagents was mixed with the component of PDA medium in different proportion (25, 50 , 75 and 100%). The medium was then sterilized by steamer for three successive days and poured into Petri-dishes (20 ml/plate). After solidification the Petri-dishes were carefully inoculated with 5 mm. discs of t he test pathogen cut from the five day old culture. PDA plates inoculated with the test pathogen, but not amended with culture filtrate of th e bioagents were maintained as control. Plates were then incubated in an incubator at 30±2°C. Five replicates were maintained for each treatment. Periodic observations on the linear growth of the tested fungus were recorded. Inhibition percentage of the mycelial growth of tested pathogen was calculated by the formula:

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

Where;

I = Percent of inhibition in growth of test pathogen,

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

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

2.4. Effect of aqueous filtrate of soaked compost on the linear growth of the causal pathogen:

One kg. compost was soaked overnight in three litre water then filtrate through two layers of Whitman 1 filter paper. The counted amounts of potato broth, dextrose and agar were added to 20, 40, 60 and 80% of the filtrate and steamed for three successive days then poured in sterilized Petri-dishes. After solidification the Petri-dishes were carefully inoculated with 5 mm. discs of the test pathogen cut from the five day old culture. PDA plates inoculated with the test pathogen, but not amended with compost filtrate (normal PDA) were maintained as control. Plates were then incubated in an incubator at 30±2°C. Five replications were maintained for each treatment. Periodic observations on the linear growth of the tested fungus were recorded . Inhibition percentage of the mycelial growth of tested pathogen was calculated as mentioned before.

2.5. Effect of the culture filtrate of *B.subtilis* and *P.fluorescens* as well as the aqueous filtrate of compost on the germination of the conidiospores of *V.dahliae*:

V.dahliae was left to grow on PDA medium for one week at 30±2˚ C . Ten ml. of any of the concentration of 0.0, 20, 40.60 and 80 % of the culture filtrate of the bioagents *B.subtilis* and *P.fluorescens* as well as the aqueous filtrate of compost were added to each *V.dahliae* Petri-dish and left for 24 h. at 30±2˚C. Five dishes were used for each treatment. The culture filtrate

of both bioagents and aqueous filtrate of compost were mixed well with the mycelial growth and the conidiospores of the causal fungus in the Petri-dishes using of sterilized camel brush to make spore suspension. One drop from the spore suspension of any concentration was added to a slide glass and one drop of lactophenol-cotton blue was added the spore suspension and examined under light microscope . The number of the germinated conidiospores in 100 conidia was counted and the average of five readings was recorded for each concentration of the tested bioagents and compost.

2.6. Effect of combination among compost , the two bioagents *B.subtilis* and *P.fluorescens* and soil solarization on management of Verticillium wilt and fruit yield:

In the present investigation, transplants of Oso Grandee strawberry cv. taken from Strawberry Development Centre, Fac. Agric., Ain Shams Univ. were used. The pathogen was isolated from strawberry crowns by tissue segment method on PDA medium. The highly antagonistic bioagents *B.subtilis* and *P. fluorescens* against the test pathogen *in vitro* were used in management of strawberry Verticillium wilt in combination with compost and soil solarization. The upper 20 cm. layer of each plot ($1m^2$), located in the experimental unit of Plant Pathol. Dept., Fac., Cairo Univ., was infested with 2 % inoculum level (grown on sterilized corn-sand medium in 500 ml. glass bottles) of the tested pathogen. The plots were divided into the following treatments:

1. Three infested plots with the causal pathogen received 2 kg compost for each plot (mixed thoroughly with the upper 20 cm soil layer) two weeks before transplanting strawberry transplants.
2. Three infested plots with the causal pathogen were infested with the bioagent *B.subtilis* ($1x10^6$ cfu/ L water) at the rate of 2 L / plot two weeks before transplanting strawberry transplants.
3. Three infested plots with the causal pathogen were infested with the bioagent *P.fluorescens* ($1x10^6$ cfu/ L water) at the rate of 2 L / plot two weeks before transplanting strawberry transplants.
4. Three infested plots with the causal pathogen were solarized with proliferated plastic sheets (50 µ thick) during end of August , 2015 for 45 days before preparing to transplanting strawberry transplants .
5. Three infested plots with the causal pathogen received 2 kg compost for each plot and infested with the bioagent *B.subtilis* ($1x10^6$ cfu/ L water) at the rate of 2 L / plot two weeks before transplanting strawberry transplants.
6. Three infested plots with the causal pathogen received 2 kg compost for each plot and infested with the bioagent *P.fluorescens* ($1x\ 10^6$ cfu/ L water) at the rate of 2 L / plot two weeks before transplanting strawberry transplants.
7. Three infested plots with the causal pathogen received 2 kg compost for each plot and solarized as mentioned before.
8. Three infested plots with the causal pathogen were infested with both bioagents $1x10^6$ cfu/ L water) at the rate of 2 L / plot from each bioagent two weeks before transplanting strawberry transplants.
9. Three infested plots with the causal pathogen were solarized as mentioned before and infested with the bioagent *B.subtilis* at the previous rate after solarization , two weeks before transplanting strawberry transplants.
10. Three infested plots with the causal pathogen were solarized as mentioned before and infested with the bioagent *P.fluorescens* at the previous rate after solarisation, two weeks before transplanting strawberry transplants.
11. Three infested plots with the causal pathogen received 2 kg compost for each plot , solarized as mentioned before and then infested with both bioagents at the previous rate after solarization and two weeks before transplanting strawberry transplants.
12. Three infested plots with the causal pathogen were left without another treatment.
13. Three un-infested plots with the pathogen were left without any treatment.

The plots were irrigated each week for two times and transplanted with apparently healthy Oso Grandee strawberry cv. (grown in fumigated nursery with methyl bromide) . The transplants (Frigo transplants) were dipped in 1% of the fungicide Maxim for 30 minutes just before transplanting (end of October, 2015) to make sour that the transplants were uninfected with any fungal pathogen. Twelve transplants were transplanted in each plot. The plots were irrigated when it was necessary and fertilized with the recommended doses as recommended by Min. of Agric. and Land reclamation.

Disease severity was assessed six months after transplanting and the average was recorded. Also, the produced mature fruits were harvested periodically and the average was recorded. Furthermore, T.S.S. of five randomly fruits were measured using hand fractometer after harvest and the averages were recorded.

2.7. Disease assessment :

The plants were rated for vascular and leaf discoloration using the devised scale (0-5) by Ulloa *et al*. (2006) after modification as follows:

Where:

0 = No discoloration on the leaves and the crown (healthy),

1 = Light discoloration evident on the leaves and as spotty areas in the cross-section of the crown,

2 = More continuous discoloration on the leaves and covering an area between one quarter and one half of the cross-section of the crown but light in colour,

3 = L eaves and vascular of moderate discoloration (moderate in color) evident in a band enci rcling almost the entire crown cross-section,

4 = Most leaves yellowish and vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a cross section of the crown, and

5 = P lants severely damaged, leaves seemed to be burned and vascular discoloration evident throughout cross-section of the crown.

Disease severity was assessed using the following formula :

$$\text{Disease severity \%} = \sum (n \times V) \ / \ 5\,N \times 100$$

Where:

n = Number of the inspected samples in each category.

v = Numerical values of each category.

N = Total number of the inspected samples.

5= The highest grade scale.

2.9. Statistical analysis:

Data were statistically analyzed using the standard procedures for split design as mentioned by Snedecor and Cochran (1967). The a verages were compared at 5% level using least significant differences (L.S.D) according to Fisher (1948).

3.Results

3.1. Isolation, purification and identification of the associated fungi to Verticillium wilt:

Isolation trials from strawberry plants (Oso Grandee cv.) showing characteristic symptoms of wilt collected from Behera, Ismailia , Men ofiya, Kalubia and Giza governorates yielded 120 fungal isolates belonging to four genera(Table, 1). The isolated fungi were purified and identified as : *Alternaria* spp. *Fusarium oxysporum, F.solani , Rhizontonia solani Verticillium dahliae* . The fungus *F.oxysporum* recorded the highest occurrence and frequency , being 37 isolates of 30 .8 % frequ ency followed by *V. dahliae* , being 26 isolates and 21.7% frequency then *R.solani* ,being 23 isolates and 19.2 % frequency . Both *F.solani* (18 i solates and 15.0 % frequency) and *Alternaria* spp.(16 isolates and 15.8% frequency) recorded the lowest occurrence and frequency. The occurrence of the isolated fungi from the five governorates were not greatly differed. The isolates of the fungus *V.dahliae* were selected and tested for their pathogenicity to choose the most virulent one .

Table 1. Occurrence and frequency of the isolated fungi from strawberry plants showing wilt symptoms collected from five governorates.

The isolated fungi	Occurrence of the isolated fungi at					Total	% Frequency
	Behera	Ismailia	Menofia	Kalubia	Giza		
Alternaria spp.	4	3	2	3	4	16	15.8
F.oxysporum	7	6	7	9	8	37	30.8
F.solani	3	5	4	3	3	18	15.0
R.solani	4	3	6	5	5	23	19.2
V.dahliae	5	4	6	6	5	26	21.7
Total	23	21	25	26	25	120	------

3.2. Pathogenicity test of the five isolates of *V.dahliae:*

Pathogenicity test of the five isolates of *V.dahliae* isolates (Table,2) reveal that they were pathogenic to Oso Grandee strawberry cv. and showing typical wilt symptoms on the foliage growth and the xylem vascular. Results also, indicate that the isolate of Ismailia governorate was t he most virulent one than the other isolates (50.9% wilt severity) and of poor plant growth vigor (+) followed by the isolate of Behera governorate

(48.6% wilt severity and + growth vigor) the isolate of Kalubia governorat (47.3 % wilt severity and + growth vigor). Meanwhile, both isolates of Giza an d Menofia governorate resulted in the lowest figures of wilt severity (34.4 and 36.2 respectively) an d good growth vigor (++). No apparent symptoms of wilt were observed on control plants and showed excellent growth vigor (+++). Therefore, isolate of Ismailia governorate was used in the following experiments.

Table 2. Pathogenicity test of the five isolates of *V.dahliae* using transplants of strawberry plants (Oso Grandee cv.) , greenhouse experiment.

Governorate's isolates	%, Disease severity	Plant Growth vigor
Behera	48.6	+
Ismailia	50.9	+
Menofia	36.2	++
Kalubia	47.3	+
Giza	34.4	++
Control	00	+++

3.3. Effect of aqueous filtrate of compost on the linear growth of the causal pathogen :

Data presented in Table (3) show that the aqueous filtrate of compost caused significant reduction to the linear growth of *V.dahliae* , seven days after incubation at 30±2°C compared with the control treatment This reduction was gradually increased by increasing the concentration. In add ition, the causal fungus failed to grow on the concentration of 80 % .

3.4. *In vitro* effect of four *Bacillus* spp. as well as *P.fluorescens* and *P.putida* on the linear growth of the causal Pathogen :

Results shown in Table (4) reveal that all the tested isolates of *Bacillus* spp. as well as *P.fluorescens* and *P.putida* resulted in significant inhibition to the linear growth of t he causal fungus, 7 days after incubation at 30±2°C compared with control treatment. This reduction was gradually increased by increasing the concentration.

Table 3. Effect of aqueous filtrate of c ompost linear growth *V.dahliae*,7 days after incubation at 30±2°C.

Conc.(%)	Average linear growth (mm)
20	72.6
40	41.2
60	26.0
80	0.0
Control	90.0
L.S.D. at 5%	2.5

Table 4. *In vitro* effect of five *Bacillus* spp. as well as *P.fluorescens* and *P.putida* culture filtrate on the linear growth of *V.dahliae* ,7days after incubation at 30±2°C.

Bioagents	Linear growth (mm) at concentration of (%) 0.0	20	40	60	80	Mean
B.coagulans	90.0	86.2	79.0	41.8	16.2	62.6
B.megaterium	90.0	82.8	72.6	36.0	11.6	58.6
B.pumilus	90.0	86.6	79.4	42.0	17.0	63.0
B.subtilis	90.0	78.2	66.0	31.6	0.0	53.2
B.thuringiensis	90.0	85.8	78.8	43.2	16.2	62.8
P.fluorescens	90.0	79.4	68.2	30.4	0.0	53.6
P.putida	90.0	82.6	70.4	33.0	10.0	57.2
Mean	90.0	83.1	73.5	36.9	10.1	----

L.S.D. at 5% for: Bioagents (B) = 2.1, Conc. (C)= 2.7 and B x C = 3.9 .

In addition, the causal fungus greatly affected by both *B.subtilis* and *P.fluorescens* , being 53.2 and 53.6 mm., respectively followed by *B.megaterium* (58.6 mm.) . Also, it failed to grow on the concentration of 80 % of both *B.subtilis* and *P.fluorescens*. Meanwhile, isolates of *B.coagulans* and *B.pumilus* were the lowest efficient in this regard , being 62.6 and 63.0 mm., respectively.

3.5. 3.5. Effect of the aqueous filtrate of compost as well as the culture filtrate of *B.subtilis* and *P.fluorsecens* on the germination of the conidiospores of *V.dahliae:*

Table (5) indicates that the aqueous filtrate of compost as well as culture filtrate of *B.subtilis* and *P.fluorescens* resulted in significant reduction to the germinated conidiospores of the causal fungus compared with the control . This reduction was gradually increased by increasing the tested concentration. In addition, aqueous filtrate of the compost was more efficient than the culture filtrate of the tested bioagents.

Table 5. Effect of the aqueous filtrate of compost and culture filtrate of *B. subtilis* and *P.fluorescens* on conidial germination, 25 h. after incubation at 30±2 C.

Treatments	% Conidial germination at conc. 0.0	20	40	60	80	Mean
Compost	91.6	69.4	50.0	16.6	0.0	45.5
B.subtilis	91.6	73.8	56.4	31.6	0.0	50.7
P.fluorescens	91.6	71.0	54.2	30.4	0.0	49.4
Mean	91.6	71.4	53.5	26.2	0.0	----

L.S.D. at 5% for: Treatments (T) = 2.7, Conc. (C)= 2.3 and T x C = 3.5 .

Table 6. Effect of combination among compost, the bioagents *B.subtilis* and *P.fluorescens* and soil solarization on the management of strawberry Verticillium wilt (Oso Grandee cv.) as well as fruit yield and it ′s T.S.S., plot experiment.

Treatments	% Wilt severity	Weight of fruits (kg)/ plot	Total soluble solids
Compost (C)	9.5	8.8	17.5
B.subtilis (BS)	9.8	8.3	17.7
P.fluorescens (PF)	9.6	8.6	17.8
Solarization (S)	9.0	8.0	17.9
C +BS	7.0	9.6	17.4
C + PF	7.7	9.7	17.6
C+S	6.2	9.5	17.8
BS + PF	7.0	9.2	17.9
BS + S	6.5	9.5	17.9
PF+S	5.8	9.6	17.0
C+ BS+PF+S	0.0	10.3	18.1
Control (Infested soil)	43.9	4.5	13.3
Control (Un-infested soil)	0.0	10.5	18.3
L.S.D. at 5 %	2.2	1.8	1.4

3.6. Effect of combination among compost , the two bioagents *B.subtilis* and *P. fluorescens* and soil solarization on management of Verticillium wilt and fruit yield:

Results shown in Table (6) indicate that combination among compost, the bioagents *B.subtilis* and *P.fluorescens* and soil solarisation resulted in significant reduction to strawberry Verticillium wilt with significant increase to the produced fruits and their total soluble solids (T.S.S.) , eit her each of them was used alone or in their different combinations, compared with control treatment (inf ested with the causal fungus). On the other hand, soil solarization was the most efficient in this regard compared with the other

two items of disease management when each of them was used alone, being 9.0, 9.5, 9.8 and 9.6% . Moreover, no apparent infection was detected when compost, the bioagents *B.subtilis* and *P.fluorescens* and soil solarization were used together and the produced fruit yield and it´s T.S.S. were, to somewhat, similar to the control treatment (uninfested soil with any fungus).The highest disease se verity and the poor fruit yield as well as T.S.S. were recorded for strawberry plants grown in soil infested with the causal fungus .

4. DISCUSSION

Nowadays, the rising awareness of the adverse effects of chemical pesticides, people are looking for organically grown vegetables , where they are consume freshly. Due to the great hazards of such pesticides on the human health, people are increasingly choosing organic foods due to the perception that they are healthier than those conventionally grown. Vegetable crops including strawberry are vulnerable to a range of pathogenic organisms that reduce yield by killing the plant or damaging the product. thus making it unmarketable (Mass, 1998).

Soil-borne diseases are among the major factors contributing to low yields of organic produce. In addition, there are several methods that can be used to protect crops from soil-borne pathogens rather than chemical control. These include the introduction of bioagents against soil-borne plant pathogens , resistant cultivars , cr op rotation , sanitary methods , organic soil amendments that stimulate antagonistic activities of microorganisms to soil-borne diseases and soil solarization (Mass ,1998 ; Abada *et al.,*2014 ; Pinkerton *et al.,*2002 and Shafique *et al.,* 2016).

Isolation trials from strawberry plants (Oso Grandee cv.) showing characteristic symptoms of wi lt collected from Behera, Ismailia , Menof iya, Kalubia and Giza governorates yielded 120 fungal isolates belonging to four genera. The isolated fungi were purified and identified as : *Alternaria* spp. *Fusarium oxysporum, F.solani , Rhizontonia solani* and *Verticillium dahliae* . The fungus *F.oxysporum* recorded the highest occurrence and frequency followed by *V. dahliae* then *R.solani*. Both *F.solani* and *Alternaria* spp. recorded the lowest occurrence and frequency. The occurrence of the isolated fungi from the five governorates were not greatly differed. The isolated fungi were previously isolated by Abada (1986); A ttia *et al.*(1989); Mass (1998) ; Fang *et al.* (2012); Perez-Jiménez *et al.*(2012) and Abada *et al.*(2014) .The isolates of the fungus *V.dahliae* were selected and tested for their pathogenicity to choose the most virulent one , where Fusarium wilt was previously investigated (Abada *et al.*, 2014). Pathogenicity test of the five isolates of *V.dahliae* revealed that they caused wilt symptoms and Ismailia isolate was the most virulent one followed by Behera then Kalubia isolate . Meanwhile, both Menofia and Giza isolates were the lowest pathogenic ones.

As dessert strawberry cultivars became more popular in commercial production, Verticillium wilt has developed into great problem. Also banning fumigants such as methyl bromide from practical use and lack of cultural practices for effective disease control, stimulate efforts to supplement chemical protection with new biological treatments (Hanson, 2000).

Many organisms, that occur naturally in the environment, have the ability to interfere with pathogens (Uppal *et al,*. 2008). All the tested isolates of *Bacillus* spp. as well as *P.flurescens* and *P.putida* resulted in significant inhibition to the linear growth of the causal fungus, 7 days after incubation at 3 0±2°C compared with control treatment. This reduction was gradually increased by increasing the concentration. In addition, the causal fungus greatly affected by both *B. subtilis* and *P.fluorescens* followed by *B.megaterium* . The pathogen failed to grow on the concentration of 80 % of both *B.subtilis* and *P.fluorescens*. Meanwhile, isolates of *B.coagulans* and *B.pumilus* were the lowest efficient in this regard. (Blumer and Haas (2 000) mentioned that a few b acterial species are kn own to produce and excrete hydrogen cyanide (HCN), a potent inhibitor of c ytochrome-c oxidase and several other meta-lloenzymes. Haas and Defago (2005) declared that particular bacterial strains in certain natural environments prevent infectious diseases of plant roots. How these bacteria achieve this protection from pathogenic fungi has been analyzed in detail in biocontrol strains of fluorescent pseudomonads. During root colonization, these bacteria produce antifungal antibiotics, elicit induced systemic resistance in the host plant or interfere specifically with fungal pathogenicity factors. 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 aqueous filtrate of compost caused significant reduction to the linear growth of *V.dahliae* , seven days after incubation at 30±2^{O}C compared with the control treatment This reduction was grad ually increased by increasing the concentration. In addition, the causal fungus failed to grow on the concentration of 80 % .The aqueous filtrate of compost as well as culture

filtrate of *B.subtilis* and *P.fluorescens* resulted in significant reduction to the germinated conidiospores of the causal fungus compared with the control . This reduction was gradually increased by increasing the tested concentration. In addition, culture filtrate of the compost was more efficient than the culture filtrate of the tested bioagents.The obtained results are of great valuable, where the treatment with such bioagents and compost must be done before transplanting strawberry transplants in soil infested with *V.dahliae* to cause their drastically effect on both the mycelial growth and the germinated conidiospores to lowering the inoculums level of the fungus. Suppression of *V.dahliae* by compost will most likely occur through biological control in the soil and in the rhizosphere. Predation, parasitism, antibiosis, and competition are all means of suppressing pathogens, which interfere with a pathogen's success at infecting the root cortex.

It has been found that compost , the bioagents *B.subtilis* and *P.fluorescens* and soil solarisation resulted in significant reduction to strawberry Verticillium wilt with significant increase to the produced fruits and their total soluble solids (T.S.S.) , either each of them was used alone or in their different combinations, compared with the control treatment (infested with the causal fungus). Soil solarization was the most efficient treatment in this regard compared with the other three items of di sease management, *i.e.* the bioagents *B.subtilis* and *P.fluorescens* as wel l as soil compost when each of them was used alone. No apparent infection was observed when the combination among the bioagents *B.subtilis* and *P.fluorescens* ,compost and soil solarization was used and the produced fruit yield and it's T.S.S. ,to somewhat, were similar to the control treatment (uninfested with the causal fungus). Strawberry plants grown in soil infested with the causal fungus recorded the highest disease severity and produced poor fruit yield of low T.S.S.Many researches are act ively trying to f ind metabolites produced by bioagents which will suppress particular diseases (Keel *et al.,* 1992 *;* Abada *et al.,*2014 ; Ragab *et al.,*2015 and Hassan *et al.,*2017). Certain biochemical changes occurring after the application of bioagents can act as markers for induced systemic resistance. These include accumulation of certain enzymes, such as peroxidase (Govindappa *et al.,* 2010). Among the new biological approaches, the stimulation of natural plant defenses is considered to be one of the most promising alternative strategies for crop protection (Walters and Fountaine, 2009). This original biological approach does not exert direct effects on the pathogen (Walters and Fountaine, 2009) but stimulates natural defenses in plants, leading to a systemic acquired resistance (Goel and Paul, 2015).

The induction of plant defense mechanisms was associated with the production of elicitors by the plant-host (endogenous elicitor) (Montesano *et al.,* 2003).

Ramamoorthy *et al.* (2001) mentioned that the treatment with biopreparation induce systemic resistance as the main mechanism of activity on a plant or might be due to *P. fluorescens* produce 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. Moreover, Meena *et al.* (2006) mentioned that the reduction in the infection by the plant pathogens and the increase in the plant length and fresh weight of the treated plants might be due to that *P. fluorescens* produces of indole acetic acid as a growth regulator as well as some antibiotic, *i.e.* pyrrolnitrin, py oluterin and 2, 4 diacetyl phloroglucino. 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 (Kloepper *et* al., 2004). Jacobsen *et al.* (2004) mentioned that Bacillus-based biological control agents (BCAs) have great potential in integrated pest management (IPM) systems; however, relatively little work has been published on integration with other IPM management tools. Unfortunately, most research has focused on BCAs as al ternatives to synthetic chemical fungicides or bactericides and not as part of an integrated management system.

It is well known that soil solarization is a special mulching process which causes hydrothermal disinfestation and other physical and biological changes in soil which are beneficial to plant health and growth. Plastic film laid over moist soil during periods of high air temperature, usually for 1–2 months, can greatly reduce or eradicate a number of pathogens and pests including fungi, bacteria, nematodes, arthropods and weeds. As it was in agreement with, Stapleton *et al.* (1985) and Pinkerton *et al.* (2002). They added that following soil solarization, growth of microflora beneficial to plant growth or antagonistic to pathogens and pests may slow the reinfestation of soil by these organisms for more than one growing season. Increased plant growth and yield of annual and perennial field, row, and nursery crops usually occur following soil solarization. In addition, the availability of increased mineral nutrients following solarization

may reduce crop fertilization requirements The highest efficiency of the combination between soil solarization and any of compost and *B.subtiis* or *P.fluorescens* may be greatly due to the drastic effect of soil solarization on the fungus prop gules make them to be we ek to resist the invasion by the tested bioagents and compost plays a su itable medium for reproduction and establishment of the added bioagents and saprophytic microbes in the s oil. IPM is a sustainable approach to managing pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health and environmental risks. Therefore, this work evaluates the integrated use of genera *Bacillus* and *Pseudomonas* as BCAs with another disease management including compost and soil solarization. This integration is important because the consistency and d egree of disease control by genera *Bacillus* and *Pseudomonas* as BCAs is rarely equal to the control afforded by the best fungicides or bactericides. In theory, integration of several tools brings stability to disease management programs. Integration of genera *Bacillus* and *Pseudomonas* as BCAs with other disease management tools often provides broader crop adaptation and both more efficacious and consistent levels of disease control. In this respect, Noble and Coventry (2003) reported that composts have also been shown to suppress several diseases in the field, although the effects have been generally smaller and more variable than in container experiments.

The disease suppressive effect of compost generally increased with rate of application. Compost inclusion rates of at least 20% (v/v) are normally required to consistently obtain a disease suppressive effect, particularly in peat-based media, but significant disease suppression has been found at lower inclusion rates in soil. Kwok, et al., (1987) declared that copiotrophic bacteria re-colonize composts most rapidly (24-48 h) after peak heating of compost. He added that the predominant biocontrol agents in this group include strains of Bacillus , *Pseudomonas* and *Pantoea* species. In addition, Lockwood (1988) reported that edaphic microorganisms stimulated by compost amendments contribute to t he suppressive activity of the amended soil through four control mechanisms ,*i.e.* antibiosis , competition, predation hyperparasitism and the induction of systemic acquired resistance in the host plant. It is supposed that *Bacillus* spp. could be have diverse plant response involved in s ynthesis and accumulation of antimicrobial phytoalexins (Hammond-Kosack and Jones, 1996), induction of hypersensitive response (He *et al*.,1993).

It has been mentioned that phytopathologists have begun to characterize the determinants and pathways of induced resistance stimulated by bioagents and other non-pathogenic microbes (Park,1995 and Bargabus, *et al.*,2004) .

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