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Effect of combination among bioagents, compost and soil solarization on management of strawberry Fusarium wilt

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Abstract: Isolation trials from strawberry plants showing mainly wilt symptoms grown at Behera, Ismailia, Kalubia and Giza governorates yielded *Fusarium oxysporum , F.solani , Macrophomina phaseolina , Pythium ultimum , Phytophthora cactorum , Rhizoctonia solani* and *Sclerotium rolfsii* . Pathogenicity test of the four isolates of *F.o*xysporum revealed that they caused wilt symptoms and Kalubia isolate was the most virulent one. In addition, inoculating different seven plants, *i.e.* bean, cucumber, eggplant, sweet pepper, strawberry, tomato and water melon with *F. oxysporum* isolate of Kalubia governorate indicated that it caused wilt symptoms to strawberry plants only. Therefore, it named *Fusarium oxysporum* f.sp. *fragariae* Winks & Y.N. Williams. Four isolates of *Bacillus* spp., *i.e. Bacillus coagulans , B.humilus , B.subtils* and *B. thringiensis* and one isolate of *Pseudomonas flurescens* were isolated from the rhizospheric soil of strawberry plants grown in a field have severe infection by Fusarium wilt were screened for their efficacy against *F. o.* f.sp. *fragariae*, *in vitro* and *in vivo*. In general, *P.flurescens* followed by *Bacillus subtilis* were the most efficient in reducing the linear growth of the pathogenic fungus. Sterilized aqueous filtrate of the tested compost resulted in significant reduction to the linear growth of the tested fungus compared with control treatment. This reduction was gradually increased by increasing it's concentration. The combination among the bioagents *B.subtilis* and *P.flurescens*, compost and soil solarization resulted in significant reduction to strawberry Fusarium 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 control treatment (infested with the causal fungus).On the other hand, compost was the most efficient in this regard compared with the other three items of disease management, *i.e.* soil solarization and the bioagents *B.subtilis* and *P.flurescens* when each of them was used alone. Moreover, no apparent infection was detected when the bioagents *B.subtilis* and *P.flurescens* ,compost and soil solarization were used together and produced fruit yield of T.S.S., to somewhat, similar to control treatment (uninfested soil with the causal fungus).

Keywords: Bacterial Bioagents, Compost, Fruit Yield, Management, Strawberry and Total Soluble Solids

1. Introduction

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, Fusarium wilt poses a serious threat to commercial strawberry production worldwide and causes severe economic losses (Attia *et al.,* 1989 ; Fang *et al.,*2011 and 2012 and Juber *et al.,* 2014).

Strawberry is grown in most arable regions of the world. The crop is enjoyed by millions of people in all kinds of climates including temperate, Mediterranean, subtropical and taiga zones.

During the last decade many complains have been received from strawberry growers due to the death of strawberry plants due to the infection by wilt beginning from beginning of March to end of the growing season.

Fusarium wilt of strawberry caused by *F. oxysporum* f. sp.

fragariae is responsible for causing yield losses in commercial strawberry production (Fang *et al.*, 2011 and 2012 and Juber *et al.,* 2014). The causal agent is a haploid fungus and is difficult to control because the pathogen survives as chlamidospores in soil for many years. Early detection and diagnosis of the pathogen in plants and soils is essential for development of an effective disease control strategy. The control of Fusarium wilt disease is currently accomplished 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 & 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 (Shalini and Srivastar, 2007 and Abada and Ahmed, 2014). Among the bacterial bioagents, genera *Bacillus 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 the causal of Fusarium 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 (Fang *et al.,*2012 ; Abada and Ahmed, 2014 and Juber *et al.,* 2014). 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.

Also, Stapleton and Devay (1986) mentioned that soil solarization has been effective as a pre-plant and as a post-plant treatment, and has been compatible with chemical soil treatments and also biological soil amendments after solarization. Soil solarization is a significant advance in the non-chemical control of many pathogens and pests.

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

2. Materials and Methods

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

Strawberry plants showing characteristic wilt symptoms were collected from Behera, Ismailia, 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 20 ± 2°C and observed periodically for growth of the fungi. Axenic culture 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 on the basis of cultural, morphological characteristics and the description of Booth (1971) and Domsch *et al.* (1980).

2.2. Isolation, Purification and Identification of the Bacterial Antagonists

Soil samples collected from the rhizospheric soil of strawberry plants grown in a field have severe infection by Fusarium strawberry 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 flurescens* 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. Instit., A.R.C., Giza, Egypt).

2.3. Pathogenicity Test

Formalin disinfested clay soil was infested by 2 % inoculum level of the four isolates of *F.oxysporum,* each alone and distributed in Plastic post (25 cm in diameter). Camarosa strawberry cv. transplants were dipped in 1% of the fungicide Rizolex-T (Tolcolfos- methyl) for 30 minutes to make sour that the transplants were uninfected with any fungal pathogen then 2 transplants were transplanted in each pot. Transplanted transplants in uninfested soil were used as control. The severity of Fusarium wilt was assessed four months after transplanting. Plant growth vigor (+= poor growth , ++= good growth and +++= excellent growth) was also noticed and recorded.

In addition, bean (Pronco cv.), cucumber (Amera cv.), eggplant(Balady white cv.) , sweet pepper (Balady cv.) , strawberry(Camarosa cv.) , tomato (GS cv.) and water melon (Giza 1 cv.) plants 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 .

2.4. Effect of the Culture Filtrate of the Bioagents on the Linear Growth of the Tested Pathogen

The effect of the culture filtrate of the four isolates of *Bacillus* spp. and *Pseudomonas flurescens* on the growth of *F.o.* f.sp. *fragariae* 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 3 days at 30 ± 2°C . The culture filtrate was filtered through Whatman 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 the test pathogen cut from the five day old culture. PDA plates inoculated with the test pathogen, but not amended with culture filtrate of the 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 test pathogens was calculated by the formula:

I = (C- T)/C X100

Where; I = Percent of inhibition in growth of test pathogen , C = Radial growth of pathogen (mm) in control , T = Radial growth of pathogen (mm) in treatment.

2.5. Effect of Filtrate of Soaked Compost on the Linear Growth of the Causal Pathogen

One kg compost was soaked overnight in one liter water then filtrate through two layers of Whatman 1 filter paper. The counted amounts of potato broth, dextrose and agar were added to 20, 40, 60, 80 and 100% 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 (Khan 2007). Inhibition percentage of the mycelial growth of test pathogens was calculated as mentioned before.

2.6. Effect of Combination among Compost, the Two Bioagents B.subtilis and P.flurescens and Soil Solarization on Management of Fusarium Wilt and Some Crop Parameters

In the present investigation, transplants of Camarosa strawberry cv. taken from Strawberry Development Center, 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.flurescens* against the test pathogen *in vitro* were used in management of strawberry Fusarium 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).

2 Three infested plots with the causal pathogen were infested with the bioagent *B.subtilis* (1x10^6 cfu/ L water) at the rate of one 2 L / plot.

3 Three infested plots with the causal pathogen were infested with the bioagent *P.flurescens* (1x10^6 cfu/ L water) at the rate of one 2 L / plot

4 Three infested plots with the causal pathogen were solarized with proliferated plastic sheets (50 µ thick) during end of August , 2013 for 45 days.

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.

6 Three infested plots with the causal pathogen received 2 kg compost for each plot and infested with the bioagent *P.flurescens* (1x10^6 cfu/ L water) at the rate of 2 L / plot.

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.

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.

10 Three infested plots with the causal pathogen were solarized as mentioned before and infested with the bioagent *P.flurescens* at the previous rate after solarization.

11 Three infested plots with the causal pathogen received 2 kg compost for each plot , solarized as mentioned before and infested with both bioagents at the previous rate after solarization.

12 Three infested plots with the causal pathogen were left without another treatment.

The plots were then irrigated and left for three days and hoe then transplanted with Camarosa strawberry cv. Transplants (frigo transplants) dipped in 1% of the fungicide Rizolex-T for 30 minutes just before transplanting (beginning of October, 2013) 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 on four randomly plants in each plot , 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 each 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 vascular (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 color,

3= Leaves and vascular of moderate discoloration (moderate in color) evident in a band encircling 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 = Plants 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

$$\text{Disease severity \%} = \frac{\sum(\text{nxv})}{5\text{N}} \times 100$$

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.

2.8. Statistical Analysis

Data were statistically analyzed using the standard procedures for split design as mentioned by Snedecor and Cochran (1967). The averages 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

Isolation trials from strawberry plants (Camarosa cv.) showing characteristic symptoms of wilt collected from Behera, Ismailia , Kalubia and Giza governorates yielded many fungal isolates. The isolated fungi were purified and identified as : *Fusarium oxysporum, F.solani ,Pythium ultimum,Phytophthora cactorum, Rhizontonia solani* and *Sclerotium rolfsii* .

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

3.2. Pathogenicity Test of the Four Isolates of F. oxysporum

Pathogenicity test of the four isolates of *F. oxysporum* (Table 1) reveal that they were pathogenic to Camarosa strawberry cv. and showing typical wilt symptoms on the foliage growth and the vascular. Results also, indicate that the isolate of Kalubia governorate was the most virulent one than the other isolates (51.3% wilt severity) and of poor plant growth vigor . Therefore, it was used in the following experiments. Also, testing of bean (Pronco cv.), cucumber (Amera cv) , eggplant (Balady white 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* reveal that the highest infection by the fungus *F. oxysporum* was occurred only to strawberry and no infection was found in case of the other plants. Therefore, the fungus *F. oxysporum* named as *Fusarium oxysporum* f.sp. *fragariae* Winks & Y.N. Williams.

3.3. In Vitro Effect of Four Bacillus spp. and P.flurescens on the Linear Growth of the Tested Pathogen

Results shown in Table (2) indicate that all the tested isolates of *Bacillus* spp. as well as *P.flurescens* resulted in different inhibitory effect to the linear growth of the causal fungus, 5 days after incubation at 30±2°C compared with control treatment. This reduction was gradually increased by increasing the concentration. In addition, the causal fungus greatly affected by both *P.flurescens* and *B.subtilis* , being 32.2 and 33.6 mm., respectively and failed to grow on the concentration of 100 % of both bioagents. Meanwhile, isolates of *B.coagulans* and *B.humilus* were the lowest efficient in this regard , being 44.3 and 43.3 mm., respectively .

Table 1. *Pathogenicity test of the four isolates of F. oxysporum using transplants of strawberry plants (Camarosa cv.) , greenhouse experiment.*

Governorates	% Disease severity	Plant growth vigor *
Behera	42.5	++
Ismailia	48.1	+
Kalubia	51.2	+
Giza	44.3	++
Control	0.0	+++

* Plant growth vigor; += poor growth, ++= good growth and +++ = excellent growth

Table 2. *In vitro effect of four Bacillus spp. and P.flurescens culture filtrate on the linear growth of F.o.f.sp. fragariae , 5 days after incubation at 30±2°C.*

Bioagents	linear growth (mm) at concentration of (%)				
	25	50	75	100	Mean
B.coagulans	73.4	46.6	37.0	20.2	44.3
B.humilus	71.2	45.8	36.8	19.4	43.3
B.subtilis	66.0	41.4	27.0	0.0	33.6
B.thuringiensis	69.6	44.2	33.2	10.0	39.3
P.flurescens	64.8	39.0	25.0	0.0	32.2
Control	90.0	90.0	90.0	90.0	90.0
Mean	72.5	51.2	41.5	23.3	----

L.S.D. at 5 % for: Bioagent s(B)= 2.5, Concentration (C)= 3.3 and B x C= 3.8 .

3.4. Effect of Filtrate of Compost on the Linear Growth of the Causal Pathogen

Table 3. *Effect of filtrate of compost linear growth of F. o.f.sp. fragariae five days after incubation at 30±°C..*

Concentrations (%)	Average linear growth (mm)
20	81.0
40	65.8
60	42.4
80	21.2
100	0.0
Control	90.0
L.S.D. at 5%	2.7

Data presented in Table (3) show that the filtrate of compost caused significant reduction to the linear growth of *F. o*.f.sp. *fragariae* , five days after incubation at 30±°C compared with control treatment This reduction was gradually increased by increasing the concentration. In addition, the causal fungus failed to grow on the concentration of 100 %.

3.5. Effect of Combination among the Bioagents B.subtilis and P.flurescens, Compost and Soil Solarization on Management of Strawberry Fusarium Wilt and fruit yield and it's T.S.S.

Table (4) shows that combination among the bioagents B.subtilis and P.flurescens, compost and soil solarization resulted in significant reduction to strawberry Fusarium 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 control treatment (infested with the causal fungus). Compost was the most efficient in this regard compared with the other three items of disease management. i.e. the bioagents B.subtilis and P.flurescens as well as soil solarization when each of them was used alone, being 11.5, 13.3, 13.0 and 12.0% ,

Table 4. *Effect of combination among the bioagents B.subtilis and P.flurescens, compost and soil solarization on the management of strawberry Fusarium wilt (Camarosa 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)	11.5	5.6	16.2
B.subtilis (BS)	13.3	4.4	16.0
P.flurecens (PF)	13.0	4.7	15.9
Solarization (S)	12.8	5.0	16.0
C +BS	9.0	6.2	16.4
C + PF	8.5	6.8	16.5
C+S	8.2	7.0	16.6
BS + PF	10.0	6.1	16.1
BS + S	10.0	6.1	16.1
PF+S	10.0	6.1	16.1
C+ BS+PF+S	0.0	9.0	16.7
Control (Infested soil)	46.8	2.3	11.0
Control (Uninfested soil)	0.0	9.0	16.7
L.S.D. at 5 %	2.1	2.9	1.7

respectively. No apparent infection was detected when the combination among the bioagents *B.subtilis* and *P.flurescens* , compost and soil solarization was used and the produced fruit yield and it's T.S.S. ,to somewhat, similar to control treatment (uninfested with the causal fungus). The highest disease severity and poor fruit yield as well as T.S.S. were noticed for strawberry plants grown in soil infested with the causal fungus .

4. Discussion

Nowadays, farmers are interested in reducing dependence on chemical inputs, so rather than chemical control such as agriculture practices, sanitation, biological control, resistant cvs. , soil solarization …ect could be exploded to play an important role in Integrated Pest Management (IPM) systems, especially in case of vegetable production. A model describing the several steps required for a successful IPM has been developed by Mc Spadden and Fravel (2002) .

Akram *et al.*(2013) mentioned that *F. oxysporum* is a soil-borne in nature and invades vascular system of a plant internally. It is better to protect the entrance point of this fungus in plant instead of changing the entire soil mycoflora. For this purpose, some microorganisms can be used to induce resistance in plants for combating with this devastating pathogen.

Isolation trials from strawberry plants showing mainly wilt symptoms grown at Behera, Ismailia, Kalubia and Giza governorates yielded *Fusarium oxysporum , F.solani , Macrophomina phaseolina ,Pythium ultimum , Phytophthora cactorum , Rhizoctonia solani* and *Sclerotium rolfsii*. the isolated fungi were previously isolated by Abada (1986); Mass (1998) and Fang *et al.* (2011) and (2012). Pathogenicity test of the four isolates of *F.o*xysporum revealed that they caused wilt symptoms and Kalubia isolate was the most virulent one. In addition, inoculating different seven plants, *i.e.* cucumber, eggplant , bean , pepper , strawberry , tomato and water melon with *F. oxysporum* isolate of Kalubia governorate indicated that it caused wilt symptoms to strawberry only. Therefore, it named *Fusarium oxysporum* f.sp. *fragariae* Winks & Y.N. Williams.

Culture filtrate of four *Bacillus* spp. and one isolate of *Pseudomonas flurescens* resulted in different degrees of inhibitory effect on the linear growth of *F.o* f.sp. *fragariae,* 5

days after incubation at 30±2°C compared with control treatment. In this respect, the fungus failed to grow on the concentration of 100 % culture filtrate of *B.subtilis* and *P. flurescens* . However, *P. flurescens* was the most efficient in reducing the linear growth of the causal fungus followed by the isolate of *B. subtilis* . Meanwhile, *B. coagulans* was the lowest effective one in reducing the linear growth of the causal pathogen then the isolate of *B. pumilus* .

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, pyoluterin 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 (Kloeppe *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 alternatives to synthetic chemical fungicides or bactericides and not as part of an integrated management system.

Sterilized aqueous filtrate of the tested compost resulted in significant reduction to the linear growth of the tested fungus compared with control treatment. This reduction was gradually increased by increasing it′s concentration.

Using of the two bioagents *B.subtilis* and *P.flurescens* , compost and soil solarization resulted in significant reduction to the severity of strawberry Fusarium wilt with significant increase to the fruit yield and it′s total soluble solids (T.S.S.) compared with control treatment. In addition, the combination between any of the tested bioagents and soil solarization was more efficient in reducing disease severity and increasing fruit yield and it′s T.S.S. than when each of them was used alone. Moreover, the combination among the two bioagents + compost + soil solarization was the most efficient in this regard , which no apparent infection by the disease was detected and the highest fruit yield and it′s T.S.S. were obtained . The highest efficiency of the combination between soil solarization and any of compost and *B.subtiis* or *P.flurescens* may be greatly due to the drastic effect of soil solarization on the fungus prop gules make them to be week to resist the invasion by the tested bioagents and compost plays a suitable medium for reproduction and establishment of the added bioagents and saprophytic microbes in the soil .

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 degree 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 (2005) 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 (1987) declared that copiotrophic bacteria recolonize 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, Lookwood (1988) reported that edaphic microorganisms stimulated by compost amendments contribute to the 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 synthesis and accumulation of antimicrobial phytoalexins (Hammond-Kosack and Jones, 1996), induction of hypersensitive response (He *et al.*,1993), production of defense-related proteins (Yu,1995) production of activated oxygen species (Baker *et al.,*1993), and modification of plant cell wall by deposition of callose (Veit *et al.*,2001).

Nonpathogenic bacteria, such as various species of the genus *Bacillus* (Kloepper *et al.,* 2004) 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) (Van Loon,2007 and De Vleesschauwer *et al.,*2009). Recently, there has been a growing interest in nonpathogenic bacteria due to their efficacy as bioagents in many crops (Kloepper *et al.,* 2004; Akram *et al*., 2013 ; Zaher *et al.*, 2013 and Abada and Ahmed, 2014). Application of some Bacillus strains to the seedlings has been found effective for suppressing soil borne diseases and has successfully induced systemic resistance in the treated plants (Kloepper *et al.,* 2004 and Szczech and Shoda, 2007).

It is presumed that the induced chitinase, β-1,3-glucanase and peroxidase in localized and split-root experiments may be involved in the reduction of vascular wilt development in tomato. Reduced disease severity coupled with enhanced enzyme production elicited by S_2BC-1+GIBC-Jamog in localized and split-root experiments indicate that its mode of action or vascular wilt suppression in tomato is through both direct biocontrol and ISR (Shanmugam and Kanoujia, 2011). Also , Yu *et al.* (2011) 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 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. In this regard, Kodama and Fuki (1982) found out that the propagules of *F.o.*f.sp.*fragariae* were not detected at 5 cm. soil depth of the solarized soil and population at 10-15 depth showed a 60 % decrease. They added that as a results disease incidence was significantly reduced in outdoor cultivation of strawberry and in closed plastic houses population density of the pathogen filled sharply and remained low for 9 months following soil solarization . Also, Stapleton *et al.*(1985) reported 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. 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. Soil solarization has been effective as a pre-plant and as a post-plant treatment, and has been compatible with chemical soil treatments and also biological soil amendments after solarization. They added that soil solarization is a significant advance in the non-chemical control of many pathogens and pests.

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) . The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes some of which may act directly to lyze invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death. A second phenotype, first referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria. Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR.

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