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PERFORMANCE OF SOME NEW BIOFORMULATIONS AGAINST TOMATO FUSARIUM WILT

Abeer E. El-Hadidy
Department of Plant Protection, Desert Research Center, El-Matareya, Cairo, Egypt
E-mail: elhadidyabeer@gmail.com

Rhizobacteria strains of *Bacillus pseudomycoides* M3, *Brevibacillus brevis* M4 and *Stenotrophomonas maltophilia* BG4 were assayed for its antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici*; a phytopathogenic fungus causing wilt in tomato and evaluated to formulate carrier-based bioformulations. The viability of these bacterial strains was monitored at different time intervals during the period of storage at room temperature in different carriers; such as peatmos, sawdust, biochar, peatmos-biochar, sawdust-biochar and kaolin. Biochar was found to be the most efficient carrier material for the three bacterial strains, followed by other carriers. Tomato seeds were coated with different carrier-based bioformulations experiments were carried out to access its effects against wilt disease. Biochar-based bioformulations showed higher colonies forming unit counts and maximum viability for bacterial strains at 180 days of storage. Minimum percentage of disease incidence and severity were observed in biochar inoculated with mixture of three bacterial strains. Maximum increase in plant growth parameters (plant height, dry and fresh weight) were ascertained amongst all bioformulations from field experiment, biochar inoculated with bacterial strains performed consistently thriving results for tomato yield. Furthermore, seed treatment with bacterial formulation induced plants to synthesize defense enzymes; such as peroxidase and chitinase, whereas an additional increase in the synthesis was observed in biochar followed by peatmos-biochar pretreated plants challenge inoculated with *Fusarium oxysporum* f. sp. *lycopersici*.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, biological control, bioformulations, *Bacillus pseudomycoides*, *Brevibacillus brevis*, *Stenotrophomonas maltophilia*

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated crops in the world. Due to its extensive cultivation in non-

traditional areas, several biotic and abiotic factors have emerged as a major constraint in its successful cultivation. *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is one of the important fungal pathogens that cause wilt resulting in substantial yield losses. It causes up to 30% of crop loss of tomato in Egypt (Amer et al., 2014). The causal agent of fusarium wilt is soil-borne pathogen, which can persist many years in the soil without a host. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is infested with the pathogen. The main disease symptoms are half-leaf yellowing, browning of vascular tissues, plant wilting, stunting and ultimately death (Maja et al., 2012). The long survival of pathogen resting structures (chlamydospores) in the soil, even in absence of host plants, limits the suppressive effect of crop rotation. Furthermore, due to the endophytic pathogen progress within vascular tissues, chemical control fails to successfully control disease and risks of development of fungicide resistance are frequent. Various chemical fungicides have been used as the main method for controlling wilt. However, there is increasing interest in alternative control methods, because of the possible harmful effects of pesticides on human health and the environment and due to the emergence of resistance to chemical fungicides among crop pathogens.

Biological control is an important alternative method for managing plant diseases. It represents a significant strategy for environmentally friendly plant disease control and has been shown to effectively control several diseases in many crops (Ben Abdallah et al., 2016). Many bacterial species associated with or surrounding the plant roots can be played effective roles in promote the growth of plants and act as biocontrol agents against potential phytopathogens through various mechanisms (Rojas-Solís et al., 2018). Rhizobacterial strains of *Stenotrophomonas*, *Brevibacillus* and *Bacillus* spp. have been used to reduce disease caused by a variety of soil-borne pathogens (Ben Abdallah et al., 2016; Bahroun et al., 2018 and Hassan et al., 2018). However, the success of biocontrol agent application largely depends on the carrier materials, which used for the bacterial inoculants protect them from various stress factors and prolong shelf life in arid soils (Egamberdieva and Adesemoye, 2016). An ideal carrier that can support the survival of a biocontrol agent, while discouraging the growth of the target pathogen (Wei et al., 2015). Inoculum carriers offer the advantage of providing a protected habitat and facilitate handling and mixing of the inoculant into the soil. Common materials that are used to deliver bacteria include peat moss, ground corncobs, clay, charcoal perlite, vermiculite and polyacrylamide (Bashan et al., 2014). More recently, biochar materials derived from woody feed stocks by low-oxygen pyrolysis have been shown to serve as effective inoculum carriers and provide several advantages as compared to other carriers (Głodowska et al., 2016 and Sun et al., 2016).

Biochar can be produced from locally available biomass and is presterilized during the pyrolysis process facilitating inoculation as the last step in the production process. There are abundant raw materials for biochar production; including agricultural and forestry residues, compost and manures, many of which are considered waste products and require disposal fees. Some biochars retain organic and inorganic nutrients from the feedstock as well as offering internal structure with pores with specific dimensions, that can provide a protected habitat for the inoculum and exclude predators. Additionally, biochar may adsorb nutrients from root exudates to support the growth of the inoculum after it is introduced into the soil (Sun et al., 2015). The objectives of this study were to evaluate solid *Bacillus pseudomycoides* M3, *Brevibacillus brevis* M4 and *Stenotrophomonas maltophilia* BG4 based bioformulations as potential biocontrol agents against fusarium tomato wilt disease and as plant growth promoting rhizobacteria under green house and field condition.

MATERIALS AND METHODS

1. Isolation and Identification of Causal Organisms

Tomato plants showing wilt symptoms were collected and kept in plastic bags from different growing areas at Ismailia governorate. Root and crown diseases infected plants were washed thoroughly with tap water. Small pieces (2-5 mm) were cut from each sample and sterilized with 1% sodium hypochlorite solution for 2 min and dried between sterilized filter papers and placed on potato dextrose agar plates (PDA) supplemented with 100 mg/L streptomycin-sulfate, which is equivalent to 100 ppm. Petri dishes were incubated at 25ºC for 48-72 h. Single spores or hyphal tips were taken from developed colonies and transferred onto PDA. The fungal isolates were identified in Plant Pathology Institute, Agricultural Research Center, Cairo, Egypt according to Booth (1977) for *Fusarium* species and evaluated for their pathogenicity to tomato plants. Pathogenicity of isolated fungi on tomato plants was proved. A highly virulent isolate of each isolated fungal strain was selected for further use. All isolates were maintained on PDA at 4ºC. Inocula for soil-infestation were prepared using barley grain medium as described by Singleton et al. (1992).

2. Bacterial Strains

Five rhizobacterial strains i.e. *B. pseudomycoides* M3, *B. brevis* M4, *S. maltophilia* M5, *S. maltophilia* BG4 and *S.toxytricini* C5, which isolated and showed high potentiality against *R. solanacearum* in a previous study (Hassan et al., 2018) were assayed against *F. oxysporum* f. sp. *lycopersici*, which is a causal of fungal wilt in tomato.

3. *In Vitro* Assay

The inhibition of mycelium growth of *F. oxysporum* f. sp. *lycopersici* by strains M3, M4, M5, BG4 and C5 was tested on PDA medium. One ml of each bacterial suspension (10^8 cfu/ml) was streaked on PDA media plates and a 6 mm agar disc of fungal isolate from fresh PDA culture was placed at the other marginal side and incubated at 25±2ºC for seven days. The radial growth of the developed fungal colony towards and away from the bacterial colony was measured. The percentage of growth inhibition was calculated using the following calculation:

$$\% \text{ Inhibition} = [(R - r)/R \times 100]$$

Where, r is the radius of the fungal colony opposite the bacterial colony and R is the maximum radius of the fungal colony away from the bacterial colony.

4. *In Vivo* Assay

Testing the efficiency of bacterial strains against *F. oxysporum* f. sp. *lycopersici in vivo* was carried out using the soil-dishes technique as described by Mosa et al. (1997). The pathogen was grown for five days on a thin layer of PDA media, in 9 cm diameter Petri dishes. Then, the fungal colony was covered by autoclaved mixture of peat moss and vermiculite (1:1 v/v). Five treated tomato seeds with rhizobacteria were sown over soil in each Petri dish using sterile tweezers to prevent cross contamination through handling. Set of dishes contained non-infested soil served as control. Treatment with the fungicide rizolex-T (2 g/kg seeds) was carried out for comparison. Thereafter, seeds covered by soil mixture, were watered daily by sterilized distilled water. Percentage of survived seedlings was recorded after 25 days from sowing date. Seedlings dry weight was also determined.

5. Bioformulation of Antagonistic Bacterial Strains

5.1. Preparation of antagonistic bacterial formulations

The highest three antagonistic bacterial strains; i.e. *B. pseudomycoides* M3, *B. brevis* M4 and *S. maltophilia* BG4 were grown in nutrient medium broth (NAM) at 28±1°C for 24 h at 150 rpm (pH 7.2). The broth culture of 10^8 cells/ml was utilized for inoculant preparation. The solid carrier materials; such as peatmos, sawdust, biochar, sawdust - biochar (1:1), peatmos - biochar (1:1) and kaolin were ground separately and air dried before mixing and curing, followed by double sterilization (121°C for 20 min). The sterile carrier materials (40 g each) were packed in recommended low density polythene bags (50–70 µm thick) of flexible sheets to protect from loss of moisture. The bags were sealed leaving about 25% airspace to give proper aeration to the inoculants. Each bacterial inoculum was mixed thoroughly with supporting carrier material under aseptic condition, sealed

and stored at room temperature. The initial count in each carrier-based preparation was made so as to obtain 10^8 cells/g at the time of storage.

5.2. Viability assessment of antagonistic bacterial strains

The viability of bacterial cells was determined in six carrier preparations, including peatmos, sawdust, biochar, sawdust - biochar (1:1), peatmos - biochar (1:1) and kaolin. The samples (1 g each) were collected from bioformulations after different time intervals up to 180 days under aseptic conditions. Suitable dilutions were spread plated on NAM amended with antibiotics (streptomycin 50 µg/ml each), and incubated at 28±1°C for 48 h. The bacterial population (CFU/g) was enumerated. The experiment was conducted in triplicate, and one bag of each carrier from each replicate was investigated after every 30 days interval.

6. Evaluation of Bioformulations Against Tomato Wilt

6.1. Greenhouse experiment

In seedbed bioformulations (peatmos, sawdust, biochar, sawdust - biochar (1:1), peatmos - biochar (1:1) and kaolin) containing a mixture of three bacterial strains, they were mixed with tomato seeds treated with powder formulations at the rate of 1% (powder formulation: seeds) to give a bacterial population of $\geq 10^7$ CFU/seed of formulation. Seeds were moistened in carboxyl methyl cellulose (CMC) solution (1%) before application of inoculum to get a thin, uniform coating of inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran et al., 1982). Two-week old tomato seedlings were transplanted in 30 cm pots filled with soil infested with *F. oxysporum* f. sp. *lycopersici*. Uninfested soil and soil infested with *F. oxysporum* f. sp. *lycopersici* without bacterial bioformulation were served as negative and positive controls. Wilt incidence and severity were calculated at 45 days after planting.

6.2. Field experiment

An experiment was conducted in a complete randomized design with three replicates in naturally infested field at Ismailia government for evaluation of bioformulations against tomato wilt disease. A standard plot size of 3x3 m^2 with 5 rows was maintained for all treatments. For each plot, 150 seedlings were transplanted. Seeds of tomato were treated with the bacterial formulations as previously mentioned in seedbed. The seedlings that obtained from untreated seeds were served as control. Soil in all treatments was amended with recommended dose of super phosphate (15.5% P_2O_5) at a rate of 250 kg/fed, ammonium nitrate (33.3% N) at a rate of 300 kg/fed and K-sulphate (48% K_2O) at a rate of 200 kg/fed. Wilt incidence and severity were recorded three months after planting as well as plant growth parameters.

7. Diseases Assessment

The percentage of infected plants with wilt and survived plants was recorded periodically up to three months after transplanting as follows:

$$\text{Infection percentage} = \frac{\text{No. of infected plants X 100}}{\text{Total plants}}$$

Assessment of wilt disease was based on amassment of foliar wilt ratings and presence of internal browning vascular system.

For foliar wilt ratings, number of symptomatic leaves and dead plants were recorded after periodically up to six from transplanting. Wilt development on each plant was rated using the scale described by Gao et al. (1995) as follows: 5= dead plant; 4= 76 to 100% of leaves with symptoms, 3= 51 to 75% of leaves with symptoms; 2= 25 to 50% of leaves with symptoms, 1= < 25% of leaves with symptoms and 0= no symptoms. The disease rating was calculated by the following formula:

$$\text{Disease index} = \frac{\Sigma \text{ (rating no. x no. of plants in the rating) x 100}}{\text{Total no. of plants x highest rating}}$$

Internal symptoms were determined based on length of vascular discoloration (cm) as described by Szczech (1999).

8. Enzymes Activity

8.1. Peroxidase

Root samples (1 g) of tomato plants grown in field experiment and obtained from different treatments and control (20 days after seedling were homogenized in 2 ml of 0.1M phosphate buffer, pH 7.0 at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28±2°C). The changes in absorbance at 420 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg $protein^{-1}$ (Hammershmidt et al., 1982).

8.2. Chitinase

Root samples (1 g) of tomato plants grown in field experiment and obtained from different treatments and control (20 days after seedling were homogenized with 0.2 M Tris HCl buffer, pH 7.8 containing 14 mM B-mercaptoethanol at a rate of 1/3 (w/v). The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used to determine the enzyme activity, according to the colourimetric method suggested by Monreal and Reese (1969) using 1% colloidal chitin. Chitinase activity was measured by the release of N-acetyl-D-glucosamine (NAG) from colloidal chitin, and expressed as acetylglucosamine released/ g fresh weight tissue/60 min.

9. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and were calculated for mean separation analyzed and subjected to Duncan's multiple range tests and comparison after analysis of variance (Duncan, 1955).

RESULTS

1. Evaluation of Bacterial Strains, *In Vitro*

Data presented in table (1) show that, only three bacterial strains *i.e.* M3, M4 and BG4 significantly reduced mycelial growth of the tested fungus isolate, while the rest of bacterial strains showed less inhibition effect.

Table (1). Antagonistic effect of bacterial strains against *F. oxysporum* f. sp. *lycopersici* grown at PDA medium

Bacterial strains	***F. oxysporum***	
	Growth*	**Inhibition (%)****
M3	0.7e	79.4
M4	0.5f	85.3
M5	1.9c	44.1
BG4	1.0d	70.6
C5	2.8b	17.6
Untreated	3.4a	-

*Fungal colony diameter

**% of inhibition calculated based on colony diameter relative to control (no bacteria)

Means in each column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (P=0.05)

2. Evaluation of Bacterial Strains, *In Vivo*

Data in table (2) indicate that three bacterial strains i.e. M3, M4 and BG4 were effective in reducing damping-off of tomato seedlings caused by *F. oxysporum*. Bacterial strains varied among them in reducing damping-off. However, the fungicides rizolex-T seed treatment performed the best reduction of damping-off caused by *F. oxysporum*. Data in table (2) also indicate that, there were various effects of the tested bacterial strains on seedling survival. Pathogen-non infested soil M3 and M4 gave the most increase in seedling dry weight. Results also indicate that the tomato seed germination test in Petri plates could be used for evaluation of the biocontrol agents against wilt pathogen.

Table (2). Evaluation of antagonistic strains against *Fusarium oxysporum* f. sp. *lycopersici* of tomato and their effect on seedling dry weight

Bacterial strains	Infested soil		Non-infested soil	
	Survived seedlings (%)	Dry Weight (mg)	Survived seedlings (%)	Dry weight (mg)
M3	83a *	17a	87a	19a
M4	82a *	16a	86a	18a
M5	71 c	14b	79c	15bc
BG4	80 b	15ab	83b	16b
C5	70c	12c	78c	14c
Rizolex[a]-T	82a	14b	76d	12d
Untreated[b]	46d	9d	75d	12d

*Significant at 0.01 level of probability

a) Seeds were treated with rhizolex-T at rate of 2 g/kg seeds. b) Seeds were treated with 0.01% MC only

3. Survival of Antagonistic Bacterial Strains in Different Formulations

Data illustrated in figs. (1 and 2) show that in all formulations of three bacterial populations, there was a declined steadily relationship over time. The bacteria survived even up to 180 days of storage with different percentages, although the population declined after 60 days of formulation. The number of viable cells detected in biochar followed by peatmos-biochar carriers was slightly higher than other formulations, after six months of storage. Only 69 and 61.1%, 71.8 and 66.6%, 75.3 and 65.7% of viable cells of M3, M4 and BG4, respectively, were detected in biochar and peatmos-biochar formulations, while other formulations gave less viable cells after six months of storage.

4. Evaluation of Bioformulation Against Tomato Wilt

4.1. Under Greenhouse Condition

In this experiment, the efficiency of bacterial formulations in controlling tomato wilt disease was evaluated. Data in table (3) show that all bacterial formulations significantly decreased wilt incidence as well as disease severity compared with the control. Data indicate that soil infested with *F. oxysporum* f. sp. *lycopersici* as control showed the highest percentage of wilt compared with non-infested soil. Generally, biochar-based formulations recorded the highest reduction of wilt incidence,

followed by peatmos-biochar and sawdust-biochar by 8.9, 10.5 and 12.4%, respectively compared with other based formulations. Data also indicated that biochar, peatmos-biochar and sawdust-biochar-based formulations have most significant values, where it reduced internal root browning as well as foliar wilt rating in infested with *F. oxysporum* comparing with other based formulations.

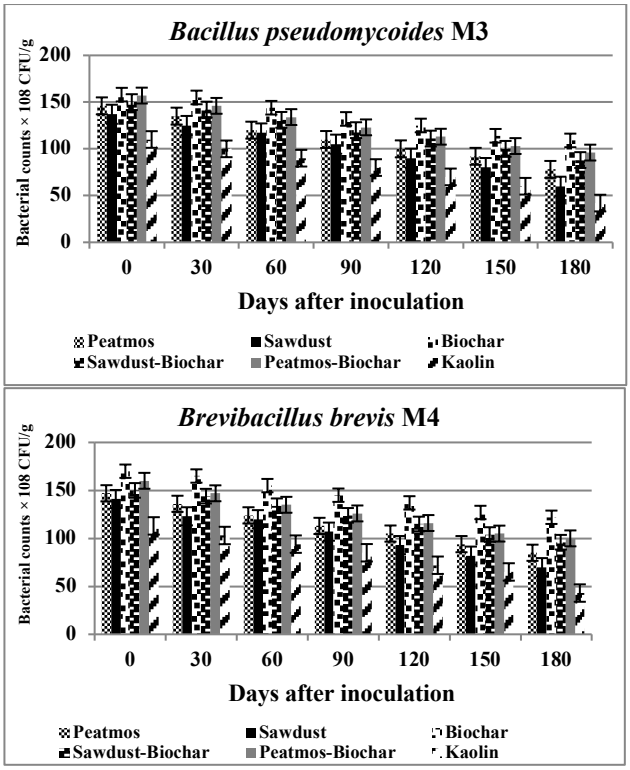

Fig. (1). Viable population of *Bacillus pseudomycoides* M3 and *Brevibacillus brevis* M4 bacterial strains in different formulations stored at room temperature (25°C±2).

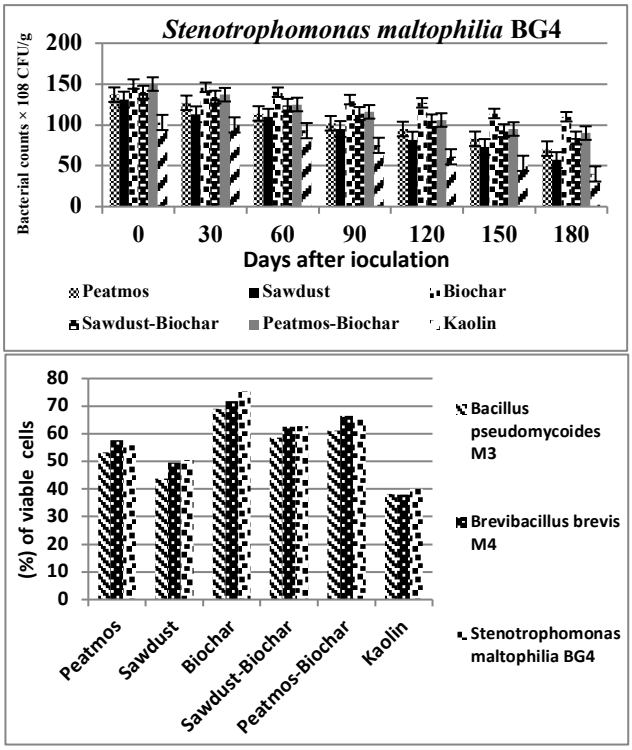

Fig. (2). Viable population of *Stenotrophomonas maltophilia* BG4 bacterial strain in different formulations stored at room temperature (25°C±2).

Table (3). Effect of some bioformulations as seed treatment on the fusarium tomato wilt under greenhouse experiment.

Bacterial formulations	**Wilt**		
	Incidence (%)	**Internal root browning**	**Foliar wilt rating**
Peatmos	23.7d	0.6c	1.3d
Sawdust	32.6c	0.7c	2.0c
Biochar	8.9g	0.0f	0.4f
Peatmos-biochar	10.5f	0.2e	0.9e
Sawdust- biochar	12.4e	0.4d	1.3d
Kaolin	36.9b	1.0b	2.7b
Positive control	69.5a	1.5a	3.8a
Negative control	0.0h	0.0f	0.0g

Means within the same column followed by the same letter are not significantly different according to Duncan's ($P \geq 0.05$)

4.2. Under Field Conditions

The effect of different formulations on the incidence and severity of tomato wilt disease under field condition is shown in table (4). The lowest incidence of wilt (8.6%) was recorded from plants treated with biochar, followed by peatmos-biochar (12.2%) and sawdust-biochar (14.5%) compared with the untreated control (73%). Data in table (4) also illustrate that there was a significant decrease of internal root browning as well as foliar wilt rating, which recorded by the three bioformulations. Biochar-based formulation was the most significant effective formulation, which recorded the lowest degree of internal root browning as well as foliar wilt rating compared with control.

Table (4). Effect of some bioformulations as seed treatment on the fusarium tomato wilt under field conditions.

Bacterial formulations	Wilt		
	Incidence (%)	Internal root browning	Foliar wilt rating
Biochar	8.6d	0.2d	0.4d
Peatmos-biochar	12.2c	0.5c	0.6c
Sawdust- biochar	14.5b	0.8b	1.0b
Untreated	73.0a	1.5a	4.0a

Means within the same column followed by the same letter are not significantly different according to Duncan's ($P \geq 0.05$)

Data presented in table (5) reveal that seed inoculation with antagonistic bacterial formulations caused a significant increase for all growth characters as well as fruits yield measured over the control. Biochar recorded the highest values in plant height, fresh weight, dry weight and fruits yield compared with other treatments and untreated samples.

Table (5). Effect of some bioformulations as seed treatment on the growth characters and yield of tomato plants under field conditions

Bacterial formulations	Growth Characters			Fruits Yield (ton/fed)
	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	
Biochar	75.3a	141.4a	22.6a	18.9a
Peatmos-biochar	71.5ab	136.5b	19.2b	17.2b
Sawdust- biochar	65.9b	122.5c	17.9c	15.8c
Untreated	38.5c	93.4d	10.2d	8.7d

Means within the same column followed by the same letter are not significantly different according to Duncan's ($P \geq 0.05$)

Seed treatment with bacterial formulation induced plants to synthesize defense enzymes; such as peroxidase and chitinase, whereas an additional increase in the synthesis was observed in biochar, followed by peatmos-biochar pretreated plants challenge inoculated with *F. oxysporum* f. sp. *lycopersici*. Plants treated with biochar-based formulation gave thr highest significant activity of peroxidase and chitinase, whereas recorded 2.4 and 3, respectively compared with other treatments and untreated (Table 6).

Table (6). Effect of some bacterial formulations on defense enzymes activity in inoculated tomato plants under field conditions.

Bacterial formulations	**Peroxidase activity (min^{-1} mg $protein^{-1}$) [a]**	**Chitinase activity (min^{-1} mg $protein^{-1}$) [b]**
Biochar	2.4a	3.0a
Peatmoss-biochar	2.2b	2.8b
Sawdust-biochar	1.9c	2.5c
Untreated	1.6d	2.0d

a) Peroxidase activity was determined in 1 g of tomato roots and expressed as changes in the absorbance
min^{-1} mg $protein^{-1}$ (Hammershmidt et al., 1982)
b) Chitinase activity was determined in 1 g of tomato roots according to the colourimetric method suggested by Monreal and Reese (1969)

DISCUSSION

Soil-borne plant diseases seriously limit agricultural production. Due to environmental concerns, sustainable agricultural practices must involve the use of environmentally friendly alternatives, such as biocontrol agents, to suppress various plant diseases. An ecofriendly approach in crop protection to reduce the damage caused by fungal pathogens with several biocontrol agents were reported in many crops (Sarma et al., 2015 and Mishra et al., 2015). In the present study, antagonistic effect of five bacterial strains was evaluated against *F. oxysporum* f. sp. *lycopersici in vitro* and *in vivo* assay. Results indicated the varying degrees of inhibition of *F. oxysporum* f. sp. *lycopersici* by all these five bacterial strains viz. *B. pseudomycoides* M3, *B. brevis* M4, *S. maltophilia* M5, *S. maltophilia* BG4 and *S. toxytricini* C5. Endophytic bacteria are part of the plant microbiome, which can promote the growth of plants and act as biocontrol agents against potential phytopathogens through various mechanisms, including the production of volatile compounds (Rojas-Solís et al., 2018). These bacterial strains were mentioned to secrete various antimicrobial secondary metabolites, siderphores and HCN (Hassan et al., 2018).

Inoculants for field-scale use have to be designed to provide a dependable source of bacteria that survives in the soil and become available to crops, when needed. The first goal when considering inoculation of plants

with bioagents is to find the best strain of bacteria or a microbial consortium for the intended effect on the target crop. The next step is to design a specific inoculant formulation for the target crop and a method of practical application, considering the limitations of the growers (Bashan et al., 2014). However, the success of biocontrol agent application largely depends on the carrier formulation. An ideal carrier that can support the survival of a bioagent, while discouraging the growth of the target pathogen is expected to enhance the performance of the bioagent in plant disease control. Considering that peatmos, which is presently used as a standard inoculant carrier, is nonrenewable resource, and its price is expected to increase in coming years, there is an urgent need to find alternatives to it. The more popular alternatives to peat inoculants are biochar, sawdust, charcoal, coir dust and composts of various origins and compositions, sugarcane filter mud bagasses, soils mixed with various organic amendments and vermiculite as a carrier. Several amendments were added to enhance common formulations of peat with various microorganisms (Bashan et al., 2014).

In this study, various agricultural and industrial wastes were evaluated as a solid-based carrier of best antagonistic three strains; i.e. M3, M4 and BG4, against tomato fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici*. Among the evaluated carriers, biochar gave the most promising results on residual cell viability at 180 days, acting as food source for bacterial strains. Biochar facilitated the growth and survival of the bacterial strains by supplying adequate levels of dissolved carbon and nitrogen. Although other carriers maintained large populations of the three bacterial strains, similar to those obtained with biochar, peatmos-biochar and sawdust-biochar, they were not deemed suitable carriers because they constituted a poor nutrient supply for these bacterial strains growth; thus, they had less of an impact on disease control (Egamberdieva and Adesemoye, 2016).

Biochar can function as an effective low-cost inoculum carrier for plant growth-promoting bacteria. The shelf life and inoculum potential of *Pseudomonas putida* UW4 were examined compared with peatmos a standard reference material (Sun et al., 2016). Biochar-based bioformulations showed higher cfu count and maximum viability for *Burkholderia* sp. strain L2 (107 cfu g^{-1}) at 240 days of storage. Maximum percentage of seed germination was also observed in biochar inoculated with *Burkholderia* sp. strain L2. Significant increase in plant growth parameters were ascertained from the pot experiment and amongst all bioformulations, biochar inoculated with strain L2 performed consistently thriving results for tomato yield (Tripti et al., 2017). Following soil inoculation, the introduced bacteria must be able to move from the carrier to the plant root surface, a process generally involving chemotaxis towards root exudates and that also may be affected by the carrier properties and ability to detach. Some reports

indicated that roots preferentially grow towards and form root hairs on and around biochar particles (Prendergast-Miller et al., 2014).

Greenhouse and field experiments in this study demonstrated that, biochar-based followed by peatmos-biochar and sawdust-biochar improved the ability of the three bacterial strains to reduce the incidence and severity of tomato fusarium wilt. There are several possible explanations for the efficiency of biochar. One possibility is that the pore structure and internal surface area of biochar may provide better colonizeable microsites for *Pseudomonas putida* UW4 than peat. Biochar also may absorb soluble organic substances from soil and support the growth of the inoculum, which may support a high abundance of *Pseudomonas putida* UW4 (Mohan et al., 2014). The principle of beneficial microorganisms carrier selection should be adopted in the future when developing new bio-products for the effective control of soil-borne plant diseases (Wei et al., 2015). Adding biochar to soil and soilless media can help protect plants against diseases caused by soil-borne pathogens. There are a number of direct and indirect mechanisms that are potentially responsible for this effect (Jaiswal et al., 2018). In summary, although some organic wastes can perform equally well or better than peat as a carrier, the main limitation is the availability of the raw material for industry (Głodowska et al., 2016). In the present study, application of M3, M4 and BG4 via a biochar, peatmos-biochar and sawdust-biochar-based bioformulation resulted significant increase in plant height, fresh weight, dry weight and fruits yield. Biochar recorded greatest values in plant growth characters and fruit yield compared with others and control. These results are in strong agreement with previous findings of other researchers (Głodowska et al., 2016 and 2017).

The formulations when applied to seed, root and soil were more effective in reducing disease severity possibly due to the all-round placement of the antagonist viz. on the seed, from which the antagonist migrated to the elongating roots (Bashan et al., 2014). Biochar-based formulations prepared with antagonistic rhizobacteria had the longest stability and biocontrol activity against *Macrophomina phasolina,* which had significant effect on chickpea growth compare to control (Shahjahan et al., 2018). In the present study, it has been observed that seeds treated with bioformulations contenting mixture of three antagonistics; i.e. M3, M4 and BG4 increased the activities of peroxidase and chitinase enzymes, which lead to the synthesis of defense chemicals in the plants against pathogen. In addition to direct antagonism and plant growth promotion, rhizobacteria increased the activities of various defense-related enzymes and chemicals in response to infection by the pathogen.

It is well known that all plants are endowed with defense genes, which are quiescent in nature and appropriate stimuli or signals are needed to activate those. Pretreatment of tomato seeds, under greenhouse conditions, with *Bacillus megaterium* var. *phosphaticum*, significantly increased the

induction of chitinase, β-1, 3-glucanase, peroxidase and polyphenol oxidase and reduced incidence and severity of wilt disease caused by *F. oxysporum* f. sp. *Lycopercisi*, compared with untreated control (Amer et al., 2014). Biochar-based formulations gave the significantly highest increase of the activities of peroxidase and chitinase enzymes. In this study, biochar successfully induced systemic resistance when peroxidase activity increased in treated eggplant against *Sclerotinia sclerotiorum* (Hassan, 2017).

CONCLUSION

In arid and semi-arid regions, soil fertility diminishes gradually due to water stress, salinity, fertility depletion, erosion, high/abrupt changes in temperature, and other climatic conditions in addition to biotic stress such as soil-borne pathogens. There is good evidence that formulations based on microbial inoculants could be very useful to suppress soil-borne pathogens and improve crop yield. Several materials have been evaluated as carriers for bacterial inoculants, successfully protecting them from biotic stress and prolonging shelf life. Those results imply that formulated microbial inoculants could be a useful approach against soil-borne diseases and for improving growth and yield of crop plants under hostile environmental conditions. In the current study, biochar was used as an inoculant carrier for *B.* M3, *B. brevis* M4 and *S. maltophilia* BG4 as an alternative to peat moss under reclaimed soil conditions. Seeds coating with biochar-based inoculant maintained a high population of M3, M4 and BG4, which ensure that the biofermulation has the longest stability and effectiveness for biological control against tomato fusarium wilt.

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فاعلية بعض مستحضرات حيوية جديدة ضد ذبول الفيزاريومي للطماطم

عبير المرسي أحمد الحديدي
قسم وقاية النبات، مركز بحوث الصحراء، المطرية، القاهرة، مصر

تم تقيم النشاط التضادي لعزلات بكتيرية *Bacillus pseudomycoides* M3, *Brevibacillus brevis* M4 و*Stenotrophomonas maltophilia* BG4 ضد الفطر الممرض *Fusarium oxysporum* f. sp. *lycopersici* المسبب لذبول الطماطم، وقد قيمت كمستحضرات حيوية. تم متابعة حيوية تلك العزلات على حوامل مختلفة مثل البيتموس، نشارة الخشب، بيوتشار، بيتموس-بيوتشار، نشارة الخشب - بيوتشار، كاولين على فترات مختلفة. أظهر البيوتشار أفضل الحوامل للثلاث عزلات ابكتيرية ويتبعه باقي العزلات. أجرى إختبار تلك المستحضرات الحيوية كمعاملة بذره ليصل تأثيرها ضد مرض الذبول. أظهر البيوتشار أعلى أعداد من وحدات مستعمرات وأعلى حيوية للسلالات البكتيرية عند ١٨٠ يوم من التخزين. أعطى البيوتشار عند حقنه بخليط الثلاث عزلات بكتيرية أقل نسبة وشدة إصابة لمرض الذبول. أوضحت النتائج أعلى زيادة في صفات النمو والمحصول لنبات الطماطم عند حقن العزلات البكتيرية على البيوتشار في الحقل. أوضحت الدراسة عند معاملة البذور بالمستحضرات الحيوية تم تحفيز إنتاج الإنزيمات الدفاعية كالبيركسديز والشيتينيز وتزيد كمياتها عند إستخدام البيوتشار ويتبعه بيتموس – بيوتشار لنباتات معدية بالفطر الممرض.