



Research Article

**ZOOLOGY**

## Impact of four biofertilizers on the abundance of insect pests of eggplant, *Solanum melongena* (L) under soilless conditions: Evaluation of the net yield of fruits and nutrient contents of the plant

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**KEY WORDS****ABSTRACT**

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Rhizobacteria,  
Rhizofungi

Soil microorganisms can improve plant health by allowing broad-spectrum resistance of the treated plants against insect herbivores. The present study was conducted to evaluate the effect of four different types of biofertilizers; *Azotobacter* sp., *Azospirillum* sp., *Bacillus megatherium*, and *Vasicular arbuscular endomycorrhizae* (VAM) on the incidence and abundance of insect pests of the eggplant, *Solanum melongena* (L) during autumn season. The studied pests were; *Bemisia tabaci*, *Empoasca* sp., *thrips tabaci*, two species of aphids, *Aphis gossypii* (Glover) and *Myzus persicae*, and red mites, *Tetranychus urticae* (koach.). The contents of Nitrogen, Phosphorous, and Pottasium (NPK%), chlorophyll and phenols in the treated plants and the net yield of fruits under soilless agriculture system were evaluated. Results revealed that biofertilizer treatments significantly decreased the abundance of insect pests infesting the green leaves of the treated plants except for *A. gossypii*. The highest values of N% and P% were recorded in plants treated with (VAM inoculation +Azt. spray) (1.08% and 0.3%, respectively). The highest percent of K% (5.75%) was recorded in plants treated with (Az inoculation +Azt spray). The Mix1 and Mix2 achieved the highest values of phenols contents (259.32 ppm and 259 ppm, respectively) compared to un-inoculated control (231.8 ppm), while Mix1 enhanced the chlorophyll content (59.86 ppm). The yield of fruits was also increased due to the application of biofertilizer inoculations and /or spray. Here, we provide the evidence that beneficial microbes modulate plant defenses against insect herbivores via triggering induced systemic resistance against insects by promoting the plant growth.

## Introduction

Fertilization is vital for plant development, plays a substantial role in plant metabolism and energy production, and increases significantly the plant yield (Sinha *et al.*, 2018). It enhances the content of Nitrogen, Phosphorus, and Potassium, that are essential nutrients for plant growth. Biofertilizers are formulations containing one or more beneficial bacteria or fungi in a carrier material, there are different kinds of substances suitable for use as carriers, i.e. clay, vermiculite, diatomaceous earth, rice or wheat bran, rock phosphate pellets, charcoal, sawdust or compost (Malusa and Vassilev, 2014). Furthermore, they can improve the soil fertility, increasing the source of available nutrients and promoting plant growth when amended to the planting substrate, seeds, or aerial parts of the plants (El-Ghamry *et al.*, 2018). The beneficial microbes can be more effective in improving the soil quality and crop yield when applied as mixtures (Parewa *et al.*, 2021). These specialized microorganisms display host specificity by producing alkaloids-based defensive compounds that frighten herbivores by forming a very intimate association with their host plants (Breen, 1994). *Azotobacter*, *Azospirillum*, *Rhizobium*, *cyanobacteria*, phosphorus and

potassium solubilizing microorganisms are some of the plant growth-promoting rhizobacteria (PGPRs) that were found in the soil under no-tillage or minimum tillage treatment which rise the host plant's ability to absorb mineral elements from the soil, specifically phosphorus (Kristek *et al.*, 2005; Dabré *et al.*, 2021). Moreover, the PGPRs have been applied for inducing plant systemic resistance (ISR) (Naeem *et al.*, 2018). Plant's growth can be supported directly or indirectly by free-living PGPR (Bai *et al.*, 2003).

Direct effects of PGPRs associates are correlated to the production of substances that regulate or enrich the nutrient uptake (Ahemad and Kibret, 2014). Indirect effects are related to the production of metabolites that decline the growing of phytopathogens and other deleterious organisms (Bhattacharyya & Jha, 2012; Russo *et al.*, 2008). These microorganisms can alter the host plant's features for insect herbivores as a result of the symbiosis by affecting the plant's nutritional quality and/or by priming effects that increase inducible and constitutive plant defenses (Gehring & Bennett, 2008; Jung *et al.*, 2012). Using host plant resistance (HPR) is an environmentally sound alternative insect pest management method that can help

prevent the difficulties caused by the indiscriminate use of chemical fertilizers and insecticides. Piercing-sucking and specific chewing insects were both positively affected by arbuscular mycorrhizal fungus (AMF), whereas generalist chewers were negatively affected (**Gange *et al.*, 2002; Gehring & Bennett, 2008**). The effects of rhizobacteria on plant-herbivore interactions are similar to those of AMF, but they are influenced by the identification of the plant, the insect species and the level of insect specialization (**Dean *et al.*, 2014; Gadhave and Gange, 2018**). Also, microorganism-plant-insect interactions impact not just the herbivores, but also higher trophic levels such as their natural enemies (**Pe´rez-Monta˜no *et al.*, 2013; Balog *et al.*, 2017**). Many insect herbivores exhibit decreased abundance in organically fertilized system as a result of growing synergies between plant diversity, natural enemies, and soil fertility (**Altieri *et al.*, 2012**). In this respect, practices that increase plant resistance and growth are important among the methods of pest control (**Biere and Bennett, 2013**). The imbalanced use of fertilizers is resulting in much succulent and excessive vegetative growth that may increase the reproductive rate of pest herbivores and damage (**Baidoo and Mochiah, 2011**).

Application of soil microbial inoculation improved plant performance and accelerated fruit yield more effectively than the use of chemical fertilizers (**Megali *et al.*, 2014**). Furthermore, it has been claimed that the nutritional quality of plants is important in insect-plant interactions because plants grown in high quality medium may develop resistance to phytophagous insects (**Bala *et al.*, 2018**). In reality, host plant allelochemicals and mineral nutrients can have a beneficial or negative impact on the biology and fecundity of insect pests (**Silva *et al.*, 2009**).

Crops grown in artificial media or combinations without soil is known as soilless culture. The creation of soilless substrates has been prompted by factors such as the difficulty and high cost of controlling soil-borne diseases and pests, soil salinity, a lack of rich soil, and droughts (**Savvas *et al.*, 2013**). The most popular artificial media are Rockwool, peat, perlite, vermiculite, sawdust, bark chips, sand, gravel, pumice, polyurethane mats, water and mixtures of them (**Olympios, 1992**).

Eggplant, *Solanum melongena* (L.) (Solanaceae) is an important vegetable crop that is grown and consumed in many countries. It is grown with other vegetables including pepper, tomato, and okra, although it can also be grown in a

monoculture system (**Ibekwe et al., 2014**). Raw eggplant is composed of 92% water, 6% carbohydrates, 1% protein and negligible fat (**San José et al., 2014**). Eggplant production is severely affected by several insects and mite pests (**Srinivasan, 2009**), such as leafhoppers which suck the nutrient sap from the xylem. Severe infestations by these pests result in the crinkling of leaves, hopper burn and cupping up symptoms (**Anand et al., 2013**). Currently, pest control is carried out using chemical pesticides; however, it is necessary to have an alternative application that is safer environmentally, and sustainable.

The effects of AMF and PGPR on insects that feed on the plant leaves in natural agricultural settings are poorly understood (**Gadhawe and Gange, 2018**). Most studies to date have been undertaken under controlled conditions in the laboratory or green-houses (**Gehring and Whitham, 2002; Katayama et al., 2011; Jung et al., 2012**). The primary hypothesis of this study was to examine if the PGPR biofertilizers; *Azospirillum* sp., *Bacillus megathrium*, *Azotobacter* sp., as well as *Mycorrhiza* sp. (VAM) are able to simultaneously improve the resistance of the eggplant planted in soilless system

under field cropping conditions against herbivores attack.

## Material and methods

### Site of the study

The experiments were carried out at a semi field research garden of Microbiology Division, Department of Soil, Water and Environmental Research Institute (SWERI), Sakha Agriculture Research Station, Agricultural Research Center (ARC), Kafr El-Sheikh Governorate, Egypt. The experiments were done during the autumn seasons of 2018 and 2019.

### Tested compounds

**Biofertilizers inoculums:** All of the microbial species of biofertilizers used in this study, including *Azotobacter* sp., *Azospirillum* sp., *Bacillus megathrium*, and vesicular *Arbuscular endomycorrhizae* (VAM), were kindly provided by the microbiology lab at the Sakha Agricultural Research Station in Kafr El-Sheikh Governorate, Egypt.

**Planting medium:** Vermiculite (soilless media for cultivation), It is a mineral of soil originated from mined rocks that is heated to produce the final product. It was packed in polyethylene bags, 2 kg mixed with 500g of compost per bag. The planting media were washed with tap water several times before planting then bags were prepared.

**Compost preparation:** it was prepared by admixing of 45 kg rice straw with 1 m<sup>3</sup> of farmyard manure. The mixture was sprayed with water to reach 60% humidity. The pile was stirred every seven days till maturation.

**Seedlings:** A popular local variety of eggplant (Anana) was selected for cultivation. The seedlings of 30 days old were brought from the Horticultural Research Institute (Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt) and were immediately transplanted.

**Organic nutrient solution:** It was a fish extract which made by weighing 10 kg of fish residues put in 100 L closed container, then about 40 L of water, 250 g of compost, and 200 ml of molasses were admixed together then air pump was operated for aerating the mixture. The aerobic fermentation process was continued for 20 days. Thereafter, one liter of this extract hydrostats was admixed with 20 L of water for irrigation. The PH of the concentrated fish extract was 9.1, the electrical conductivity (EC) of it was 8.3 ds/L, then it was diluted to become 7.9, and EC was 1.025 ds/L.

Relative humidity during the experimental study ranged from 69.3% to 76.1%. Average daily minimum and

maximum air temperatures ranged from 21-23°C and 31-34°C, respectively.

The seedlings of eggplant (*S. melongena*) were planted as one seedling in each planting bag (Fig.1). The planting bags were arranged in rows at distance of 50cm between each plant, the seedlings were irrigated with tap water for three consecutive days then with the fish extract solution after three days of planting onset. The nutrition dose was consequently increased after each growth phase (seedling, flowering and fruiting). The inoculants of the biofertilizers were applied twice, the first one was during planting of the seedlings by dipping the seedling root tip at the liquid of the biofertilizers for 30 min at the transplanting stage (**Kavitha et al., 2003**) then 2 ml of the liquid culture (30 ml/L of water) of the biofertilizer inoculums was added nearest each plant. The total count of each biofertilizer was  $35 \times 10^8$  cfu/ml. The first foliar spray of *Azotobacter* (Azt) was sprayed two weeks after planting while the second spray was four weeks after planting. The spraying process was carried out during the early morning. All treatments were tabulated in Table (1).

### **Insect pest survey**

The investigation and counting of the insect pests of the eggplant and their

natural enemies was performed to determine their abundance and infestation.

### Sampling techniques

The insect samples were taken at 7 days intervals using two methods of collection. Hand collection and visual record was conducted in the field directly, at each sampling date, 72 leaves were randomly chosen (6 plants x 12 replicates). The insect samples were investigated and collected from each plant, representing three different levels of the plant height. Sampling was carried out in the early morning (from 7 am to 9 am) where the insects tend to be inactive. All harmful and beneficial arthropod species were recorded and counted as mean per 6 plants, then transferred to the laboratory to be identified.

The studied pests which were monitored as adults and nymphs were jassids, *Empoasca* sp, white fly, *Bemisia tabaci*, aphid, *Aphis gossypii*, the green aphid, *Myzus persicae*, thrips, *Thrips tabaci*, and spider mites (*Tetranychus urticae* koch.).

### Chemical composition of green leaves of eggplants

Chemical analysis of the eggplant green leaves was conducted at the laboratory of Plant Nutrition Department, Soil, Water and Environment Research Institute, Sakha agricultural Research

Station, Kafr El-Sheikh governorate (Egypt). Leaf samples were collected at 17<sup>th</sup> of June after two months of planting then dried, grinded thoroughly and wet digested using sulphuric acid method of N, P, and K determination. Powdered plant samples were digested according to **(Peterburgski, 1968)**.

Nitrogen (N) content was determined by semi-micro Kjeldahl procedure **(Bremner, 1965)**. Phosphorus (P) was determined by spectrophotometrically methods of **Peterburgsk (1968)** and Potassium (K) content determined by flame photometer method according to **Rnngana (1977)**. Total polyphenols content was determined using the microscale protocol named Folin-Ciocalteu colorimetry **(Waterhouse, 2002)**.

Chlorophyll content of green leaves of the eggplant was assessed after 60 days of planting using SPAD -50% a portable leaf chlorophyll meter **(Minolta (Marquard and Tipton, 1987))** on a fully expanded leaf. The mean of three chlorophyll measurements was used.

### Yield Assessment

Mature fruits of the eggplant were harvested twice a week, placed in labelled paper bags and weighed immediately after harvest. This was done during the whole fruiting period of the

plant then the mean of the total weight per plant per season for each treatment were calculated.

### Statistical analysis

SysTest 10 for Windows (SPSS Inc., 2000) was used for the statistical analysis. The data of biofertilizers

treatments and the yield of eggplant fruits at each treatment were analyzed via one-way analysis of variance (ANOVA), followed by a comparison of the means with Duncan's multiple range Test (Duncan, 1955), The significance level was  $p < 0.05$ .

**Table (1):** Different treatments of the experimental design of different applications (2 ml/pot) of four biofertilizers applied as inoculations or sprays and control of the eggplant seedlings.

NO.	Treatments (T1-T11)
T1	AZ inoculation ( <i>Azospirillum</i> sp. inoculation)
T2	AZ inoculation + Azt. spary ( <i>Azospirillum</i> sp. inoculation + <i>Azotobacter</i> sp. spray)
T3	Azt inoculation ( <i>Azotobacter</i> inoculation)
T4	Azt inoculation + Azt. spary ( <i>Azotobacter</i> sp. inoculation + <i>Azotobacter</i> spray)
T5	VAM inoculation (endomycorrhizae inoculation)
T6	VAM inoculation + Azt. spary (endomycorrhizae inoculation + <i>Azotobacter</i> spray)
T7	B inoculation ( <i>Bacillus</i> sp. inoculation)
T8	B inoculation + Azt. spary ( <i>Bacillus</i> sp. inoculation + <i>Azotobacter</i> spray)
T9	Mix1 ( <i>Azospirillum</i> + <i>Azotobacter</i> + <i>Bacillus</i> ) inoculation
T10	Mix2 ( <i>Azospirillum</i> + <i>Azotobacter</i> + <i>Bacillus</i> inoculation+ <i>Azotobacter</i> spray)
T11	Azt. spray ( <i>Azospirillum</i> spary)
T12	Uninoculated control

AZ, *Azospirillum* sp.; Azt, *Azotobacter* sp. VAM, endomycorrhizae B, *Bacillus megatherium*; Mix1(AZ + Azt + B inoculation); Mix2 (AZ + Azt+ B inoculation + Azt spray).



**Fig. (1):** A: The experimental design of eggplant bags, B: Spraying of *Azotobacter* biofertilizer, C: Fruiting stage of the eggplant.

## Results

### Impact of the tested biofertilizers on the incidence of six sucking pests infesting eggplant during Autumn 2018/2019

Data recorded in Table (2) showed the effect of fertilization with *Azospirillum* sp., *B. megathrium*, *Azotobacter* sp. and mixtures of them as well as *Mychorrhiza* sp. with/or without *Azotobacter* spray on the population abundance of six species of sucking insect pests. The major pests identified on the eggplant during the study period were whitefly, *B. tabaci* (Genn.) (Homoptera: Alyrodidae), two species of aphids, *A. gossypii* (Glover) and *M. persicae* (Sulzer) (Homoptera: Aphididae), Jassid, *Empoasca* sp. (Homoptera: Cicadellida), *T. tabaci* (Thysanoptera: Thripidae) and red mites, *T. urticae* (Koch.) (Acarina: Tetranychidae) (Fig.2).

#### 1. *Bemisia tabaci*

The application of the studied biofertilizer inoculums with/or without *Azotobacter* liquid spray on the eggplant decreased the number of the two stages (adults and nymphs) of *B. tabaci*. Number of nymphs showed a notable decrease due to these treatments, VAM inoculation, B inoculation, B inoculation + Azt spray, Mix1 and Mix2 treatments which attained significant decreases lower than uninoculated control (one way ANOVA,  $F=2.2$ ,  $df=11$ ,  $P=0.05$ ).

The treatments of Mix1 gave the lowest abundance of *B. tabaci* (0.68 pests/3leaves  $\pm$  0.08) compared to (5.16 pests/3leaves  $\pm$  1.1) for untreated control but in the case of adults there were non-significant decrease due to treatments compared with control (one way ANOVA,  $F=1.7$ ,  $df=11$ ,  $P>0.05$ ).

#### 2. *Empoasca* sp.

The treatment of AZ inoculation, AZ inoculation + Azt spray and Azt inoculation + Azt spray induced a significant decrease in the abundance of *Empoasca* sp (one way ANOVA,  $F=3.3$ ,  $df=11$ ,  $P<0.05$ ). AZ inoculation attained the highest reduction effect, where it gave (7.5 pests/3leaves  $\pm$  0.96) compared to (14.34 pests/3leaves  $\pm$  0.56) for untreated control.

#### 3. *Tetranychus urticae* (Koch.)

Typically, most applied treatments negatively affected the abundance of *T. urticae* (Koch.). The treatment which attained the highest significant decrease in the abundance of *T. urticae* (Koch.) was AZ inoculation (0.12 pests/3leaves  $\pm$  0.04) compared to uninoculated control (1.87 pests/3leaves  $\pm$  0.23) (one way ANOVA,  $F=3.6$ ,  $df=11$ ,  $P<0.05$ ).

#### 4. *Aphis gossypii*

The applied treatments didn't attain a significant decrease in *A. gossypii* than untreated control (one way ANOVA,  $F=23.4$ ,  $df=11$ ,  $P > 0.0001$ ). In contrast,



AZ inoculation, VAM inoculation and VAM inoculation + Azt spray treatments exhibited high abundance over untreated control.

### 5. *Myzus persicae*

On the other hand, *M. persicae* drastically affected by the applied biofertilizer treatments which had a significant decline on it (one way ANOVA,  $F=4.34$ ,  $df=11$ ,  $P<0.05$ ). The treatments of AZ inoculation, VAM inoculation, B inoculation +Azt spray, Mix1 and Mix2 caused the same lower abundance ( $0.01$  pests/3leaves  $\pm 0.02$ ) than untreated control ( $1.2$  pests/3leaves  $\pm 0.1$ ).

### 6. *Thrips tabaci*

*Thrips tabaci* had the same trend regarding inoculation with all applied biofertilizers with or without Azt spray, all studied treatments achieved a decrease in the abundance of *T. tabaci* compared to uninoculated control. However, there were no significant differences between all treatments (one way ANOVA,  $F=1.04$ ,  $df=11$ ,  $P>0.05$ ).

### **Effect of studied biofertilizers, on the Chlorophyll, Phenols, Nitrogen, Phosphorous and Potassium contents of the green leaves of the eggplant.**

The results illustrated in Fig. (3) showed that biofertilization with *Azospirillum* sp., *Bacillus megathrium*, *Azotobacter* sp. and mixtures of them as well as *Mycorrhizae* sp. with/or without Azt

spray treatments induced higher contents of N% in green leaves than uninoculated control. Moreover, some inoculated treatments exhibited significant differences over control, such as, (Azt inoculation + Azt spray), Azt inoculation and (VAM inoculation + Azt spray) which had significant differences in Nitrogen contents (N%) over uninoculated control (one way ANOVA,  $F=2.3$ ,  $df=11$ ,  $P<0.001$ ). Typically, the studied biofertilizers showed a significant enhancement in the Phosphorus content of the green leaves (P%) of treated plants over untreated control (one way ANOVA,  $F=14.04$ ,  $df=11$ ,  $P<0.05$ ) (Fig. 4). The highest values of P% were recorded due to the treatment of VAM inoculation followed by VAM inoculation accompanied with *Azotobacter* spray (0.3% and 0.25%, respectively). The effect of the biofertilization treatments on the content of Potassium (K%) was generally positive (Fig. 5). The differences of the content of K% in the green leaves of the treated plants over untreated control were mostly significant (one way ANOVA,  $F=15.86$ ,  $df=11$ ,  $P<0.0001$ ). The highest content of K% was achieved with the treatments of AZ inoculation with *Azotobacter* spray (AZ inoculation +Azt spray) followed by VAM inoculation with *Azotobacter* spray (5.75% and 4.48%, respectively)

compared to (3.12%) for uninoculated control.

Phenols content of the green leaves of the eggplant sharply increased due to the application of the tested biofertilizers whereas all differences over control were significant (one way ANOVA,  $F=1.23$ ,  $df=11$ ,  $P<0.0001$ ) (Fig. 6). The Mix1 and Mix2 attained the highest values of phenols (259.32 ppm and 259.0 ppm, respectively) compared to 231.8 for uninoculated control.

Data represented in Fig. (7) indicated that the bio-inoculation with/without Azt spray treatments significantly increased the chlorophyll contents over un-inoculated control (one way ANOVA,  $F=73.1$ ,  $df=11$ ,  $P<0.0001$ ). The best treatment that increased the chlorophyll

content was Mix1 treatment (59.86 ppm) compared to (39.93 ppm) for uninoculated control.

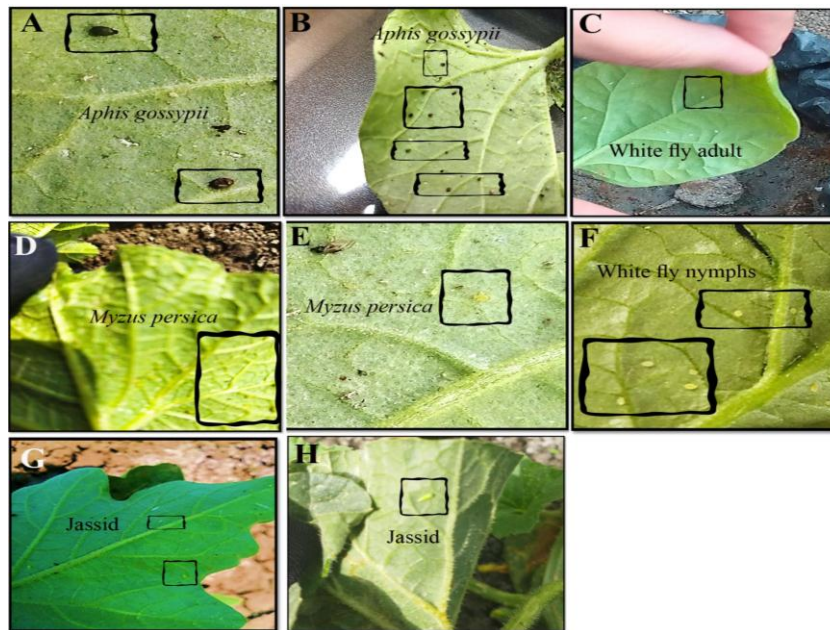
### Effect of biofertilizer applications on the eggplant's fruit yield

Data showed that the inoculation with/without Azt spray treatments attained higher increases in the fruits yield over un-inoculated control (one way ANOVA,  $F=5.11$ ,  $df=11$ ,  $P<0.05$ ). The best treatments gave a higher fruit yield were Mix2 (465.94 g/plant), VAM inoculation + Azt spray (286.16 g/plant) and Mix1 (284.09 g/plant) compared to uninoculated control (117.81 g/plant) (Fig.8).

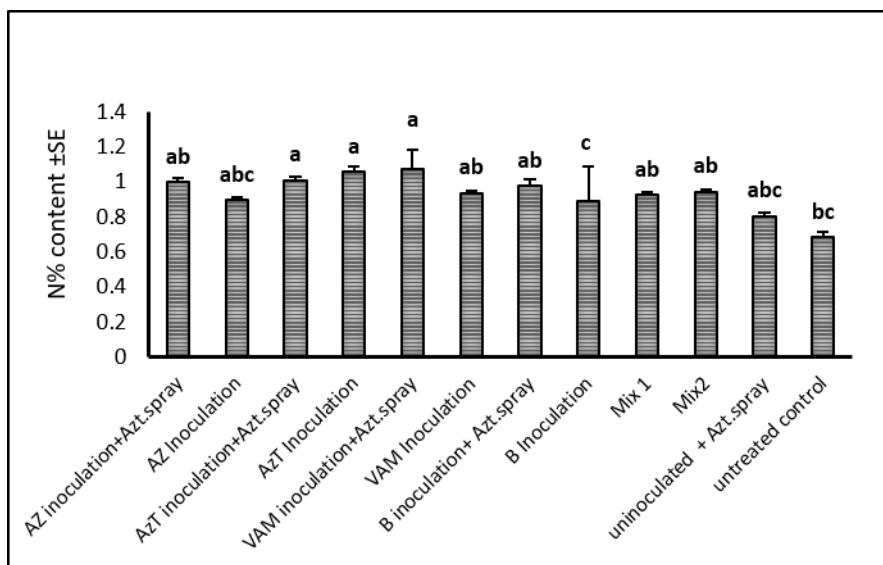
**Table (2):** Impact of four different bio-fertilizers on the population abundance (Mean± SE) of six sucking pest species infesting eggplants (*Solanum melongena* L.) during Autumn 2018/2019.

Treatments	<i>B. tabaci</i>		<i>Empoasca</i> sp.	<i>T. urtica</i> <i>koach</i>	<i>A. gossypii</i>	<i>M. persica</i>	<i>T. tabaci</i>
	<i>adults</i>	<i>nymphs</i>					
AZ inoculation	1.03±0.32	2.37±1.0 <sup>ab</sup>	7.50 ±0.96 <sup>b</sup>	0.12 ± 0.01 <sup>c</sup>	0.30 ± 0.24 <sup>b</sup>	0.01±0.02 <sup>b</sup>	0.37±0.14
AZ inoculation +Azt spray	1.24±0.29	3.27 ±0.37 <sup>ab</sup>	8.50±1.6 <sup>b</sup>	0.57±0.03 <sup>bc</sup>	0.81±0.05 <sup>b</sup>	0.02±0.03 <sup>b</sup>	0.44±0.12
Azt inoculation	1.50±0.09	2.90±0.83 <sup>ab</sup>	10.33±1.3 <sup>ab</sup>	0.72± 0.09 <sup>bc</sup>	0.02±0.05 <sup>b</sup>	0.08±0.05 <sup>b</sup>	0.19±0.07
Azt inoculation + Azt spray	1.72±0.31	2.50±0.53 <sup>ab</sup>	8.20±1.3 <sup>b</sup>	0.35±0.16 <sup>bc</sup>	0.24±0.05 <sup>b</sup>	0.34±0.09 <sup>a</sup>	0.56±0.14
VAM Inoculation	2.04±0.73	4.06± 1.3 <sup>ab</sup>	10.89±0.68 <sup>ab</sup>	0.61±0.14 <sup>bc</sup>	1.45±0.68 <sup>a</sup>	0.01±0.02 <sup>b</sup>	0.27±0.05
VAM inoculation + Azt spray	2.00±0.25	1.35±0.23 <sup>b</sup>	10.35±1.0 <sup>ab</sup>	0.24±0.04 <sup>c</sup>	14.34 ±2.7 <sup>a</sup>	0.10±0.02 <sup>b</sup>	0.57±0.32
B inoculation.	2.50±0.77	1.33±0.35 <sup>b</sup>	10.27±1.2 <sup>ab</sup>	0.97±0.12 <sup>abc</sup>	0.28 ± 0.05 <sup>b</sup>	0.02±0.03 <sup>b</sup>	0.56±0.14
B inoculation+ Azt spray	1.55±0.43	1.18 ±0.17 <sup>b</sup>	9.39 ±0.92 <sup>ab</sup>	0.78±0.08 <sup>bc</sup>	0.19 ±0.07 <sup>b</sup>	0.01±0.02 <sup>b</sup>	0.30±0.12
Mix1	1.03±0.32	0.67±0.09 <sup>b</sup>	13.99±1.7 <sup>a</sup>	0.39 ±0.06 <sup>bc</sup>	0.11 ± 0.02 <sup>b</sup>	0.01±0.02 <sup>b</sup>	0.35 ± 0.16
Mix2	0.81±0.17	0.68±0.08 <sup>b</sup>	10.43±1.2 <sup>ab</sup>	0.43±0.09 <sup>bc</sup>	0.37 ± 0.07 <sup>b</sup>	0.01±0.02 <sup>b</sup>	0.60 ±0.04
Azt spray	2.24 ±0.3	5.11±1.1 <sup>a</sup>	14.34±0.58 <sup>a</sup>	1.28±0.23 <sup>a</sup>	0.04 ± 0.03 <sup>b</sup>	1.0 ±0.1 <sup>a</sup>	0.21 ± 0.05
untreated control	3.14 ±0.58	5.16±2.9 <sup>a</sup>	14.35±1.6 <sup>a</sup>	1.87±0.19 <sup>ab</sup>	0.50± 0.16 <sup>b</sup>	1.2±0.06 <sup>a</sup>	0.78 ± 0.36
	NS						NS

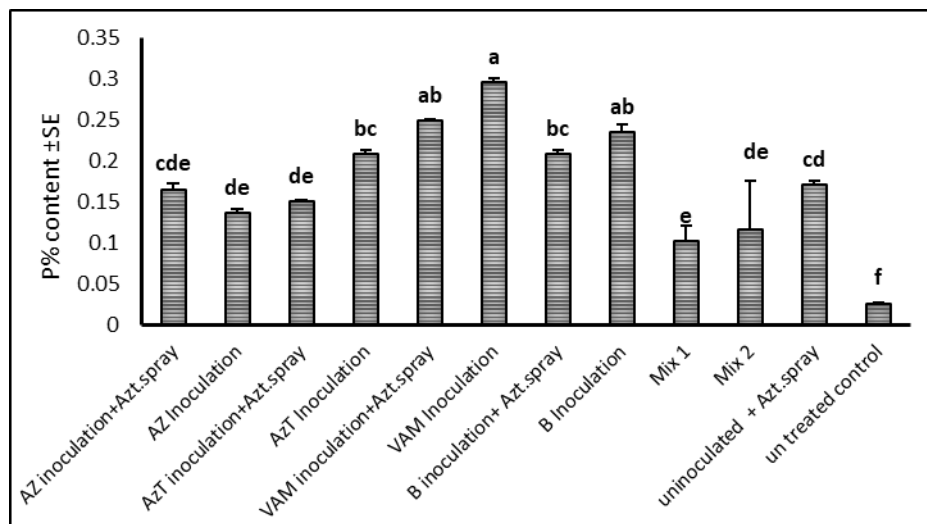
Means followed by different letters are significantly different based on Duncan's multirange Test ( $p < 0.05$ ),  $a > b > c$ . SE, standard error of means. Each record is a mean of pest number/3leavesof the plant  $\pm$ SE. NS: Means within a column without letters are not significantly different ( $p \leq 0.05$ )



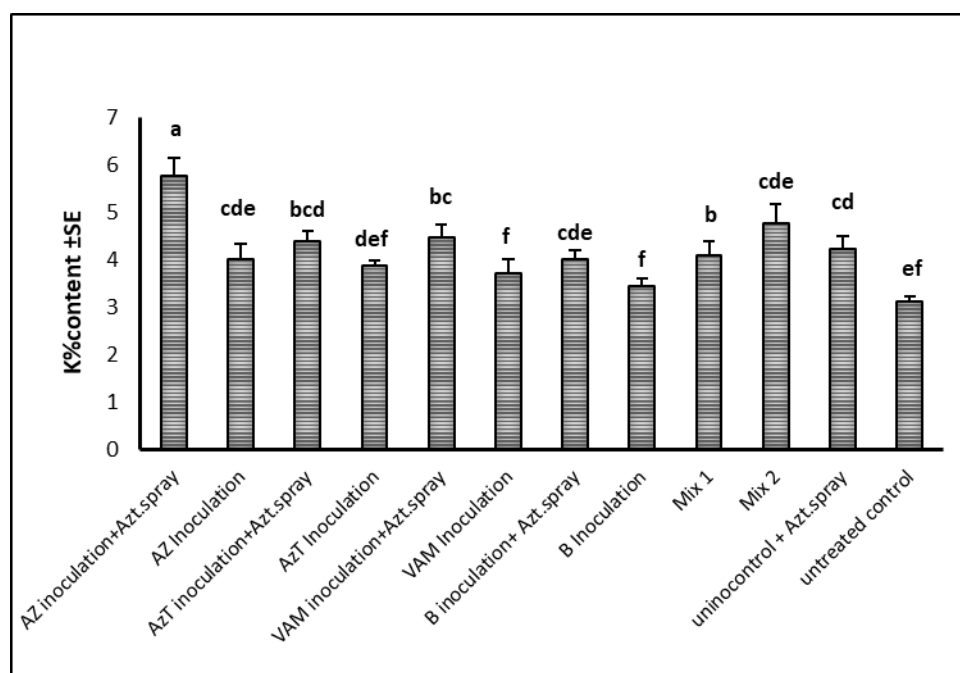
**Fig. (2):** Some of the studied insect pests associated with the eggplant green leaves



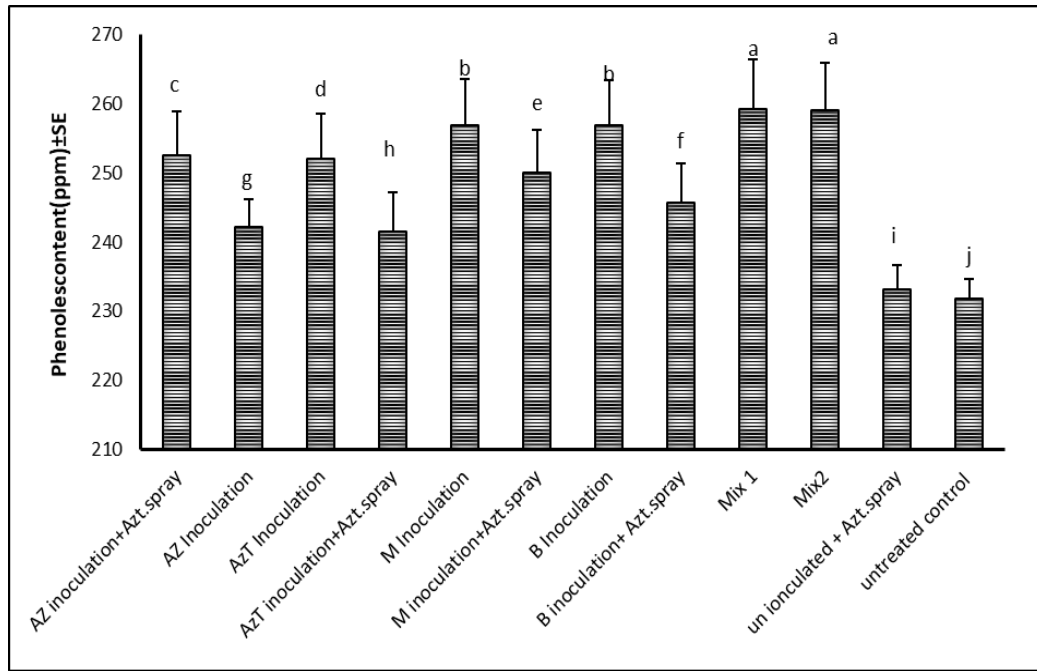
**Fig. (3):** Effect of four different biofertilizers and their mixtures on Nitrogen content (N%) of green leaves of the treated eggplants (*Solanum melongna* L.).



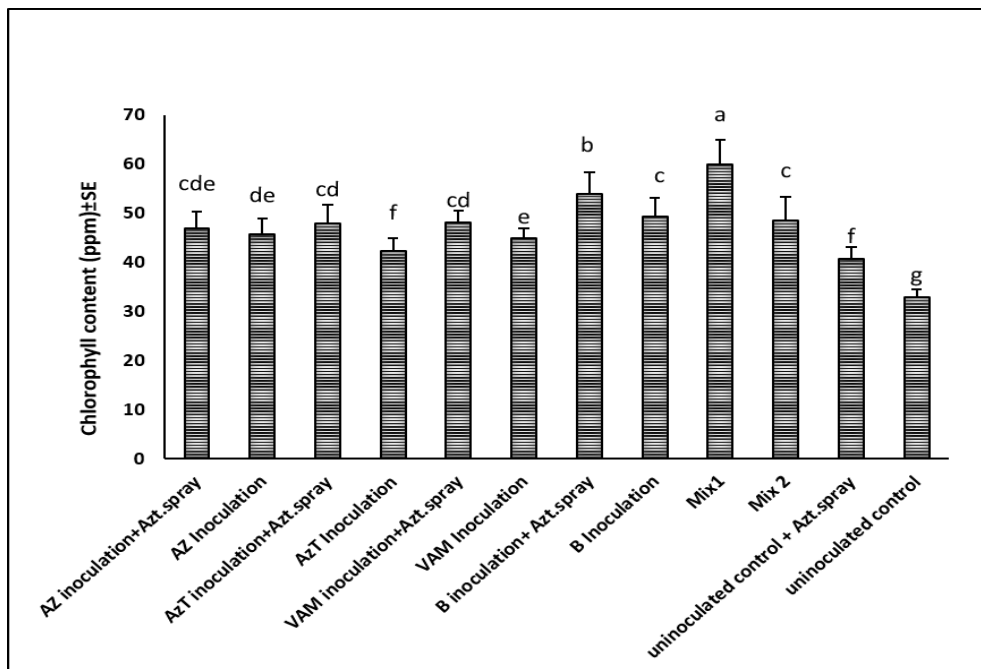
**Fig. (4):** Effect of four different biofertilizers and their mixtures on Phosphorous content (P%) of green leaves of the treated eggplants (*Solanum melongena* L.).



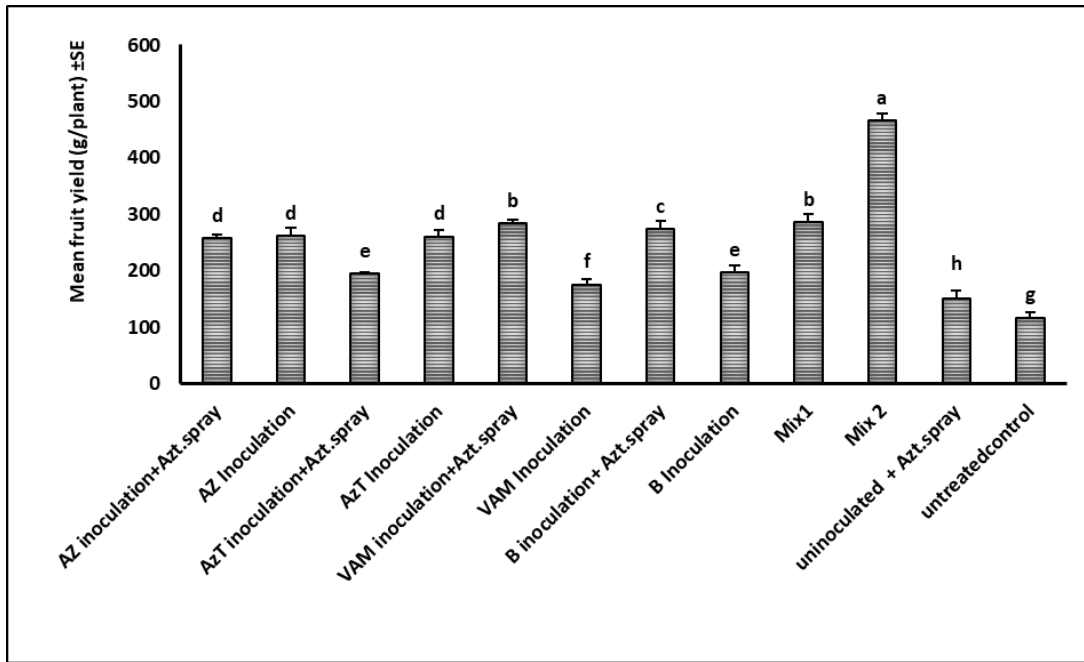
**Fig. (5):** Effect of four different biofertilizers and their mixtures on Potassium content (K%) of green leaves of the treated eggplants (*Solanum melongena* L.).



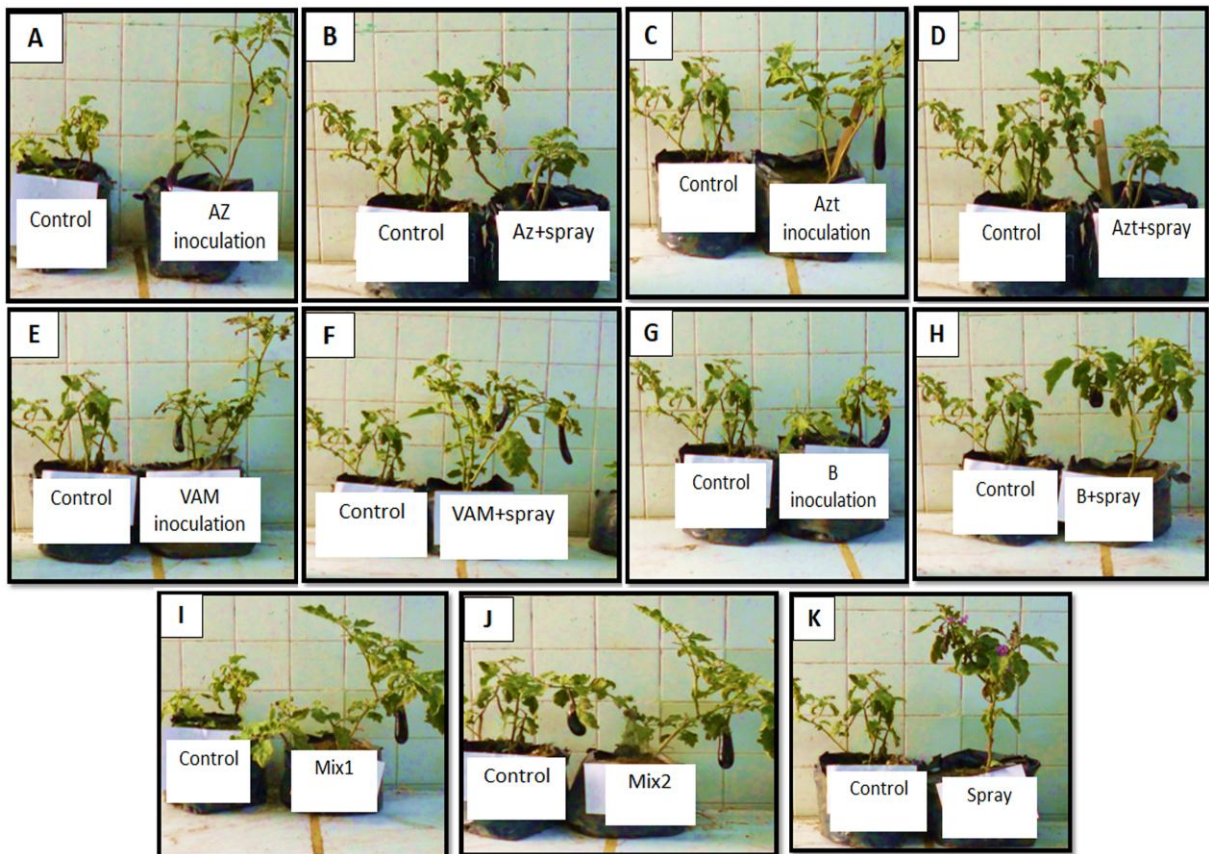
**Fig. (6):** Effect of four different biofertilizers and their mixtures on Phenol content (ppm) of green leaves of the treated eggplants (*Solanum melongena* L.)



**Fig. (7):** Effect of four different biofertilizers and their mixtures on the chlorophyll content (ppm) of green leaves of the treated eggplants (*Solanum melongena* L.)



**Fig. (8):** Effect of four different bio-fertilizers and their mixtures on the fruit yield (g)/plant (Mean  $\pm$  SE) of the eggplant in Autumn 2018/2019.



**Fig. (9):** The fruiting stage of the treated eggplants with four different biofertilizers and their mixtures compared to untreated plants (control).

## Discussion

Increased plant resistance to herbivorous insects is being developed as a result of the need for healthier food. The plant defense mechanisms are characterized by a combination of constitutive and inducible responses. Therefore, certain protective or curative procedures could be obscure using different non-chemical formulations to reduce the pest population and subsequent damage. In the current study, the applied biofertilizer formulations had significant reduction effects on the abundance of the investigated insect pests; *B. tabaci*, *A. gossypii*, *M. persica*, *Empoasca* sp. and red mites, *T. urtica* (koch.).

The present data investigated that the population abundance of the nymphs of *B. tabaci* were significantly decreased after the application of VAM inoculation, B inoculation, B inoculation + Azt spray, Mix1 and Mix2 treatments. In the case of adults of *B. tabaci*, there was a significant variation in the population abundance among all treatments. The higher impact of the applied treatments on the reduction of the population of nymphs of *B. tabaci* in contrast with adults might be due to the sessile living habit of the nymphs on the plant leaves during their entire life span before emergence to flying adults.

Moreover, the present investigation revealed the higher negative impact of (Mix1) on the abundance of the nymphs of *B. tabaci*, which might be speculated as a result of the synergistic effects of the three tested biofertilizers, *Azotobacter*, *Azospirillum* and *Bacillus* as mixtures than as single inoculants. Previous studies investigated that mixtures of biofertilizers increased the polyphenols compounds in the treated plants, which helped in the protection of the plant from high infestation of the sucking insects. The present results were in accordance with the data reported by **Dugassa-Gobena et al., (1998)**, who found that mixed inoculations achieved a good reduction of the incidence of bean aphid better than single inoculations. This was attributed to host plants, which received nutritive and protective compounds. **Ravi et al., (2006)** demonstrated that the integration of several nutrition sources progressively released the vital plant nutrients over the course of the growing period, which may have increased the establishment of induced resistance and subsequently assisted in tomato's ability to avoid whitefly attack. In this work, applications of combinations of three different types of biofertilizers have been used to achieve this goal. Suitable application of certain amounts of Nitrogen fertilizers would

be beneficial to manage insect herbivores; this might be achieved in our study during applications of mixtures of the three different types of biofertilizers. This speculation was in the same line with those of **Ai et al., (2011)** who suggested that proper application of Potassium and Nitrogen fertilizers should be beneficial to control the insect herbivores such as cotton aphid and plant growth at seedling stage of Bt-cotton field in central China. **El-Naggar et al., (2014)** investigated the positive effects of biofertilizers on the reduction of the population of the whitefly by reducing their oviposition rate. **Ravi et al., (2006)** also, observed that occurrence of sucking pests as whiteflies and a leaf hopper was decreased on plots treated with organic manures. In addition, they came to the conclusion that organic amendments significantly raised the overall amount of phenols and the activity of enzymes like polyphenol oxidase and peroxidase in the treated plants. Typically, **Godase and Patel (2002)** reported that the incidence of whitefly was significantly increased at the higher level of nitrogenous fertilizer compared to organic manures amended plots.

The present data revealed that AZ inoculation attained a significant

reduction effect against *Empoasca* sp. In contrast, AZ inoculation, VAM inoculation and VAM inoculation + Azt spray treatments exhibited a higher abundance of *A. gossypii* in comparison with the untreated plants. These results were similar to with the findings of **Herms (2002)** who assumed that the populations of these insect pests on their host plants are thought to be raised in response to rising Nitrogen levels through a variety of methods.

In the case of *T. tabaci*, our records showed that there were no significant differences in their abundance between different treatments and control. This is might be due to the potential factors that should be considered, such as the nutrient limitation of other sucking pests on biofertilized plants and plant defense compounds. For example, aphids and white flies are feeding deeply from the plant's phloem, in contrast with thrips, which feed superficially from the outer plant cells and thus may meet some limitations of nutrients or digestion of some compounds on biofertilized plants (**Hempel et al., 2009; Klopffholz et al., 2011; Ruscitti et al., 2011**). On the other hand, *M. persicae* was drastically affected by most of the applied biofertilizer treatments. The treatment of AZ inoculation, VAM inoculation, B inoculation + Azt spray, Mix1 and Mix2



attained the lowest abundance of *M. persicae*. However, **Dugassa-Gobena et al., (1998)** reported that by comparing to a single inoculation, the mixed inoculation successfully reduced the bean aphid population. This was attributed to host plants, which acquired nutrients and defensive chemicals that boosted the host plant's resistance to aphids by acting through a variety of biologically active alkaloids (**Edwards et al., 2010**). However, by boosting the activity of pest microbial antagonists and diversifying pest and predator species, organic fertilizers may help to reduce pest infestations (**Edwards et al., 2010**). **Yardımcı and Edwards, (2003)** found that organic fertilizer-grown tomatoes had fewer populations of aphids than synthetic fertilizer-grown tomatoes in the second year after application, suggesting that organic fertilizers may have the ability to reduce insect attacks in the longterm. Moreover, **Girardi et al., (2003)** hypothesised that possible changes in the bio-chemical substances and enzyme activity could be the likely cause of the lower population of thrips and whiteflies in the crop modified with organics. Also, they discovered that reducing the application rates of NPK fertilizers decreased the foliage's succulence, which may be another reason for the low occurrence of sucking insects.

In the present study, the inoculants of biofertilizers were first applied during planting of the seedlings by dipping the seedling root tip at the liquid of the biofertilizers for 30 min. at the transplanting stage. This application induced a higher resistance of the treated plants against herbivorous pests. **Bala et al., (2018)** investigated that treating plant seeds with bio-fertilizers increases their induced nutritional composition and the biological interactions in the soil, which in turn increases the developing plant's induced resistance (IR) against insect pests. As biologically induced secondary metabolites, phenol, flavonoids, and anthocyanin are cytotoxic and interact with several insect pest enzymes. Moreover, these compounds protect the plant against aphids by influencing the growth, development and feeding behavior of the insect (**Wójcicka 2010; Rashid et al., 2017**). **Pourya et al., (2020)** detected a significant relationship between Bio-farm biofertilizer-treated wheat and life table parameters of its aphid pest. The low fecundity of wheat aphid, *Sitobion avenae* fed on Bio-farm treated wheat plants was deduced by the high level of secondary metabolites, including flavonoid and total phenol in wheat leaves. These microorganisms can stimulate plant growth through the

regulation of nutrient and hormonal balance, phytohormones, the synthesis of certain chemicals or enzymes that can stimulate plant growth, the solubilization of minerals like Phosphorus, and the development of stress resistance (**Parewa et al., 2021**).

The present data investigated that the best treatment increased chlorophyll contents was Mix1 treatment compared to un-inoculated control. Whereas, the inoculation of Azt spray treatments surprisingly raised the phenol content of green leaves of the eggplant over un-inoculated control. Furthermore, the Mix1 and Mix2 attained the highest values of phenols compared to un-inoculated control. **Bhangu and Virk (2019)** itemized that the photosynthetic pigments and vegetative growth of soybean plants were improved by increasing the levels of nutrient fertilizer applications. the inoculation with or without spray treatments totally enhanced K% contents over un-inoculated control, AZ inoculation accompanied with spraying of Azt spray (AZ inoculation + Azt spray) induced the highest value of K% content compared to uninoculated control. In the case of N% and P%, the inoculation and/or Azt spray treatments attained the higher increases over un-inoculated control. VAM inoculation and VAM

inoculation + Azt spray gave the highest value of N% compared to uninoculated control. These results are consistent with those of **Swain et al., (2003)** and **Wange and Kale (2004)**, who found that Nitrogen is necessary for the synthesis of proteins, enzymes, and chlorophyll. Furthermore, Phosphorus supports plant growth and development, plays a significant role in crop production, and is required for the generation of phosphoproteins, phospholipids, ATP, and ADP (**Thilakarathna et al., 2016**). It is beneficial to legume growth because it promotes extensive root production and thus ensures a good yield (**Hefzy et al., 2015**). Organic fertilizers influence both yield and plant micronutrient contents and thus help sustain crop productivity (**Mottaghian et al., 2008**).

The present study showed that the best treatments produced the high fruit yield were Mix inoculation + Azt spray (465.94 g/plant), Mix inoculation (284.09 g/plant) and VAM inoculation + Azt spray (286.16 g/plant) compared to uninoculated control (117.81 g/plant). These observations were similar to that recorded by **Gomaa et al., (2002)** who stated that the combined inoculations are better than the single inoculation only. In the present study, the pots inoculated of Mycorrhiza achieved the

highest fruit yield of eggplants than other treatments. This appears to be due to their synergistic effect and better root proliferation, more nutrient and water uptake, higher plant growth, more photosynthesis, and food accumulation. These findings are consistent with those of **Swain *et al.*, (2003)** and **Wange and Kale (2004)** in okra. The combination of inoculation of biofertilizers consistently recorded better performance when compared with the single inoculation in okra. **Rajae *et al.*, (2007)** reported that free-living Nitrogen fixing micro-organisms, such as *Azotobacter* sp. and *Azospirillum* sp., enhanced root-development, increased water and mineral uptake, and produced plant hormones that might be responsible for growth of eggplant plant. Similarly, **Malik *et al.*, (2005)** revealed that *Azotobacter* culture application resulted in measurable improvements in grain yield, chlorophyll content, and postponed flowering. Furthermore, increased retention of nutrients, particularly P and K, which provided as irrigation nutrient solution with biofertilizers, which enhanced nutrient availability in the organic matter, were the main causes of the rise in nutrient content in soilless culture.

### Conclusion

Overall, this study offers new thoughts on the potential application of biofertilizers as a promising method for controlling sucking pests in organic soilless production.

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تأثير أربعة أنواع من الاسمدة الحيوية على تعداد الآفات الحشرية للباذنجان ، *Solanum melongena* (L) تحت ظروف الزراعة بدون تربة : تقييم صافي لمحصول الثمار والمحتويات الغذائية للنبات

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<sup>2</sup> قسم الميكروبيولوجى بمعهد بحوث الاراضى والبيئة مركز البحوث الزراعية بسخا

<sup>3</sup> شعبة علم الحشرات قسم علم الحيوان بكلية العلوم جامعة طنطا

يمكن للكائنات الحية الدقيقة في التربة بما في ذلك البكتيريا والفطريات الجذرية تحسين صحة النبات بطرق مختلفة من خلال الأنشطة المعززة للنمو. قد تسمح هذه الميكروبات المفيدة بمقاومة واسعة الطيف للحشرات اكلة النباتات. أجريت الدراسة الحالية لتقييم تأثير أربعة أنواع مختلفة من الأسمدة الحيوية، *Bacillus megatherium endomycorrhizae*، *Azospirillum* sp.، *Azotobacter* sp.، و (VAM) والتي تم تطبيقها كالفقاعات مع أو بدون رش لها أيضا على وجود وتعداد الآفات الحشرية للباذنجان ، *Solanum melongena* (L) خلال موسم الخريف. وكانت الآفات المدروسة هي *Aphis* ، *Bemisia tabaci* ، *Empoasca* sp. ، *Thrips tabaci*، نوعان من حشرات المن ، *Aphis gossypii* ، *Myzus persicae* ، والعنكبوت الأحمر ، *Tetranychus urticae*. وتم تقييم المحتوى الغذائي (%NPK) من النيتروجين ، الفسفور والبوتاسيوم، كذلك الكلوروفيل والفينولات للأوراق الخضراء للنبات وصافي محصول الثمار في ظل نظام الزراعة بدون تربة. كشفت النتائج أن معاملات الأسمدة الحيوية قللت بشكل كبير من تعداد الآفات الحشرية التي تصيب أوراق الباذنجان باستثناء *Aphis gossypii*، والتي كانت اعلى تعداد مقارنة بالمعاملة بدون الأسمدة الحيوية . بشكل عام، زاد محتوى (%NPK) في الأوراق الخضراء للباذنجان بشكل ملحوظ بسبب تلقيح ورش الأسمدة الحيوية حيث سجلت معاملة VAM in.+Azt. spray 0.3% . 1.08% اعلى قيمة لكل من N,P ، اما محتوى البوتاسيوم سجلت (5.75 % Az in.+Azt spray اعلى قيمة بينما زادت قيم الكلوروفيل بشكل إيجابي نتيجة تطبيق الأسمدة الحيوية، وأظهرت معاملة Mix1 على اعلى قيمة للكلوروفيل مع اختلاف كبير بينها وبين المعاملة بدون تسميد (59.86 ppm) . زاد تركيز الفينولات بشكل كبير في النباتات المعاملة ب Mix1 و Mix2 (259.32 ppm & 259 ppm) مقارنة بالمجموعة الغير معاملة باى اسمدة حيوية. علاوة على ذلك ، تم زيادة انتاج الثمار بشكل كبير بسبب الأسمدة الحيوية ، حيث سجلت المعاملة التي تكونت من خلط ثلاثة أنواع من الأسمدة أعلى قيمة. من هنا ، نقدم دليلا على أن الميكروبات المستخدمة كاسمدة حيوية تعتبر كدفاعات للنبات ضد الحشرات اكلة النباتات عن طريق تحفيز المقاومة الجهازية المستحثة ضد الحشرات من خلال تعزيز نمو النبات.