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Biological Control for some Insects by Using Plant Growth Promoting Bacteria in Laboratory and Field Conditions

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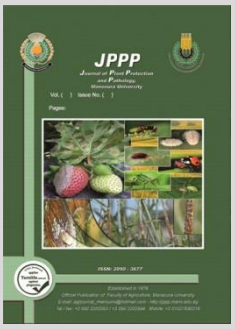

ABSTRACT

The cotton leafworm and aphid insects are major pests in Egypt that attack many host plants. Pesticides use can cause different problems in the plant system. So, it is very important to use nature products to be safe as a alternatives to synthetic pesticides. This research aimed to evaluate the efficacy of three bacterial types: *Lysinibacillus macroides*, *Brevundimonas olei* and *Acinetobacter sp.* in laboratory and field conditions throughout two seasons 2021 & 2022. *L. macroides* and *B. olei* showed more toxicity and LC_{50} levels than *Acinetobacter sp* with > 90 % corrected mortality towards the cotton leafworm and bean aphid after 3 days exposure time under laboratory conditions. Under field conditions, there were two insect pests fall armyworm (*Spodoptera frugiperda*) and aphid insects (*Myzus persica* (Sulzer) that investigate pepper plants in Ismallia Governorate. The results also showed a high efficacy of two bacterial strains, *L. macroides* and *B. olei* against *M. persicae* and *S. frugiperda* with more than 80% and 60% reduction percent respectively. The data stated that, *L. macrolides* strain was the most effective on suppressing the aphid and fall armyworm insects population. Some bacterial enzymes and biochemical products produced by bacteria used, such as protease, chitinase, catalase, glutathione peroxidase, polyphenol oxidase (PPOs) and HCN. They play a role in insect population reduction in laboratory and field conditions throughout integrated crop management.

Keywords: *Lysinibacillus*, *Brevundimonas*, Biocontrol, Bacterial enzymes, *Spodoptera sp.*, *Aphis sp.*

INTRODUCTION

The cotton leafworm and aphid is very important insect that is distributed in the world. Immature stages of these pests can feed on a lot of plant species belonging to 40 families and the percent of development has important nutritional component (Fahmi *et al*., 2019). Aphids (Hemiptera: Aphididae) are important insects in legume crops. They do a harm effects toward the plant by sucking of plant sap. In addition to be a carrier of many viruses. These insects could be control by chemical insecticides. This chemical causes harmful in the environment, residue of the product, the resistance insects, and negative effects of beneficial insects. In recent years, there a development of novel bio-insecticides as important for good environment to be used in the integrated pest control (Yankova *et al*., (2021). Chemical-based pesticides are very harmful to human and the environment. The biocontrol method will decrease our dependence on harmful chemicals and pesticides. Biopesticides are many pesticides derived from natural sources as plants, bacteria, fungi, viruses, etc., to control weeds, insects and pathogens Haarshitha (2022). Industrialization and urbanization have significantly expanded the usage of synthetic chemicals. Long-term use of toxic chemicals and indiscriminate, on the other hand, has a detrimental influence on the human and environment; as a result, public awareness about the harmful consequences of chemicals is growing by the day. Exploiting viable alternatives to alleviate these problems has become a big task and because of their safe and environmentally benign nature, beneficial microorganisms are gaining popularity (Ahsan and Shimizu, 2021).

Novel bioinsecticides is a friendly for insects control methods in the environmental system. New bacterial species are being identified and produced into new products with novel mechanisms of action. *Photorhabdus* spp. and *Xenorhabdus* spp., *Serratia* species, *Yersinia entomophaga*, *Pseudomonas entomophila*, and the recently discovered *Betaproteobacteria* sp., *Burkholderia* spp., and *Chromobacterium* spp. are other important examples. Finally, *Actinobacteria* sp. such as *Streptomyces* spp. and *Saccharopolyspora* spp. have gotten a lot of economic interest for their capacity to create a variety of insecticide-like metabolites. (Ruiu, 2015).

Bacillus cereus and *Bacillus lentus* cultures showed the highest effective protein hydrolysis (Dominika Ciurko *et al*., 2021). Antioxidant enzyme as Catalase, that acts as a catalyst to convert H_2O_2 to oxygen and water. Unbalanced activity of antioxidants and reactive oxygen species causes oxidative stress. Oxidative stress does and spreads many ailments (Sathiya Jeeva *et al*., 2015). The copper-containing enzyme, or polyphenol oxidase PPOs, is present in bacteria, fungi, plants, and animals. They are bi-copper metalloenzymes that catalyse one or two electron oxidations of phenols in the presence of oxygen (Panadare and Rathod, 2018). *Lysinibacillus macroides* and *Brevundimonas olei* can use as bio-pesticides, to reduce chemical pesticides harms and keep environment, human health and plants safe, Usta (2021) said that *Lysinibacillus* sp. species had a high potential to be used as bioinsecticide against *Varroa*

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destructor mite. Vnikova *et al*., (2015) reported that the Gram-negative bacteria dominated the bacterial community associated with *Varroa mites*, as evidenced by members of the *Brevundimonas* and *Rhizobium* genera. Several of the discovered species have never been connected with the *Varroa mite* or the honey bee, and others are members of unique bacterial taxa.

The purpose of this research is to test the insecticidal activity of various bacterium types against some insects in the vitro and in the vivo.

MATERIALS AND METHODS

Three strains, *Lysinibacillus macroides*, *Brevundimonas olei* and *Acinetobacter sp.* were studied to obtain an extensive overview for the occurrence of insecticidal activities against cotton leafworm (*Spodoptera littoralis*) and bean aphid (*Aphis craccivora*) with the leaf dip technique within strains under laboratory condition and and spriyng two insets .

Biological activity of bacterial strains under laboratory conditions

A. Biological activity of bacterial strains against cotton leafworm (*Spodoptera littoralis*) insects

Rearing of cotton leafworm insects

The selected insects for this study were cotton leaf worm, *Spodoptera littoralis* (Boisd.). A stock culture of *Spodoptera littoralis* was obtained from sensitive laboratory strain was reared for several generations for free from any contamination of insecticidal or microbial, at the plant protection department, Desert Research Center, Egypt. These insects were used in our study. Egg-masses were reared on leaves of castor bean, *Ricinus communis*, according to El-Defrawi *et al.,* (1964) under constant conditions: 26±1°C and 70±5% relative humidity. Egg-masses were collected daily and kept in plastic containers until hatching. Larva was reared on caster bean leaves that were provided daily. The pupae were collected and placed in clean Jars in moist saw dust placed at the base to provide the pupation site. Adults were provided with 10% sugar solution. All stages of cotton leafworm were cultured and tested at 26±2°C and 70± 5 % R.H.

Bioassay of the bacterial strains against cotton leafworm insects

Tests for treatments were carried out using different bacterial strains. Leaf-dipping technique was used according to El-Sheikh *et al*. (2013) and Ali *et al*., (2021). Bacterial strains were utilized to study the toxic potential against *Spodoptera littoralis* larvae, newly molted third-instar larvae (≤ 3-day-old) were fed on castor bean leaves which were evaluated with each treatment. The solvent was evaporated to leave a thin film of the matrerials. A hundred larvae were used for each treatment in five replicates in 20 larvae for each replicate. The larvae were put in clear glass jars (400 ml) provided with treated leaves and covered by pieces of muslin (Abouelghar *et al*., 2013). The dead larva was scored each day, the dead larvae were recorded documented if no movement was seen. The control treatment was the larvae that was fed on leaves with water only. Mortality percentages were recorded in one, two, three and fourth days of treatments, and were corrected by Abbott's formula (Abbott, 1925). Bacterial strains were tested against cotton leafworm for their toxicity. The strains which gave promising results in these preliminary tests were subjected to the same tests using a series of concentrations (100, 75, 50 & 25%). All the LC_{50} values of the tested extracts were calculated as mg / ml.

B. Biological activity of bacterial strains against bean aphid *Aphis craccivora* insects

Rearing of bean aphid insects.

Aphis craccivora was reared under laboratory condution of plant protection dep., Desert Research Center, Cairo Egypt. Aphids were obtained from infested faba bean plants. Seeds of faba bean were planted in plastic pots (11 cm x 14 cm) that were put in a wooden screen cage (1×1×1m) that covered with an anti-aphid screen in the laboratory. After that, the collected aphids were placed on the growing seedlings. On new un-infested faba bean seedlings, the artificial infestations by aphids were successively repeated (Ali 1999, El-Solimany and Abolfadel 2022).

Bioassay study of the bacterial strains against bean aphid:

The leaf dipping technique was used (Ali, 1999). The third node of plant leaves were used from the terminus of broad bean *Vicia faba* seedlings and were dipped in bacterial strains solution dissolved in water for 10 seconds. A control leaf was dipped in solvent. The bacterial strains were compared with Protecto formulation (bioinsecticide) which was dissolved in water. After drying at ambient temperature a single leaf was put in a petri dish (9 cm diameter). Ten adults at the age 2-3 days old were starved for an hour and were placed on the treated leaf and the dish was covered. Dead adults and offspring were recorded after one and two days as exposure time.

C. Statistical analysis and toxicity lines:

Obtained data with different treatments were subjected to statistical analysis to determine the potential activity of bacterial strains as spots as insecticides. In the present investigation all mortalities were corrected according to Abbot's formula (1925). Mortality curves (LC-P Lines) were drawn according to the method developed by Finney (1971) and computed according to the POLO software program.

Microbiological study

Lysinibacillus macroides, *Brevundimonas olei* and *Acinetobacter* sp. were identified as phenol degrading bacteria from olive mill effluent samples, Ramsay *et al*. (1983) presented the procedure, and Bergy's Manual of Determinative Bacteriology (1994) was used to identify the bacteria. According to (Lane, 1991), 16SrRNA sequencing studies verified the discovery, (Omar and Ibrahim, 2023).

A. Determination of bacterial enzymes

Protease enzyme detection

The individual strains were spread onto plates of skimmed milk agar medium component were ($NaCl_2$ 5%, $NaCl_2$ 3%, Agar 2%, Skimmed Milk 1%, pH 7 and Distilled water 100 ml, they autoclaved separately at 12°C for 15 minutes. After cooling to 45°C both were mixed and plated then incubated at 30°C for 24 to 48 hours), according to (Aruna *et al*., 2013).

Chitinase enzyme detection

Basic chitin medium (BCM) (tryptone 1% (w/v), yeast extract 0.5% (w/v), NaCl 1% (w/v), agar 1.5% (w/v), and chitin 0.25% (crab shell)) was used to evaluate strains

with chitinolytic activity. Aliquots of cell suspension (20 ul) were placed on the surface of (BCM) agar and incubated at 300 °C for up to 1 week; the presence of clearing zones encircling the colony showed the presence of chitinase activity. According to Liu *et al*., (2014), colloidal chitin was used in this study instead of crude chitin because it is more soluble and accessible, promotes chitinase synthesis, and is hidrolised more rapidly by bacterial chitinases.

Glutathione Peroxidase detection

Glutathione peroxides and Catalase were conducted in Desert Research Center, Central Lab., by HPLC Ultimate 3000 Thermo dionex, Germany. It is a member of the glutathione peroxides family of enzymes that detoxify peroxides in the cell. Peroxides can breakdown into extremely reactive compounds. Free radicals lead to damage to the cell. The conversion of H_2O_2 to water is catalyzed by peroxides enzymes. The equivalent stable organic peroxides (R-O-O-H) glutathione (GSH) as a source of alcohols (R-O-H) reducing. This evaluate according to Aebi, (1984).

Catalase enzyme detection

Catalase is an antioxidant enzyme found in almost all aerobic cells. It's one among the body's defensive mechanisms against H_2O_2, a powerful oxidant that can cause intracellular damage. The catalase test joins the Antioxidant Biomarkers family, providing yet another oxidative stress tool research, according to Paglia and Valentine (1967). Both bacterium individuals were cultivated at 30°C for 7 days after being inoculated in nutrient broth medium with 6% NaCl was used (Jacobs and Gerstein, 1960). To measure catalase activity in this approach, a 100-µL bacterial solution was placed in a Pyrex tube (the diameter was 13 mm and the height was 100 mm). Following that, 100 µL of undiluted H_2O_2 was added to the solutions and properly mixed before being incubated at room temperature, when the reaction is finished, the height of the O_2-forming foam that, according to (Iwase *et al*., 2013).

Cellulase enzyme detection

A quantity approach Medium LuriaBertani (LB),forb acterial activity increase, 100 µl of the solution was put onto1 L of carboymethyl cellulose (CMC) agar medium plates cont aining 0.5gKH2PO4, 0.25g MgSO4, 0.25g cellulose, and 2g gelatin.The plates were thenincubated12h at 37°C, according to Oxoid, (1982) and Yin *et al.,* (2010).

Polyphenol oxidase (PPOs) activity detection

A spectrophotometer at absorption 420 nm was used to measure enzyme activity in a 3 ml reaction combination containing 0.1 ml substrate (0.1 M 4-methylcatechol, catechol, or L-Dopa) and 0.1 ml enzyme preparation. (0.1 M phosphate buffer, pH 6.5). A spectrophotometer (Jenway Model 6105 UV/ Vis spectrophotometer) and a 1 centimetre light path quartz cuvette were used to quantify the activity of PPOs. The void was included. (0.1 mL substrate and 2.9 mL buffer). PPOs activity was measured in duplicate, with one enzyme unit equaling the quantity of enzyme needed to produce a 0.001 absorbance increase in one minute at 420 nm, (Guven *et al*., 2017).

HCN detection

Lorck's (1948) method was used to assess HCN generation in all genotypes. To summarize, nutritional broth was enriched with 4.4 g glycine/L, and organisms were spread on modified agar dish. The surface of the plate was coated with Whitman filter paper No. 1 that had been soaked in a solution of 2% sodium carbonate in 0.5% picric acid. Plates were covered with parafilm and kept at 28 2°C for 4 days. The development of an orange to crimson hue suggested HCN production.

Field study

A farm in Ismailia Governorate (30°35'00"N 32°16'00"E), Pepper seeds (Top Star) were provided by Agriculture Research Center, Agriculture and Land Reclamation (MALR) Ministry, Cairo - Egypt. The experiment statistical design was split plot design with three replicates of pepper plants in (2×3 m^2). Seeds of pepper plants were sown at May, 2022. The growth parameters of plant were taken (lengths / Cm, fresh and dry weights / Kg/Feddan) these estimated as stated by (Black *et al*., 1965). The following fertilizers added before preparation the soil. 20 m^3 organic manure, Super phosphate (15.5% P_2O_5) Phosphate fertiliser (15.5% P_2O_5) was applied at a rate of 300 kg/feddan, 50 kg of potassium sulphate (50.0% K_2O) was applied at flowering stage, 25 kg Magnesium sulphate, 100 kg agricultural sulfur, and nitrogen fertiliser (Ammonium sulphate) (20.5% N) was applied at a rate of 400 kg/feddan of recommended dose (half of the amount was incorporated in dry soil 15 - 20 days after sowing, the other half dose was added 45 – 50 days after first addition and the rest was added one week pre flowering stage). The irrigation system used in the experiment was drip irrigation using four-liter-per-hour drippers once a day for 30 minetes. Plastic pipe lines with a 16 mm diameter and a 43 m height made up the lateral sides.100 centimetres separated them (the pipe) from one another, the distance between the plants in the row was 30 cm, and the distance between the treatments was 1 m. Every replication has a lateral side of 10 m and 20 plants. Each treatment comprised three duplicates and 60 plants. Table 1 describes the physical and chemical parameters of the experimental soil (1). Plant samples were properly cleaned and dried at 70ºC/ 3 days, including straw and seeds. Plant materials were wet digested with H_2O_2 and H_2SO_4 using the Nicholson (1984) technique. The concentrates of bacteria utilised were (10^8 CFU) for both bacteria used separated, and they were added separately by dilution 1 bacterium: 3 equal volume of water. The nutritional broth culture (Jacobs and Gerstein, 1960) was employed in a 250 ml Erlenmeyer flask with 100 ml of nutrient broth medium to establish individual bacterial growth. Both cultures developed to maturity after being inoculated and incubated at 30°C for 7 days (10^8 CFU). Protecto, a bio pesticide, was applied at a rate of 2 mL per litre.

Table 1. The properties of Physical and chemical Ismailia experimental soil

	Particle size distribution (%)				EC	pH		
	Sand	Silt	Clay	Texture class	(dS m^{-1})			
Soil depth (cm) 0-30	87.5	7.3	4.9	Sand	0.68	7.76		
	Soluble cations and anions in soil extract saturated (meq/l)							
	Soluble anions					Soluble cations		
	Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^{--}	HCO_3^-	SO_4^{--}	Cl^-
	0.25	0.19	1.44	0.92	-	0.54	1.61	4.65

Insect observations

To investigate the insects that attack pepper plants, samples of 20 leaves from each replication were randomly selected from plants and placed in various levels and directions (Sharaby *et al*., 2015).Samples of infected leaves were taken before each subsequent spray to gauge the pre-treatment count and every 15 days to gauge the level of the infestation. To be examined under a binocular microscope, the samples were stored in paper bags in a refrigerator. They were separated, identified and counted Using the algorithm, the percentage of infestation reduction (R%) was determined by the formula (Topps and Wain, 1957).

$$R\ \% = \frac{C - T}{C} X\ 100$$

Where:

C: Number of insects recorded in the control samples.
T: Number of insects recorded in treatment samples.

Foliar spraying treatments:-

Two bacterial strains *Lysinibacillus macroides* and *Brevundimonas olei* were sprayed on pepper plants. It was kindly obtained from Soil Fertility and Microbiology- Dep. Desert research center. The concentration 10 ml/L was used in foliar spray in both seasons.

Protecto formulation was used as bio insecticide.

Control (without spraying).

Microbiological analysis in soil rhizosphere of Pepper

The following media were used to count various Densities of microorganisms in soil rhizosphere samples:

The M.P.N approach and Cochren's tables were used to count nitrogen fixers using Ashby's medium (Abd- El - Malek and Ishac, 1968), (Cochran, 1950). Overall microbiological counts were performed using nutrient agar medium (Jacobs and Gerstein, 1960). Bunt and Rovira (1955) medium agar was employed for phosphate dissolving bacteria counts.

Dehydrogenase activity in the rhizosphere of soil: Soil Dehydrogenase activity (gTPF/g dry soil/24hr.) was measured using 2, 3, 5-triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF), Casida (1977).

Statistical analysis

Using the Statistix version 9 computer software, the present work data was quantitatively analyzed, and the differences between the means of the treatments were significant, as they were greater than the least significant differences (L.S.D) at the 5% level. (Analytical software, 2008).

RESULTS AND DISCUSSION

Biological activity of bacterial strains under laboratory conditions

A. Biological activity of bacterial strains against cotton leafworm (*Spodoptera littoralis*) insects.

Toxic effects of bacterial strains against cotton leafworm insects

The data given in table (2) show the toxic effects of bacterial strains towards cotton leafworm (*Spodoptera littoralis)* with the leaf dip technique. A high degree of mortality percentage were provided for three bacterial strains through 3 days exposure time in laboratory conditions. The results show that two strains (*Lysinibacillus macroides* and *Brevundimonas olei*) showed highly toxicity with > 90 % corrected mortality after three days exposure period towards the *Spodoptera littoralis* (98.0 & 91.0%), respectively. The strain *Acinetobacter sp.* which achieved activity less than 50 % mortality (43.0%). In agreement with our results, Alfazairy *et al*., (2013) showed that *B. thuringiensis var. mexicanensis*) is a very effective microbial control agent on laravae of cotton leafworm. Heba *et al*., (2016) showed that five bacterial isolates (244, 482, 483, 722 and 723) showed ≥ 90 % mortalities percentage on cotton leafworms. The data were acceptance with Sarita *et al*., (2022) showed biocidal effects against *S. litura* of , *Enterococcus casseliflavus, Enterococcus mundtii, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas paralactis and Pantoea brenneri* that isolated from adults of *S. litura* and showed higher larval mortality in *K. pneumoniae* and *P. paralactis.* The data was acceptance with Terry *et al*., (2014) which discussed the commercial formulation of bacteria, *Bacillus thuringiensis* , *Lysinibacillus (Bacillus) sphaericus*. and *Bacillus sp.* is the only commercial strains of entomopathogenic bacteria that are produced in mass production techniques.

Table 2. Toxicity of bacterial strains against cotton leafworm larval stage.

Treatments	Exposure time (Days) %Corrected Mortality		
	1 days	2 days	3 days
Lysinibacillus macrolides	23.0	55.0	98.0
Brevundimonas olei	15.0	45.0	91.0
Acinetobacter sp.	5.0	25.0	43.0

Generally, it could be concluded that *Lysinibacillus macroides* and *Brevundimonas olei* were showed the most potential since they gave > 90 % corrected mortalities against *Spodoptera littoralis*. So, more details studies were need about two strains to calculate LC_{50} values.

Toxicity lines and LC_{50} values for most toxic treatments against cotton leafworm

Table (3), presents the efficiency of two tested strains *Lysinibacillus macroides* and *Brevundimonas olei* on the 4th instar larvae of *S. littoralis*. LC_{50} values of two tested treatments on 4th instar larvae were determined as 107.3 and 125.0 mg/ml, in *Lysinibacillus macroides* and *Brevundimonas olei* after three days exposure period, respectively. The Slope was 5.4 and 4.0 in *Lysinibacillus macroides* and *Brevundimonas olei*, respectively. The isolate *Lysinibacillus macroides* was the most toxicity than *Brevundimonas olei* against the 4th instar larvae of *S. littoralis* through the days exposure times. According to these findings, Seham and Fatma (2022) showed that the entomopathogenic bacteria *Bacillus thuringiensis* var. *kurstaki* (BtK) were the most activity for the sugar beet population against cotton leafworm. Approved with our data Alfazairy *et al*., (2013) showed that the Egyptian cotton leafworm expresses a protein toxin that is active against lepidopterous insects, so four local *Bacillus thuringiensis* (Bt) isolates that had been serologically identified as Bt var. kurstaki (Btk2, Btk3, and Btk66) and Bt var. mexicanensis (Btm27) were tested in the lab as biocidal control agents on this pest. Moreover, PCR amplification results demonstrated that 4D20 and Btk66 both include the cry2 type gene, which is active in Lepidoptera and Diptera, and that Btk66 also has the cry7 and cry8 genes, which are

active in Coleoptera. Btk66 and Btm27 had the greatest effectiveness as microbial control agents against *S. littoralis* among the six strains. The current research is the first to show that Btm27, a strain of *B. thuringiensis* known as *B. mexicanensis*, is a highly effective microbial control agent against cotton leafworm larvae.

Table 3. Cumulative LC_{50} values (mg/ml) of two bacterial strains against cotton leafworm with leaf dip technique.

Treatments	Exposure time (Days) %Corrected Mortality					
	1 days		2 days		3 day	
	LC_{50}	P	LC_{50}	P	LC_{50}	P
Lysinibacillus macroides	25361.2	5.4	330.4	1.7	107.3	5.4
Brevundimonas olei	4.44E+08	1.5	901.9	1.9	125.0	4.0

B. Toxic effect of bacterial strains against bean aphid

The data presented in table (4) shows that effective three bacterial strains (*Lysinibacillus macrolides*, *Brevundimonas olei* and *Acinetobacter sp.*) against adult and nymphal aphid stages. The toxicity against nymphs and adults of bean aphid insect increased as exposure time after treatment increased.

Effects on nymphs

Bacterial isolate (*Lysinibacillus macroides*), showed the highest insecticidal effect against nymphal stages since it gave 100% mortality followed by *Brevundimonas olei* which gave mortality > 90% with 94.6% corrected mortality after three days exposure. Other bacterial isolate (*Acinetobacter sp.*) showed mortality less than 50 % with 48.0% corrected mortality after three days exposure. It is clear that, Shaohui *et al.,* (2022) said that the metabolites of two bacteria, *Photorhabdus luminescens* and *Xenorhabdus bovienii, had* toxic effects on the black pecan aphid, *Melanocallis caryaefoliae,* and the black margined aphid, *Monellia caryella.* These data was agreement with Ali *et al*., (2017) showed toxic effects for some bacterial isolates on nymphs and adults of mealy bug after three days with 90 % corrected mortality percentage.

Effects on adults

The potential activities of bacterial strains (Lysinibacillus macroides and Brevundimonas olei) were estimated to show maximum toxicity with > 90 % percent mortality after 24h exposure time towards the aphid adult stage. The strains *Acinetobacter sp.*, which achieved activity less than 50 % mortality. With the intention of using bacteria as efficient biocontrol agents that were acceptable with our results, Paliwal *et al*., (2021) discovered new insights into aphid sensitivity to bacterial infection. We screened a range of environmental bacteria isolates for their abilities to kill target aphid species. Tests demonstrated the killing aptitude of these bacteria against six aphid genera (including *Myzus persicae*). *Pseudomonas fluorescens* PpR24 proved highly toxicity to aphid insects. Shaohui *et al*., (2022) cleared toxicity of the metabolites of *Photorhabdus luminescens* and *Xenorhabdus bovienii,* symbionts as bacterial strains against the blackmargined aphid, *Monellia caryella.*

Table 4. Toxicity of bacterial strains against bean aphid nymphs and adults with leaf dip technique.

Treatments	Exposure time (Days) %Corrected Mortality					
	Nymphs			Adults		
	1 days	2 days	3 day	1 days	2 days	3 day
Lysinibacillus macrolides	40.2	77.8	100.0	36.0	68.0	98.2
Brevundimonas olei	33.6	60.0	94.6	28.0	55.8	90.2
Acinetobacter sp.	22.1	34.2	48.0	17.4	28.0	44.1

From the previous data, it is clear that (*Lysinibacillus macroides* and *Brevundimonas olei*) gave the most toxic effect against the aphid nymphal and adult stages. So the two strains were chosen for detailed studies and a series of concentrations (100, 75, 50 & 25%) were tested to calculate different toxicological parameters. The data proved that the nymphal stage was more sensitive than adult stage towards the bacterial strains.

Toxicity lines and LC_{50} values for most toxic treatments against bean aphid

Effects on nymphs

Concerning the bacterial strains, table (5) indicates that the toxicity of the two strains can be arranged discerningly according to the LC_{50} levels as follows; *Lysinibacillus macroides* (34.6) and *Brevundimonas olei* (38.5) mg/ml, respectively against bean aphid nymphs after 3 days exposure.

Effects on adults

The data in table (5) prove that the bacterial isolate of *Lysinibacillus macroides* after 3 days exposure was more effective than the other bacterial isolate *Brevundimonas olei* against adult of *Aphis craccivora*. After three days exposure, the calculated concentrations of the bacterial strains (*Lysinibacillus macroides*) and *Brevundimonas olei* were 36.8 & 38.9 mg/ml at the LC_{50} levels, respectively.

Table 5. Cumulative LC_{50} values (mg/ml) of two bacterial strains on nymphs and adults of bean aphid with leaf dip technique.

Treatments	Exposure time (Days) %Corrected Mortality											
	Nymphs						Adults					
	1 days		2 days		3 day		1 days		2 days		3day	
	LC_{50}	P	LC_{50}	P	LC_{50}	P	LC_{50}	P	LC_{50}	P	LC_{50}	P
Lysinibacillus macrolides	151.2	1.3	44.7	2.5	34.6	3.6	363.0	0.74	59.3	2.08	36.8	3.35
Brevundimonas olei	235.6	1.3	91.1	2.2	38.5	2.8	469.3	1.1	100.4	1.68	38.9	2.90

Concerning the LC_{50} value, isolate *L. macroides* was more active than *B. olei* against adult of *Aphis craccivora* through three days exposure. Ramasamy *et al*., (2020) created this study isolates against *Aphis gossypii* and *Aphis. punicae* . The exposed aphids died that back to the Bt toxins, according to bioassay results, and *B. thuringiensis* infection caused the physical alterations that were seen. These strains of *B. thuringiensis* were re-isolated from the deceased aphids, and the results provided here supported Koch's hypotheses. In light of this, native Bt strains have the potential to significantly reduce aphid populations and are a crucial part of integrated pest control. To identify and create a bioinsecticide that can be administered systemically to

control *A. gossypii* and *A. punicae*, more research is required.

Under laboratory conditions, the bioassay indicates that both bean aphid and cotton leafworm insects are susceptible to (*Lysinibacillus macroides* and *Brevundimonas olei*) strains. Two bacterial strains gave mortalities in acceptable LC_{50} values to be applicable under field conditions.

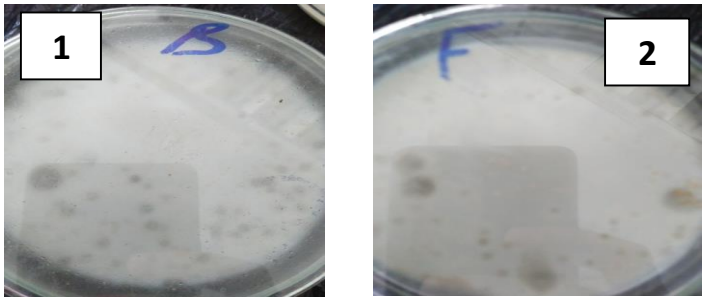
Microbiological study

The two isolates used were separated from olive mill effluent samples and identified as *Lysinibacillus macroides* strain LMG 18474 (NR 114920.1) and *Brevundimonas olei* MJ15 (NR 117268.1), (Omar and Ibrahim, 2023).

Determination of bacterial enzymes

Protease enzyme detection

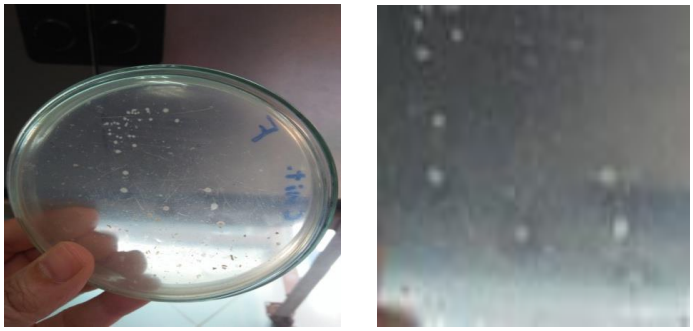
Both bacteria produced protease enzymes as shown in picture (1) and Table (6), by analysis of skimmed milk agar plates with zone of inhibition around bacterial colonies growth after incubation for 24 hours. Hydrolysis in the culture was by proteolytic bacteria. After incubation, the bacterial colonies showed zone of inhibition around every colony which mean protein degradation. The clear area depends on protease production, (Ling, 2019). Mechri *et al*., (2017), who used methods of statistical to assess *Lysinibacillus fusiformis* strain C250R produces protease in a submerged fermentation technique. The adjusted conditions resulted in a protease production of 3100U / mL, which was 4.5 times greater than the starting settings (680U / mL). Additionally, from strain C250R, a novel extracellular 51kDa-protease named SAPLF was isolated and biochemically characterised. Its action is highest at 70°C and pH 10. At 70°C and 80°C, its half-life periods were 10 and 6 hours, respectively. According to Poszytek *et al*. (2019), the *Brevundimonas* sp. LPMIX5 has a wide range of enzymatic activity, metabolic diversity, and the capacity to develop in a variety of environments. This strain has proteolytic, cellulolytic, lipolytic, xylanolytic amylolytic, and capabilities, it may make use of a variety of unique carbon and energy sources, as well as more complex industrial by products like molasses and dairy waste.



Picture 1. Detection of Protease enzyme activity as clear of zones by *Brevundimonas olei* (1) and *Lysinibacillus macroides* (2)

Chitinase enzyme detection

Picture (2) and Table (6) show *Brevundimonas olei* was positive produce for chitinase, which gave zone of inhibition around bacterial colonies, while *L. macroides* was negative producer. This agree with Ashour *et al*., (2018) isolated that the *Brevundimonus diminuta* KT277492 is a novel isolated from Egyptian soil. As a consequence, the maximal enzyme output of chitinase was 832.87 I UL^{-1}, representing an 8.767-fold improvement in enzyme production. This may be agrees with Singh *et al*., (2013) who study producing chitinase by strain *Lysinibacillus fusiformis* B-CM18 was isolated from rhizosphere and identified. It was discovered to produce a number of PGPR actions. A multivariate response surface method was also used to evaluate the effect of different factors on chitinolytic activity and enzyme production. A central composite design was used to achieve the highest chitinase production at optimum values of the process variables, temperature (20 - 45 °C), sodium chloride (2 -7%), starch (0.1 - 1%), and yeast extract (0.1 - 1%), added in the minimal medium supplemented with colloidal chitin (1-10%, w: w). The minimal medium was made up of chitin (6.09%), NaCl (4.5%), starch (0.55%), and yeast extract (0.55%) at 32.5 °C. was used to obtain the greatest chitinase production (101U ml^{-1}).



Picture 2. Detection of chitinase enzyme activity as clear of zones by *Brevundimonas olei*

Catalase antioxidant enzyme detection

Table (6) shows the amount technique of catalase antioxidant enzyme determination was high positive for both bacteria employed. According to Chantarasiri *et al.*, (2017), all isolated bacteria in Thailand being tested for ligninolytic features, based on the results of Remazol Brilliant Blue R decolorization in BSGYP medium, with nine bacteria were identified as ligninolytic bacteria. During 72 hours, the isolate BR2308 had the highest breakdown percentage of commercial lignin by 42.46 ± 2.79.

Temperature and pH were calculated to be ideal for industrial lignin decomposition at 8.0 and 30°C, respectively. The crude enzyme's ligninolytic performance for laccase, lignin peroxidase, and manganese peroxidase was examined, and it had 1.930.26 U mL^{-1} productive laccase activity. The isolate BR2308 was identified as *Lysinibacillus sphaericus* based on morphological and genotypic characteristics. It has the potential to be used in *Sesbania aculeata* biological pre-treatment.

Glutathione peroxidase detection

Table (6) shows Glutathione peroxidase produce by *Brevundimonas olei* up to (19.45338 U/L), this agree with Kim *et al*., (2021) who estimated Following that, the Axenic M. aeruginosa was co-cultured with synthetic bacterial communities taken from 15 distinct freshwater samples, each with variable degrees of H_2O_2-production and catalase activity. According to nanopore-based bacterial community studies, these growth-promoting effects were likely ascribed to a high abundance of *Alphaproteobacteria* (*Brevundimonas* and *Ochrobactrum* species), which shielded M. aeruginosa cells from H_2O_2 toxicity. Furthermore, after H_2O_2 detoxification, these bacterial populations had higher amounts of catalase activity and faster O_2 production rates. Glutathione peroxidase determination produce by *L. macroides* up to (0.542 U/L), this result agree with Ahsan and Shimizu (2021) studied that the *Lysinibacillus* has a lengthy history of being well-known for its ability to kill a variety of insects, Researchers' focus has recently turned to *Lysinibacillus* sp. as potential substitutes for agrochemicals for their ability to promote plant development and control disease.

Cellulase enzyme detection

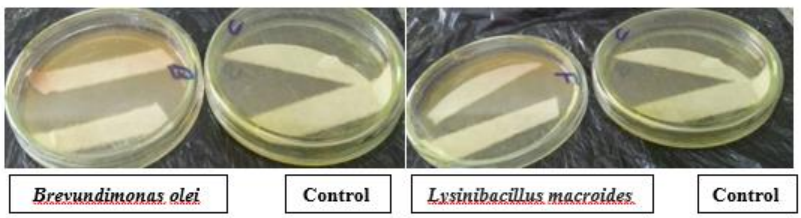
Table (6) shows *Brevundimonas olei* and *L. macroides* were grown on nutrient then put 1ml of each culture to carboxymethyl cellulose (CMC) media. They gave negative results in this experiment. These results were disagree with Mahalik *et al*., (2018) studied that the secondary metabolites, enzymes, and proteins are abundant in microorganisms. Cellulase is a key component of the enzyme cocktail utilised for the breakdown of lignocellulosic biomass, and the capacity to produce cellulase has been identified in *Lysinibacillus* sp., and in contrast to Khianngam *et al*., (2014), Based on the decolorization of CMC-basal agar media with Congo red as a colour identifier, the bacteria were grown to have the ability to degrade carboxymethyl-cellulose (CMC). The breakdown capacity of cellulase was determined to be between 1.56 and 4.14 IU/ml. Based on phenotypic characteristics and 16S rRNA gene sequence sequencing, all isolates were Gram positive rod-shaped *Bacillus*, *Paenibacillus*, and *Lysinibacillus* bacteria. In broth medium, their cellulase activity varied from 0.0390.002 to 0.2330.005 IU/ml. Cellulose production may be related to species differences within the same strain.

Polyphenol oxidase (PPOs) detection

Table (6) shows both strains gave positive result for produce (PPOs). *L. macroides* gave (1.3 mM) and *Brevundimonas olei* gave (5.4 mM). This agree with Seleim *et al*., (2014) evaluated that the effect of bio-agents in induction of Peroxidase (PO) and polyphenoloxidase (PPOs) enzyme activity in plant, *Pseudomonas putida* and *Pseudomonas fluorescens* effects, as well as their combination, were investigated. The study also discovered that the activity of PO and PPOs in tomato plants treated with the PGPR isolates *P. fluorescens* and *P. putida* increased considerably. Peroxidase and polyphenol-oxidase activity are biochemical indicators for biocontrol effectiveness in tomato bacterial wilt management. These results are consistent with those of Rana *et al*. (2011), who investigated the efficacy of plant growth-promoting rhizobacteria (PGPRs) in activating plant immune enzymes in wheat (*Titicum aestivum*) at an early period in the field. Defense enzyme activity was significantly increased in wheat leaf homogenates. Peroxidase (PO) and Polyphenol oxidase (PPOs) activities were increased by 42.5 - 56.9% in *Providencia* sp. (AW_5) alone and in conjunction with *Bacillus* sp. (AW_1) and *Brevundimonas* sp. (AW_7), as well as a twofold increase in Phenylalanine ammonia-lyase. These findings indicate that *Providencia* sp. (AW_5), alone or in conjunction with AW_1 and AW_7, has the ability to boost defensive enzymes in wheat plants in the field. Vanitha *et al*, (2009) estimated that the PPOs, an enzyme that contains copper, aids in the cell's defence against pathogen infection. Although reports have suggested that PPOs do a role in pathogen defence, the physiological activities of PPOs in plants are yet unknown. PPO's antinutritive effect in tomato pathogen defence, where PPOs can be generated to high levels, has been well reported. The coordination of PPOs induction with a number of other recognised tomato defence proteins were also present the significance of PPOs in defence.

HCN detection

Picture (3) and Table (6) showed positive results of production of HCN from both bacteria which gave orange color, while the yellow color was in control treatment. Adrian *et al*., (2017) indicated cyanogenesis is a chemical defensive tactic that involves the production, storage, and discharge of hydrogen cyanide (HCN, hydrocyanic, or prussic acid) towards an assaulting opponent. Because of HCN's extreme volatility and toxicity, it requires chemical stabilisation for storage as well as protection against accidental self-poisoning. According to Ahsan and Shimizu, (2021), *Lysinibacillus* has long been known for its insecticidal ability against numerous insects, especially mosquitoes, which are the carrier of several human illnesses.



Picture 3. HCN detection

Table 6. Results of enzymes determination

Strains Enzymes	*Lysinibacillus macroides*	*Brevundimonas olei*
Protease enzyme	+++	+++
Chitinase enzyme	++	Negative
Catalase antioxidant enzyme	+++	+++
Glutathione peroxidase enzyme	+	+++
Cellulase enzyme	Negative	Negative
Polyphenol oxidase (PPOs)	+	+++
HCN production	++	++

Field study

Insect control

Under field conditions, there were two insect pests fall armyworm (*Spodoptera frugiperda*) and aphid insects (*Myzus persica* (Sulzer)) that investigate pepper plants in Ismallia Governorate. Two bacterial strains *Brevundimonas olei* and *Lysinibacillus macrolides* were used that predicated toxic effects against test insects under laboratory conditions by using spraying on vegetative plants in many sprayers.

Effect of foliar spraying on fall armyworm (*Spodoptera frugiperda*)

As shown in tables (7) the results indicated that differences between the treatments in reduction percentage and infestation levels of fall armyworm of pepper plants. Top Star cultivar was significantly lower in the treated plants with foliar spraying than control ones. The highest reduction was obtained from *Lysinibacillus macroides* treatment followed by *Brevundimonas olei* strains. Among the natural products tested, the strain *Lysinibacillus macroides* produced the greatest reduction (91.2 & 84.8%) in the leaves and larvae of fall armyworm. *Brevundimonas olei* isolate reduced the infestations in acceptable effect (75.2 & 70.1 %), respectively. Protecto formulation gave highly effects against fall armyworm with 100% reduction percentage. The results were in agreement with EL-Fergani's, (2019) field trials with Avaunt® and Protecto® (*Bacillus thuringiensis* kurstaki) for the control of *Spodoptera littoralis* Boisd on the sugar beet root variety (Karam) in Egypt. The field evaluation demonstrated that Avaunt® and Protecto® reduced the larval population of *S. littoralis* relative to the control area. Farmers in Egypt can use Protecto® and Avaunt® to cotton leafworm through integrated crop management. The previous information was the same information that Paliwal *et al*., (2021) used to determine the effects of applying bacterial isolate PpR24 to plants immediately before introducing aphids to the plants. It is evident from Shikano *et al*. (2017) that bacteria, plants, and insects interact with one another. Current and upcoming studies on the effects of microbe-induced modifications to plant defences and nutrient quality on the behaviour and fitness of insect herbivores. By inhibiting plant defenses and detoxifying protective phytochemicals, microorganisms that are linked with herbivore and parasitoid insects might increase insect fitness. By changing the quality and defense of the plant, microorganisms can control or impact insect behavior and fitness. Plant-beneficial microorganisms can stimulate plant development and alter the nutritional and phytochemical makeup of plants, which can either favorably or unfavorably affect insect fitness. Finally, we propose that entomopathogens have a mechanism of action that influences plant defenses indirectly by changing insect physiology or directly as entophytes.

Table 7. Cumulative infestation of fall armyworm (*Spodoptera frugiperda*) in pepper groves treated with two bacterial strains, throughout seasons 2021/2022

Foliar Treatments	Infestations*			
	Leave		Larvae	
	Mean**	R%	Mean**	R%
Lysinibacillus macrolides	1.2 b	91.2	3.1 b	84.8
Brevundimonas olei	3.4 c	75.2	6.1 c	70.1
Protecto	0.7a	98.0	0.6a	100.0
Control	13.7		20.4	

R % = Reduction percentage

*** Cumulative number of infested fruits all over the growing season (8 dating samples)**

***Means having similar letters in the same column are not statistically differed at *P*≥0.05.**

Effect of foliar spraying on aphid insects (*Myzus persica* (Sulzer))

The data in the tables (8) revealed that, *Lysinibacillus macroides* and *Brevundimonas olei* strains showed significant results in controlling aphids as compared to the control treatment. Results showed significant differences on the mean number of *M. persicae* recorded from two strains under field conditions. The differences can be attributed to different modes of action of the products and also the time after spraying. The results also showed a high efficacy of bacterial strains against *M. persicae.* The data stated that, *Lysinibacillus macroides* isolate was the most effective in suppressing the aphid insects population. The reduction percent of *L. macroides* showed more than 80% against nymphs and adults of aphid insect (87.4, 85.1 & 80.2%), respectively as compared to the control treatment after spray applications. *Brevundimonas olei* isolate recorded more than 60 % on reduction percent of control leave, nymphs and adults of aphid insect (73.6, 68.6 & 65.4%) respectively. The efficiency of *Lysinibacillus macroides* isolate was more than *Brevundimonas olei* isolate against aphid insects under field conditions. Yankova (2021) showed that *B. amyloliquefaciens* A1 and *P. rettgeri* K10 microorganism had aphicidal effects against black bean aphid (*A. fabae*) and pea aphid (*A. pisum*) on beans and peas trees.

Table 8. The reduction percent of aphid (*Myzus persica* (sulzer)) on infested Pepper groves treated with two bacterial strains, throughout seasons 2021/2022

Foliar Treatments	Reduction Percent Infestations*					
	Leave		Nymphs		Adults	
	Mean**	R%	Mean**	R%	Mean**	R%
Lysinibacillus macrolides	1.1b	87.4	2.8b	85.1	3.2b	80.2
Brevundimonas olei	2.3c	73.6	5.9c	68.6	5.6c	65.4
Protecto	0.4a	99.0	0.5a	100.0	0.6a	100.0
Control	8.7d		18.8d		16.2d	

R % = Reduction percentage

*** reduction percent infested fruits all over the growing season (8 dating samples)**

****Means having similar letters in the same column are not statistically differed at *P*≥0.05.**

Plant growth parameters

Addition of bio control role of both used bacteria; there were biofertilizer role and biotreatment Omar and Ibrahim (2023) which tested before in other researches, these results gave safety uses of these bacteria. Table (9) shows that foliar treatment of *L. macroides* and

Brevundimonas olei bacteria as biological controllers to reduce insects resulted in a much larger increase in fresh and dry weights, as well as length metrics, in Pepper plants compared with control. The most successful spray treatment with *B. olei* and *L. macroides* yielded, the following results of fresh weights: 63.8 and 51.2%, respectively at first spray and 62.7 and 14.9%, respectively at second spray.

Table 9. Plant growth parameters

Treatments		Fresh weight (Kg/Feddan)	Dry weight (Kg/Feddan)	Whole plant (Cm)
First spray	Control (untreated)	16.66^{b}	2.982^{bc}	33.6^{c}
	Lysinibacillus macroides	25.2^{a}	4.55^{ab}	38.5^{b}
	Brevundimonas olei	27.3^{a}	7^{a}	42^{a}
	Pestcide	10.5^{c}	1.05^{c}	27.3^{d}
LSD(0.05)		2.2138	2.4908	2.3983
Second spray	Control (untreated)	36.85^{c}	10.066^{b}	39.9^{c}
	Lysinibacillus macrolides	42.35^{b}	15.288^{a}	50.4^{b}
	Brevundimonas olei	59.99^{a}	15.4^{a}	59.3^{a}
	Pestcide	26.11^{d}	7.308^{c}	35^{d}
$LSD_{(0.05)}$		2.4908	1.8828	1.7585

The whole plants were increase by using bio-pesticide (*B. olei* and *L. macroides*) at first spray up to 14.5 and 25%, respect. At second spray the increases were 48.6 and 26.3%, resp., while treatment of pesticide was decreased the results of fresh weights and whole plants at first spray to 36.9 and 18.75 %, resp. At second spray, reduce of fresh weights and whole plants were 29.1 and 12.28%, resp.

These results agree with Sethi and Gupta (2013) which compared that the Treatment with pesticides caused a temporary harmful effect on the soil's microbial biomass carbon. In soil treated with bio-pesticide, the carbon content of the soil's microbial biomass increased over time, which is beneficial for agricultural productivity. With the application of bio-pesticides, biomass carbon increased. When compared to other pesticides employed, soil treated with pesticide (Victor) had a significantly lower level of microbial biomass carbon.

Microbiological analysis in soil rhizosphere of Pepper

The results of the microbial count, nitrogen fixers, number of soluble phosphate bacteria and dehydrogenase activity measurement in pepper rhizosphere were improved by using of *Brevurdimonas olei* followed by using *Lysinibacillus macroides* in first and second sprays, Table (10). Both of used bacteria gave good results, but *Brevurdimonas olei* was superior on *Lysinibacillus macroides*. AL-Ani *et al.*, (2019) which evaluated that the increase crop yield, numerous insecticides have been used. This has grown to be a significant environmental issue. An experiment determination was carried out the impact of commonly used pesticides on soil microorganism counts and microbial activities in the form of CO_2 generation. The findings showed that the three types of pesticides considerably reduced soil bacteria, fungus, and actinomycetes microbiological activity and numbers. The kind and quantity of insecticide used, as well as the length of the incubation period, all had an impact on the result that was observed. The amount of pesticides added to the soil had an adverse relationship with the microbial activity and the quantity of bacteria, fungus, and actinomycetes.

Table 10. Effect of bioinsecticides used on bacterial rhizosphere counts of Pepper plant

Treatments		Total count of bacteria (CFU/ g soil X 10^5)	Nitrogen fixers (MPN/ g soil X 10^3)	PDB (CFU/ g soil X 10^5)	Dehydrogenase activity (µg TPF /g dry soil/ 24h)
First spray	Control (untreated)	41	8	9	27.5^{ef}
	Lysinibacillus macrolides	55	11	21	30.5^{d}
	Brevundimonas olei	67	15	33	45^{b}
	Pestcide	35	3	7	24.2^{g}
Second spray	Control (untreated)	73	11	11	29.2^{de}
	Lysinibacillus macrolides	109	20	25	40.5^{c}
	Brevundimonas olei	120	17	35	49.8^{a}
	Pestcide	38	5	8	26.9^{f}
$LSD_{(0.05)}$		-	-	-	2.2175

CONCLUSION

Natural pestecticides are being increasingly in integrated pest management programs for invasive pests. Academic and industrial interest must be increased to discover and create novel bioinsecticides by scientists working in the area of insect pathology. The recent study indicated that the local bacterial strains *Lysinibacillus macroides* and *Brevurdimonas olei* showed high insecticidal effect suitable for controlling aphid and leafworm for preadult and adults insects and it could be recommended of using them as alternatives of chemical insecticides. The two strains can be also using as plant growth-promoting some enzymes and biochemical products produced by these bacteria, such as protease, chitinase, catalase, glutathione peroxidase, and polyphenol oxidase (PPOs) and they produce also HCN. The local bacterial strains *Lysinibacillus macroides* and *Brevurdimonas olei* can be used in integrated crop management.

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المكافحة البيولوجية لبعض الحشرات باستخدام البكتيريا المحفزة لنمو النبات في الظروف المعملية والحقلية

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الملخص

تعتبر كلا من دودة ورق القطن وحشرات المن من الآفات الرئيسية في مصر والتي تهاجم العديد من العوائل النباتيه. يمكن أن يؤدي استخدام مبيدات الآفات المصنعه الي العديد من الاضرار في انظمه النبات المختلفه. لذلك ، من المهم جدًا استخدام المنتجات الطبيعية لتكون آمنة كبديل لمبيدات الآفات المصنعه. يهدف هذا البحث إلى تقييم فعالية ثلاثة سلالات بكتيريه وهما : *Lysinibacillus macroides* و *Brevundimonas olei* و *Acinetobacter* sp. تحت الظروف المعملية والحقليه خلال موسمي ٢٠٢١ و ٢٠٢٢. واوضحت النتائج تحت ظروف المعمل ان كلا من السلالاتين *L. macroides* و *B. olei* لها سمية ومستويات LC_{50} أكثر من السلاله *Acinetobacter* sp بنسبه موت مصححه اعلي من ٩٠٪ ضد كلا من دودة ورق القطن وحشره المن وذلك بعد ٣ أيام من التعرض . ولقد اوضحت النتائج تحت الظروف الحقليه انه توجد نوعان من الآفات الحشرية هما دودة الحشد (*Spodoptera frugiperda*) وحشرة المن (*Myzus persica* (Sulzer)) علي نباتات الفلفل بمحافظة الإسماعيلية. كما أظهرت النتائج كفاءة عالية لسلالتين من البكتريا هما *L. macroides* و *B. olei* ضد *M. persicae* و *S. frugiperda* مع نسبه خفض في الاصابه أكثر من ٨٠٪ و ٦٠٪ على التوالي. حيث اوضحت النتائج أن السلالة *L. macrolides* كانت الأكثر فاعليه في خفض تعداد كلا من حشرات المن ودودة الحشد الخريفية. وأوضحت الدراسه ان بعض الإنزيمات البكتيرية والمنتجات الكيميائية الحيوية التي تنتجها البكتيريا مثل إنزيم البروتياز ، والكيتيناز ، والكتاليز ، والجلوتاثيون بيروكسيديز ، وبوليفينول أوكسيديز (PPOs) و HCN كلها ذات دور هام في الحد من تعداد الحشرات تحت ظروف المعمل والحقل وذلك من خلال برنامج الإدارة المتكاملة للمحاصيل.