TOXIC EFFECT STUDY OF *BACILLUS THURINGIENSIS* (*B.T.*) ISOLATE AND *ARTEMISIA JUDAICA* L., PLANT EXTRACT AGAINST POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* (ZELLER) (LEPIDOPTERA: GELECHIIDAE)

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Potato tuber moth (PTM), *Phthorimaea operculella* (Lepidoptera: Gelechiidae) is one of the serious worldwide pest of potato (*Solanum tuberosum* L. Solanaceae). This pest by making irregular tunnels leaves excreta behind and led to a considerable yield loss. Trials were conducted to evaluate the effect of *Bacillus thuringiensis* isolate against newly emerged larvae of PTM. It had been found that the total larval death recorded 14 and 58% of larval death at 1.25 and 10 CFU/ml, respectively. The potential of extract of *Artemisia judaica* against PTM larvae showed increase in larval mortality from 34 to 76% at 125 to 1000 ppm, respectively. In this study, the effect of *Artemisia judaica* extract on *Phthorimaea operculell*a larval instar was more effective than the effect of *Bacillus thuringiensis* isolate. In order to get the best results when using two bio-control agents in IPM strategies, bacteria should be used first and then the plant extract.

Keywords: *Bacillus thuringiensis*, *Artemisia judaica, Phthorimaea operculella*, biological control agents

Bacterial infections in insects can be broadly classified as bacteremia, septicemia, and toxemia. Bacteremia occurs when the bacteria multiply in the insect's hemolymph without the production of toxins. This situation occurs in the case of bacterial symbionts and rarely occurs with bacterial pathogens (Durasula et al., 1997). Septicemia occurs most frequently with pathogenic bacteria, which invade the hemocoel, multiply, produce toxins, and kill the insect (Wang et al., 1993). Toxemia occurs when the bacteria are confined to the gut lumen and produce toxins (Garczynski and Endo, 1991). The spore forming bacilli have received the most attention as biological control agents.

Many of them produce proteinaceous insect selective protoxins during sporulation. One member, *Bacillus thuringiensis*, has been used as a microbial pesticide against several insect pests, particularly lepidopterans. *B. thuringiensis* is a gram-positive spore forming bacterium that produces a parasporal crystal protein inclusion during its sporulation. *B. thuringiensis* has become the leading biopesticide since the beginning of the 1960s. The toxicology of *B. thuringiensis* is complex and its potency against particular insects varies with the strain of *B. thuringiensis* used.

Several studies were conducted in this area, Haghighian et al. (2008) and Hasheminia et al. (2011), mentioned that, the insecticidal effects of *Artemisia judaica* L. extract containing growth retardation, antifeedant, and larvicidal effects. Khosravi and Sendi (2013) observed that *A. judaica* extract affected the nutritional indices and also showed antifeedant activities on *Glyphodes pyloalis* Walker. Anshul et al. (2015) showed that methanolic extract of powdered *A. judaica* leaves adversely affect *Helicoverpa armigera*. The extract affected larvae by inhibition and disruption of the growth, development and histopathological and biological parameters of *H. armigera*. The essential oil of *A. judaica* has been demonstrated to possess insecticidal activity and repellence against several insects, such as *Callosobruchus maculatus* (Fab.) and *Sitophilus oryzae* L. (Aggarwal et al., 2001 and Abd-Elhady, 2012). Many studies reported that plants are considered as one of the richest sources that can be used as pest control agents (Nakatani et al*.,* 2002). In Egypt, some attempts have been done to monitor insecticidal activity of different plant extracts against many insects (Farag, 2002 and Sadek, 2003).

Potato tuber moth (PTM), *Phthorimaea operculella* Z., (Lepidoptera: Gelechiidae) is one of the serious worldwide pest of potato (*Solanum tuberosum* L. Solanaceae), this pest by making irregular tunnel leaves excreta behind and leads to a considerable yield loss (Herman et al., 2005). It causes serious damage to potato crops in fields and storage (Arnone et al., 1998). Presence of PTM in storage causing yield losses up to 100% (Joshi, 1989 and Rondon, 2010).

Many different countries search for less dangerous pesticides by using the naturally occurring herbs that can be applied effectively in habitats (Rawi et al., 1995, 1996 and Sadek, 2003).

The current aims were conducted to study the role of certain *Bacillus thuringiensis* (*B.t.*) isolate and *Artemisia judaica*, plant extract as a biological control agent against *Phthorimaea operculella.*

MATERIALS AND METHODS

1. Tested Insect

The original colony of potato tuber moth (PTM) (*Phthorimaea operculella*, Gelechiidae; Lepidoptera) was supplied from the Plant Protection Research Institute, Agricultural Research Centre, Giza,

Egypt. Mass rearing was carried out in the laboratory of the Economic Entomology Unit, Department of Plant Protection, Desert Research Center.

The infested potato tubers, containing different immature stages, were kept in glass jars (20×10 cm) with fine layer about 3-4 cm of clean, sterilized sand and fresh potato tubers in each jar, for the completion of the development of the immature stages, and covered with a piece of cotton cloth and held by rubber band. Pupae were collected every 48 hours by sieving the sand layer. Collected pupae were kept in glass tubers (3×10 cm) and covered with pieces of cotton cloth till emergence of moths. Emerged adults were collected by an aspirator and confined in an oviposition cage. The oviposition cage consisted of a glass jar (30 cm height × 25 cm diameter) and covered by a piece of cloth (Dapalan), fixed on the top of the cage and held by rubber band to serve as oviposition sites. Within each cage, a piece of cotton moistened with 20% sugar solution was used for moth feeding, held by rubber band inside the cage and changed daily. The cotton cloth containing the eggs were removed daily and replaced by another clean one. The egg sheets were inserted in 2% sodium hypochlorite and fresh water, respectively, and then were dried well for 1-3 hours and kept in clean Petri dishes (12 cm).

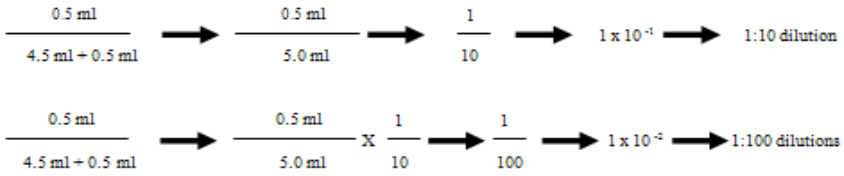
2. Soil Samples

Soil samples were collected randomly from different fields in El-Bahariya Oases. Surface materials of the soil was removed; and with a sterile spatula, about 100 g sample of soil was taken from at least 5 cm in depth. The soil samples were preserved in sterile plastic bags and stored for 2-12 months at 4°C until analyzed. The collection sites had no history of treatment with *B.t*.

3. *Bacillus thuringiensis* (*B.t.*) Isolation Technique

Based on the acetate selective method described by Smith and Couche (1991), soil samples (0.5 g) were added, each to 10 ml of LB broth buffered medium with 0.25 M sodium acetate buffer at pH 6.8 in a sterile conical flask under aseptic conditions in a laminar flow workstation. The flask was incubated in a controlled environment incubator shaker, Edmund Bühler (TH25) operated at 300 rpm and 30ºC for four hours. In this method, germination of *B.t*. spores was selectively inhibited by sodium acetate buffer (0.25 M), while most of the undesired spore-formers germinated. Then, suspensions were allowed to stand for 10 minutes; the upper layer of suspended samples were transferred to a sterile test tube with screw cap, followed by heat treatment at 80°C for three minutes in a water bath. Heat treatment was made to eliminate all vegetative cells and non-sporulated soil microorganisms present in the samples. The samples were left to cool at room temperature before inoculating 1 ml of the supernatant, using sterile pipettes onto agar plates and distributed over agar surface homogeneously. The plates were incubated overnight at 30°C; then random colonies of *B.t*. from agar

plates were transferred onto T3 - plates using sterile loop. Transferred colonies were left for 2-3 days at least to allow complete sporulation and crystal formation characteristic for *B.t.* isolate. Careful aseptic techniques were done for investigating the germinated colonies using a laminar flow workstation. Examination of germinated colonies was done using stained smears method. The germinated colonies were fixed to clean slides and stained according to Smirnoff (1962) stain method. For culturing the obtained isolates, the method Shake Flask Fermentation described by Morris et al. (1996). Small quantities of *B.t.* can easily be recovered by the lactose-acetone co-precipitation procedure of Dulmage et al. (1970), determining the number of bacteria that are present in the isolates described by Dulmage (1971), the total number of bacteria in the original solution was determined by counting the number of colony forming units (CFU's) and comparing them to the dilution factor. After that, serial dilutions from the original bacterial suspension were obtained. Tube 1 containing 4.5 ml of water; in addition to 0.5 ml of the undiluted bacterial suspension to yield a total volume of 5.0 ml.



For each dilution, the number of colony forming units (CFU's) on the plates was counted. Typically, numbers between 30 and 800 are considered to be in the range of statistically accurate data. To calculate the number of bacteria per ml of diluted sample, the following equation was used:

$$\frac{\text{Number of CFU}}{\text{Volume plated (ml) x total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{ml}}$$

4. Plant Materials

Samples of *Artemisia judaica* were collected from three different regions (Elmejrh, Alhubaiqah and Ras Saada) in Nuweiba Desert region, South Sinai, Egypt, during September and November, 2016.

5. Preparation of Plant Extracts

A known weight of the powdered plant material (about 20 g) was packed in a containers of glass separately and socked in 200 ml of the solvent (95% ethyl alcohol) for 7 days and shook for 15 minutes every day using an electric shaker. The alcoholic extracts were collected and then evaporated under vacuum at a temperature not exceeding 30°C in a rotary evaporator. The

residue left after evaporation was used for biological tests (Yazdani et al., 2014). The concentrations used for treatment of pink bollworm were 100, 200, 400 and 800 ppm.

6. Toxicity Test on Larval Stage

6.1. Using *B.t.* isolate

Larvae of *Ph. operculella* (after 5 days from egg hatching) were fed on thin slices of potato tuber, which were dipped in several concentrations of isolates (1.25, 2.5, 5, and 10 CFU/ml). Control slices were dipped in water. After drying at room temperature, a single segment was placed in a Petri- dish (10 cm diameter) and ten starved larvae (for two hours) were introduced. The dish was then covered. For each concentration, 10 replicates of 10 larvae each were tested. Numbers of alive and dead larvae were recorded daily till pupation.

6.2. Using *A. judaica* extract

The same way of treatment was applied by using different concentrations of extracts (125, 250, 500 and 1000 ppm).

7. Statistical Analysis

Data obtained in different tests were subjected to statistical analysis to evaluate the relative efficiency of the isolates. Mortalities were corrected for the natural mortality according to Abbott's formula (1925).

The corrected percent = (Observed %-Control %) x 100/ (100-Control %)

Concentration/mortality regression lines were drawn on probit logarithmic graph according to the method developed by Finney (1971). The LC_{50} and LC_{90} values were calculated according to probane program.

RESULTS AND DISCUSSION

1. Toxic Effect of *B.t.* Isolate on Potato Tuber Moth Larvae

Feeding of the larvae of *Ph. operculella* (Table 1) revealed adverse effects on the total percentage of larval mortality, which was concentration dependent. Whereas the total larval death recorded 6% in control trials, treatment revealed 14 and 58% of larval death at 1.25 and 10 CFU/ml, respectively. At any of the tested concentrations, the highest percentage of mortality occurred within the first three days following application, then the larval mortality started to decrease. As, for example, at 5 CFU/ml, the larval mortality started by 4% after the first day of treatment then recorded 6, 24, 4 and 4% during days, respectively.

Table (1). Toxic effect of *B. t.* isolate on *Ph. operculell*a larvae.

Conc. (CFU/ml)	Mortality % after indicated days										Total mortality %	
	1	2	3	4	5	6	7	8	9	10	Obs.	Corr.
0.00	0	2	0	0	0	2	0	1	1	0	6	0.0
1.25	0	4	6	0	0	0	0	4	0	0	14	8.2
2.50	2	4	14	2	0	2	0	0	0	2	26	20.6
5.00	4	6	24	4	0	2	0	0	2	0	42	37.0
10.00	8	10	30	6	0	2	0	0	0	2	58	53.6

The effect of *B.t.* isolate on PTM could be detected on the basis of the calculated LC_{50} and LC_{90} values, which recorded 7.95 and 48.00 CFU/ml, respectively (Fig. 1).

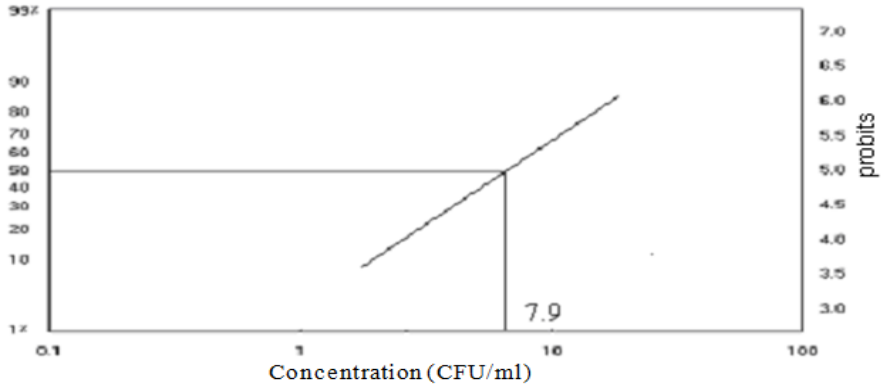


Fig. (1). Concentration / mortality regression lines for *Ph. operculella* larvae treated with *B.t.* isolate.

Larval mortality, according to Yoshinori and Kaya (1993), is probably due to either the septicemia in which the bacterial spores invade the hemocoel, multiply, produce toxin and subsequently kill the insect; or due to the toxemia in which the bacteria produce toxin and confined to the gut lumen. Abdel-Aziz (2000) attributed the larval mortality to such septicemia case. Mortality in infected larvae may also be due to the deficiency in the excretory system, due to Malpighian tubules infection (Lotfy, 1988). These factors individually or together may explain larval mortality.

The high percentage of larval mortality, three days post treatment, revealed higher susceptibility level of early larval instars of *Ph. operculella* to different concentrations of *B.t.* isolates. The higher susceptibility of young larval instars may be either due to the binding of the bacterial endotoxin to the brush border membrane of the midgut epithelium (Van Rie et al., 1990) or due

to certain physiological differences between the early and late instars, where in late instars certain enzymes are secreted due to which tolerance to the bacterial infection may be developed (Goldberg et al., 1974). Such results are in harmony with Desuky (1998), who found that when the 2nd larval instar of the cotton leaf worm was fed on both clover and cotton leaves, accumulative mortality percentage increased by time elapsed after spraying by Delfin till 24 hours, then decreased, whereas in case of the 4th larval instar, the accumulative mortality percentage decreased.

2. Toxic Effect of *A. judaica* Extract on Potato Tuber Moth Larvae

Exposure of potato tuber moth larvae to plant extract procedure revealed adverse effects on the total percent of larval mortality, which was concentration dependent. Increase in larval mortality was recorded at 125 to 1000 ppm by 34 and 76%, respectively. The data show that, the action of *A. judaica* active ingredient exerted cased the drastic larval death in the first and second days, after that the mortality decreased (Table 2).

Table (2). Toxic effect of *A. judaica* extract on *Ph. operculella* larvae.

Conc. (ppm)	Mortality % after indicated days										Total mortality %	
	1	2	3	4	5	6	7	8	9	10	Obs.	Corr.
0	0	2	0	0	0	2	0	1	1	0	6	0.0
125	16	8	4	0	2	0	2	2	0	0	34	28.8
250	24	8	2	0	2	0	2	0	2	0	40	35.0
500	30	12	8	0	4	2	0	0	2	0	58	53.6
1000	46	12	6	4	4	2	0	2	0	0	76	72.2

The effect of *A. judaica* extract on PTM could be detected on the basis of the calculated LC_{50} and LC_{90} values, which recorded 3.70 and 33.21 ppm, respectively (Fig. 2).

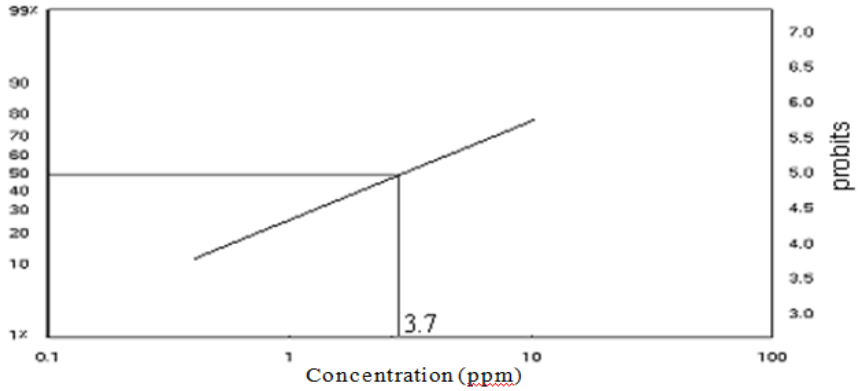


Fig. (2). Concentration/mortality regression lines for *Ph. operculella* larvae treated with *A. judaica* extract.

Mohamed and Abdelgaleil (2008) found that, the essential oils of *A. judaica* showed the highest activity with *Mentha microphylla*. Abdelgaleil et al. (2008) mentioned that, the essential oil of *A. judaica*, grown on Sinai Peninsula of Egypt, was extracted via hydrodistillation. Chromatographic separation on repeated silica gel columns led to isolate two compounds namely piperitone and *trans*-ethyl cinnamate. Insecticidal, antifeedant and antifungal properties of the isolated compounds were examined. Both compounds showed pronounced insecticidal and antifeedant activity against the third instar larvae of *Spodoptera littoralis* (Boisd). The insecticidal potency of *Artemisia* spp. extracts of other tested plants can be attributed to several factors, including plant specific differences of the extracted active ingredients, types of the extracted products, differences in their mode of action, method of penetrations and the behavioral characteristics of the studied pests (Schmutterer, 1990 and Roger et al., 1995). It is now well established that in many plants including the tested plants especially *Artemisia* spp., the activity is due to the presence of saponin (Rawi et al., 1996), triterpenoid (Schmutterer, 1988) and alkaloids components (Kogam, 1986). Tannins compounds (Klock and Chan, 1986) effect seems to be very specific dependent.

As shown in this study, the effect of *A. judaica* extract on *Ph. operculell*a larval instar was more effective than the effect of *B.t.* isolate on the insect larvae. In order to get the best results when using the two bio-control agents in IPM strategies, the bacteria should be used first and then the plant extract.

The antibacterial properties of artemisinin had been tested on a wide range of bacteria (Sack, 1975), *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Mycobacterium intracellulare* (Slade et al., 2009). Meyer and Afolayan (1995) and Ibrahim et al. (2011) found that, artemisinin and its precursor were effective against gram-positive bacteria, and their antibacterial

activities were similar to that of streptomycin, a bactericidal antibiotic. Artemisinin derived from field grown *Artemisia annua* plants was also reported to have antimicrobial activity (Ross et al., 2005 and Crespo-Ortiz and Wei, 2012). The susceptibility activity of gram-positive strains to artemisinin and precursor derived from *in vitro A. annua* plantlets, which had not been reported before confirmed that the *in vitro* plantlets could produce bioactive compounds that were similar to that found in the field grown mature plants of *A. annua.* These artemisinin and precursor produced from the *in vitro* plantlets also possess antimicrobial activity comparable to streptomycin.

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دراسة التأثير السام لبكتريا باثليس ثيرونجينسيس ومستخلص نبات البعيثران (الشيح الخليلي) علي فراشة درنات البطاطس

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أجريت هذه الدراسة بغرض تقييم فعالية تأثير سلالة من البكتيريا الممرضة للحشرات *باثليس ثيرونجينسيس* على يرقات درنات البطاطس. وأظهرت النتائج أن هذه العزلة لها تأثير على اليرقات حديثة الفقس، حيث أحدثت نسب وفيات تراوحت من ١٤-٥٨٪ عند استخدام تركيزات من ١.٢٥ ـ ١٠ CFU لكل مل، على الترتيب. وعند تقييم تأثير سمية مستخلص نبات البعيثران (الشيح الخليلي) على يرقات درنات البطاطس وجد أنها زادت بزيادة التركيز من ١٢٥إلى ١٠٠٠ جزء في المليون من ٣٤٪ إلى ٧٦٪ بالتتابع. وقد أثبتت هذه الدراسة أن تأثير مستخلص نبات الشيح على اليرقات المختبرة أقوى من تأثير عزلة البكتريا، لذلك للحصول على نتائج أفضل في برامج للمكافحة الحيوية يفضل استخدام العزلة البكتيرية أولًا.