



## ROLE OF *BACILLUS THURINGIENSIS* ISOLATE AS BIOLOGICAL CONTROL AGENT AGAINST WHITEFLY, *BEMISIA TABACI* (GENN.) AND THE SIDE EFFECT ON THE PREDATOR, *EUSEIUS SCUTALIS*

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**ABSTRACT :** *The potential of a certain Bacillus thuringiensis isolate against first-instar nymphs and newly emerged adults (i.e., sucking stages) of Bemisia tabaci, could be detected on the basis of the calculated LC<sub>50</sub> values which recorded 17.7 and 18.21 CFU/ml respectively. This means that, The effect of the bioinsecticide was stronger on adult individuals. On the other hand the calculated LC<sub>50</sub> values on newly emerged adults of Euseius scutalis by indirect and direct exposure were 4.89 and 27.5 CFU/ml respectively; this means that indirect exposure was more effective than direct exposure. Biological control agents should be directed to reduce the pest and preserve the predator (natural enemy).*

**Key words:** *Bacillus thuringiensis, Bemisia tabaci, Euseius scutalis, bioinsecticides.*

### INTRODUCTION

Sweet potato whitefly, *Bemisia tabaci*, Genn, is a polyphagous and multivoltine insect pest responsible for high economic losses in many crops with great economic impacts on many crops such as cotton, vegetables, fruit crops and ornamentals, (Khanjani, 2007 & Omid Bakhsh *et al.*, 2010). Whiteflies damage plants directly by sucking plant sap causing the silvering of leaves, irregular colour of fruits and growth stunting especially in young plants, and indirectly, whiteflies transmit several plant viruses (Lapidot and Polston, 2006). *B. tabaci* transmits plant viruses in seven distinct groups including: potyviruses, geminiviruses, carlaviruses, closteroviruses, nepoviruses, luteoviruses and DNA-containing rod-shaped virus (Thompson, 2011). Also, they excrete honeydew which stimulate the growth of sooty mold hindering the photosynthesis process (Byrne and Bellows, 1991).

*Euseius scutalis* (A-H), is a common phytoseiid mites in Egypt. Predatory mites of the family Phytoseiidae are of economic importance because they are efficient bio-agents that can be used against insect and mite pests in many crops in the open fields and in the greenhouses worldwide (Fouly *et al.* 2013).

Many phytoseiid species are facultative predators (generalists), not only on spider mites but also on other sources of food such as whiteflies, pollen (Fouly and Hassan 1991; Gnanvossous *et al.*, 2005; and Al-Shammery, 2011), and thrips (van Houten *et al.*, 2005; Messelink *et al.*, 2005; and Winner *et al.*, 2008). The two phytoseiid mites *Neoseiulus cucumeris* (Oud.) and *Neoseiulus barkeri* (Hughes) are known to play a natural important role in controlling the spider mites of the family Tetranychidae and Eriophyidae as well as the whiteflies and thrips on vegetables (Fouly *et al.*, 2011).

Alternative methods for insect control could provide acceptable levels of pest control with lower hazards. One of these alternatives is the use of bioinsecticides that contain microorganisms have valued importance because their toxicity to animals and human is very low. Compared to insecticides, (Al Arabiat *et al.*, 2018 and Al-Momany and Al-Antary, 2008),. The most widely used in the world are preparations of *Bacillus thuringiensis* (*B.t.*) (Federici, B.A. 1999),. The insecticidal activity of (*B.t.*) is due to containing parasporal inclusions produced during sporulation. Bioinsecticides based on the proteinaceous-endotoxin of (*B.t.*) constitute part of a more ecologically rational pest control strategy (Mehrabi, *et al.*, 2018), *B. thuringiensis* infects Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Phthiraptera, Orthoptera, and Mallophaga. (Federici, 1999, and Al Arabiat *et al.*, 2018).

This study conducted to evaluate the direct effect of certain *Bacillus thuringiensis* isolate on *Bemisia tabaci* and its predator *Euseius scutalis*; as well as the indirect effect on the predator fed on treated whitefly.

## MATERIALS AND METHODS

### Mites and insects collection:

Phytoseiid mite species, *Euseius scutalis* (Athias-Henriot) and *Bemisia tabaci* were collected from leaves and twigs of castor plants, *Ricinus communis*.

### Rearing of *Euseius scutalis*:

*Euseius scutalis* reared on leaf discs of castor plants *Ricinus communis* one square inch each was used for rearing predator during its whole the test. Adult were singly transferred from the culture to the aforementioned leaf discs which were kept on a moist cotton pad in Petri

dishes (15 cm in diameter). (Fouly *et al.*, 2013).

### Rearing of *Bemisia tabaci*:

The whitefly strain was transferred to the laboratory and reared on seedlings of castor planted in small pots 25cm (diameter) and kept under plastic greenhouse conditions of  $27\pm 2$  °C,  $70\pm 5$  RH, and photoperiod 14:10 (light: dark). The plant pots were carried to cages (60×60×120 cm). Lateral sides of the cages were covered by thin gauze (10×10) mesh led to suitable ventilation. Whitefly cultures were established by transferring 50-60 adults to each cage. The insects were screened on the upper surface of the plants using circular clips cages (2 cm in diameter, 3.14 surface area) for 72 hours.

### *B.t.* Isolation Technique:

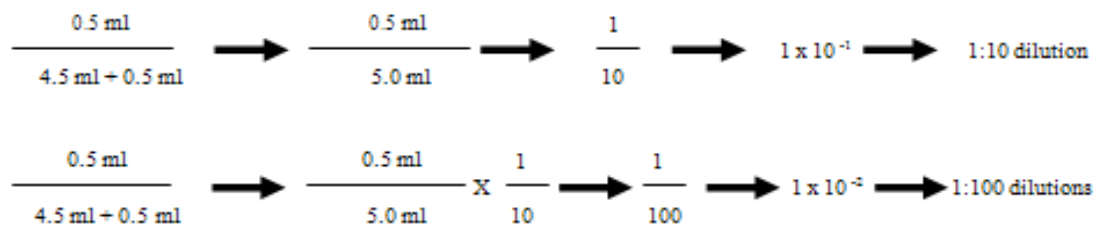
Soil samples were collected randomly from different fields in El-Bahariya Oases., Surface materials of the soil was removed; and with a sterile spatula, about 100 gm sample of soil was taken from at least 5 cm in depth. The soil samples were preserved in sterile plastic bags and stored for 2 - 12 months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

Based on the acetate selective method described by (Smith and Couche 1991), soil samples (0.5g) were added, each to 10 ml of LB broth buffered medium with 0.25M 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 30°C for four hours. In this method, germination of *B.t.* spores was selectively inhibited by sodium acetate buffer (0.25M), while most of the

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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 (Smirnof 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), determine 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 contains 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) 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:

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

**Toxicity Test:**

**Application on whitefly:**

The leaf dipping technique was used (Yang *et al.*, 2010). Plant leaves were dipped in four concentrations of the tested isolate (1.25, 2.5, 5, and 10 CFU/ml), control leaves were dipped in water. After drying at room temperature, 10 starved 2d- old first- instar nymphs and newly emerged adults (male and female) (i.e., sucking stages) of *B. tabaci*, was placed on the treated leaf, Mortalities were recorded after 12, 24, and 48 hours. Each experiment had 5 replications.

## Method of application on phytoseiid mite:

### 1- Direct effect:

The residual film technique was used. 3 ml of the desired concentration were evenly spread on a Petri dish surface (9 cm in diameter). The solvent allowed being evaporated leaving a film of several concentration of bacterial isolate (1.25, 2.5, 5, 10 CFU/ml), Pair of newly emerged adults (male and female) of *E. scutalis* were exposed to the thin film for 24 hour, and feeding on appropriate quantity of untreated *Bemisiatabaci*, (Frag, 1986). The control specimens were treated with water, each concentration was replicated 5 times. Inspection was carried out daily and Mortalities were recorded after 12, 24, and 48 hours.

### 2- Indirect effect:

Newly emerged adults (male and female) of *E. scutalis* were fed on whitefly previously treated with sublethal concentrations of bacterial isolate, (1.25, 2.5, 5, 10 CFU/ml) (Baoying *et al.*, 2001), the control specimens were treated with water. Mortality percentages of the predator were recorded.

## Statistical analyses

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 (Abbot's formula 1925).

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

### Toxicity Test:

As shown in chart (1) the effect of tested concentrations of *B. thuringiensis* isolate on 2 day old first- instar nymphs of *B. tabaci*, could be detected on the basis of the calculated  $LC_{50}$  and  $LC_{90}$  values which recorded 17.7 and 41.84 CFU/ml respectively, slope of the concentration-mortality line was 3.4174. While In case of *B. tabaci* adults  $LC_{50}$  and  $LC_{90}$  values was recorded as 18.21 and 43.36 CFU/ml respectively, where, its slope line recorded 3.40. The various concentration of *B.t.* isolate was more toxic for nymph stages than adult one Chart (2).

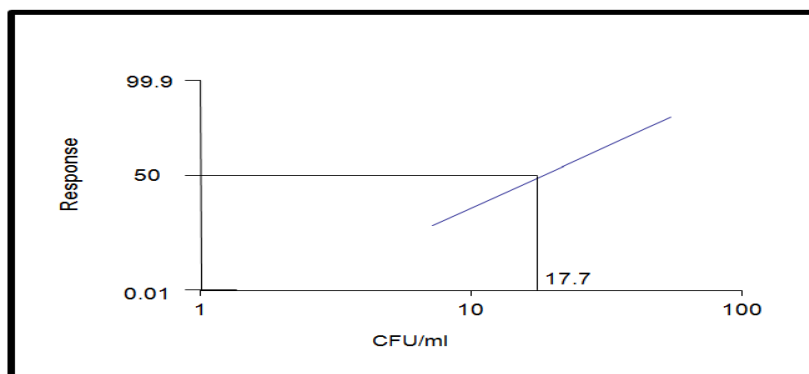
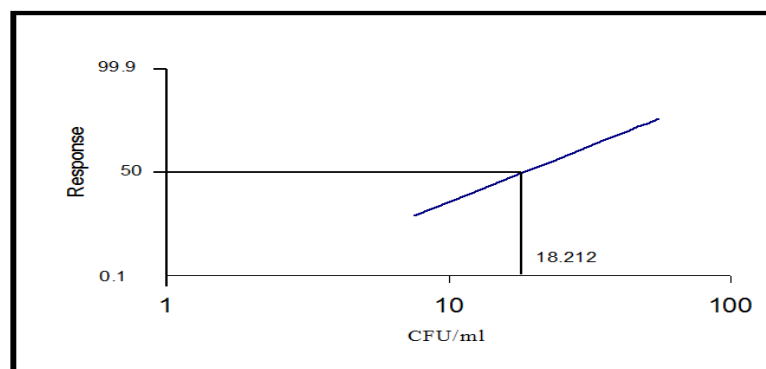


Chart (1): Concentration / mortality regression lines for *B. tabaci* nymphs treated with tested concentrations of *B. t.* isolate.



**Chart (2): Concentration / mortality regression lines for *B. tabaci* adults treated with tested concentrations of *B. t.* isolate.**

The obtained data in this study on the effect of *B. t.* isolate on 2 day old first-instar nymphs of *B. tabaci*, are in agreement with those reported by (Radwan *et al.*, 1984). A possible explanation for the mortality rates found could be the contact action of the tested isolate concentration. (Wilkinson and Ignoffo 1975) suggest the usefulness of *B.t.* preparation as a control measure to reduce the whitefly population. The genus *Bacillus* e.g. *B. pumilus*, *B. pasteurii*, *B. thuringiensis* and *B. Subtilis* which are often utilized as a bio-control agent (Al Arabiat *et al.*, 2018 and Kloepper *et al.*, 2014), These bacteria can promote growth and provide plant protection by antibiosis and/or ISR elicitation in many crops. For example reduced insect herbivores in cucumber (Zehnder *et al.*, 1997 and Al-Momany and Al-Antary, 2008), however ISR cause reduction of *P. syringae* and *pv. Tabaci* in the field and greenhouse (Park and Kloepper, 2000) and *Peronospora tabacina* (Zhang *et al.*, 2002).

The indirect tested concentrations of *B. t.* isolate on newly emerged adults of *E. scutalis* could be detected on the basis of the calculated  $LC_{50}$  and  $LC_{90}$  values which recorded 4.89 and 289.68, CFU/ml respectively, slope of the concentration-mortality line was 0.7276. (Chart 3), While the calculated  $LC_{50}$  and  $LC_{90}$  values by

direct exposure (chart 4), recorded 27.5 and 107.546. CFU/ml respectively, and its slope line was 2.18. This means that indirect exposure was more effective than direct exposure.

Field applications of thuringiensin were successful against the citrus red mite *P.citri* (Hall, *et.al.*, 1971) and *Tetranychus pacificus* (Hoy and Ouyang, 1987). Later, (Royalty *et al.*, 1990) conducted experiments by testing two different formulations of thuringiensin against the two spotted spider mite *T. urticae*. The results indicated that thuringiensin might be a potential acaricide. In particular young instars are susceptible, since these have a high growth rate. Various physiological processes in young organisms require higher RNA synthesis than in the older slower growing stages. A major drawback is that thuringiensin is toxic for a wide range of organisms. Not only are spider mites affected, but also beneficial mites, such as *Phytoseiulus persimilis*. The chemical is apparently a nonselective acaricide that should not be used in combination with predatory mites. The spore-crystal complex of *B. thuringiensis* has been tested on spider mites by (Krieg, 1972), but no mortality was observed. However, (Chapman and Hoy, 1991), conducted experiments in which *T. urticae* and

*Metaseiulus occidentalis* were treated with a commercial preparation of *B. thuringiensis* var. *tenebrionis*. This variety of *B. thuringiensis* shows an effect on beetles and is recommended for use against the Colorado potato beetle, *Leptinotarsa decemlineata*. No effect was noted on the twospotted spider mite, this toxic effect could be enhanced by starving the mites: the authors assumed that starvation may lead to a higher uptake of the material, or that the mites were more exposed to the preparation as starving mites tend to move faster. It is also possible that starvation acts as a stress factor. The authors have no explanation for the toxic effect on the predatory mite: the preparation did not contain the  $\beta$ -exotoxin (thuringiensin) known to be toxic for mites. In more recent years, isolates of *B. thuringiensis* have been found that do show toxicity

towards spider mites and house dust mites (Payne, et al., 1993 and 1994). It has been suggested to isolate the  $\delta$ -endotoxin of these isolates and to formulate it as an acaricide. One may also transfer the gene, encoding for this specific  $\delta$ -endotoxin into a crop plant in order to protect the crop against spider mite infestations. An interesting discovery is the isolation of a *B. thuringiensis* strain from dead two spotted spider mites, *T. urticae* (Jung et al., 2007). The results of this study were identical to (El-Banna et al., 2002), showed that the indirect exposure technique of both Neem Azal formulation and organophosphorus insecticide [Actellic compound] on *Aphis craccivora* Kock and its predator *Coccinella undecimpunctata* is more effective than the direct exposure technique.

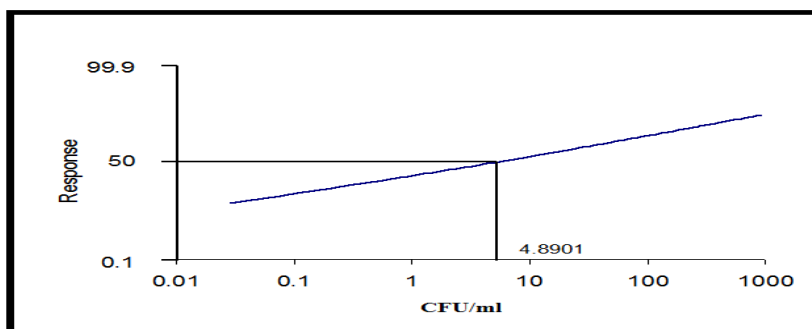


Chart (3): Concentration / mortality regression lines for *E. scutalis* adult indirect treated with tested concentrations of *B. t.* isolate.

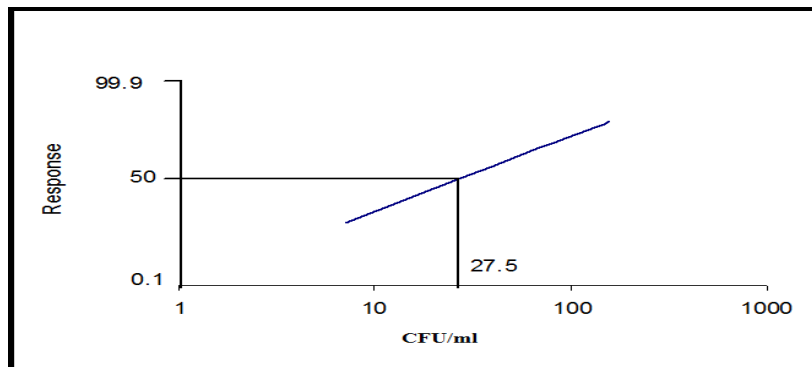


Chart (4): Concentration / mortality regression lines for *E. scutalis* adult direct treated with tested concentrations of *B. t.* isolate

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دور إحدى عزلات بكتريا *Bacillus thuringiensis* كوسيلة مكافحة بيولوجية للذبابة البيضاء *Bemisia tabaci* وأثرها الجانبي علي المفترس *Euseius scutalis*

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الملخص العربي

أجريت هذه الدراسة بغرض تقييم سمية عزله من البكتيريا الممرضة للحشرات باسيليس ثيرونجينسيس عليحوريات العمر الاول والاطوار البالغه حديثه الفقس للذبابة البيضاء علي أساس حساب  $LC_{50}$  والتي سجلت 17.7 و 18.21 CFU/ml علي التوالي. وعند تقييم تأثيرسمية العزله البكتيرييه علي الطور البالغ للمفترس الاكاروسي *E. scutalis* سجلت قيم  $LC_{50}$  بطريقتي التعريض الغير مباشر والمباشر 4.89 و 27.5 CFU/ml علي التوالي. وهذا يعني أن تأثير المبيد علي المفترس في الطريقة الغير مباشرة يكون أكثر سمية عن المعاملة بالطريقة المباشرة. ويوصى البحث بضرورة استخدام عوامل المكافحة البيولوجية للحد من الآفات والحفاظ علي المفترس (العدو الطبيعي).

السادة المحكمين

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**Role of bacillus thuringiensis isolate as biological control agent against .....**