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EFFICIENCY OF *Bacillus thuringiensis* AND *Bacillus larvae* ON *Ceratitis capitata* (WIED) LARVAE (DIPTERA : TEPHRITIDAE) IN NORTH SINAI GOVERNORATE .

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ABSTRACT

Bacillus thuringiensis and *Bacillus larva*e were tested as biocontrol agents against *Ceratitis capitata* larvae, Results showed that when larvae of *C . capitata* fed after five days from egg hatching at any of the tested concentrations (1.25,2.5,5,10) CFU/ml, the highest percentage of mortality occurred with the first three days following application, then the larval mortality started to decrease. The LC_{50} of the most potent isolates (*B. thuringiensis*) was 3.5, this was followed by *B. larvae* 8.)

Keywords: El-Aresh Rafah-Sinai Governorate, Peach trees, *Ceratitis capitata, B. thuringiensis, B. Larvae* , biocontrol agents ,LC_{50}.

INTRODUCTION

Peach crops are considered as one of the major sources of income for many farmers. The cultivated area in the Governorate of North Sinai, is about 192000 acres, of which 93 thousand acres used for the benefit of fruit crops, accounting for 48% of the total area under cultivation . peach occupied only 59 048 acres. Peach tree are infesting by many different and serious insect pests causing a lot of quantitative and qualitative damage. such wide range of pests attack and infest peach trees and affect tree health, productivity in addition to fruits quality.

The Mediterranean fruit fly*, Ceratits capitata* (Wied) (Diptera :Tephritidae) is considered as one of the most serious world wide pest, infesting more than 300 fruit plant species (Liquido *et. al*.,1991). In Egypt this pest cause considerable damage and inflicts significant economic losses in fruit orchards (Hafez and Fares,1967).

High levels of *Ceratits capitata* adults in trap catch were associated with a significant reductions in fruit yield (Aida *et. al* ,2009). Unfortunately, studies about Mediterranean fruit fly are still in lack .

The main aim of this work is studying the effect of bacterial isolated from Sainai soil against *Ceratits capitata* larvae.

The pest attacks citrus, delicious fruits mainly stone fruits and other cultivated hosts (Saafan *et.al.*,2006).

Bacillus thurinegiensis is a gram-positive spore-forming bacterium that produces a parasporal crystal protein inclusion during its sporulation. It is classified as a member of genus *Bacillus* that contains *B. subtilis, licheniformis, inegaterium*, *cereus and anthracis*. It is very related to *B. cereus* but distinguished by the presence of a parasporal inclusion body (crystal) and also, by flagella antigen. Its vegetative cells are characterized by

rod shape, peritrichous flagella and produced thin wall oval subterminal spores (BuIla *et al*., 1980) .

Salama *et al.,* (1986) *B. thuringiensis* is present in various soil types in Egypt. *B.thuringiensis* strains are characterized by vegetative cells, spores and presence of parasporal inclusion bodies (crystals). Crystals appeared in different shapes and sizes for different strains i.e. bipyramidal , spherical , pyramidal, cubical, and irregular crystals (Ohba and Aizawa,1986, Brownbridge,1991) The main aim of this work is studying the effect of bacterial isolated from Sinai soil against *Ceratits capitata* larvae.

MATERIALS AND METHODS

Soil samples:

Soil samples were collected randomly from different fields cultivated with peach trees, surface materials of the soil were removed, with a sterile spatula, about 100 gram sample of soil was taken from at least 5 cm in depth. the soil samples were preserved in sterile plastic bags and stored at 4°C until analyzed.

Isolation of *Bacillus sp:*

Suspension of (10 gm soil +90 ml of sterilized water) was shacked for an hour and allowed to stand for 10 minutes, the upper layer of suspended sample was transferred to a sterile test tube with screw cap followed by heat treatment at 80C for 15 minutes in a water bath . Heat treatment was made to eliminate all vegetative cells and non-sporulated soil microorganisms present in the sample. The sample was left to cool at room temperature about ten minutes before inoculating. 0.1 ml of the supernatant using sterile pipettes onto agar plates and distributed over agar surface(M1) homogeneously (surface spread).The plates were incubated overnight at 30ºC, then random colonies of *Bacillus SP*. from agar plates(typical colonial morphology) were transferred onto the suitable medium for the growth of *B. thuringiensis* (M2)plates using sterile loop until examination and identification(Chilcton and Wigley,1993) .

Enumeration of bacteria :

The method described by Dumlage (1971) was used to determine the number of bacteria that are present in the isolates. Serial dilution of a solution containing an unknown number of bacteria were made. The diluted bacteria were plated on media that support the growth of the bacteria. Moreover, the total number of bacteria in the original solution was determined by counting the number of Colony Forming Units (CFUs) and comparing them to the dilution factor. and the volume of the plated diluted suspension to determine the number of bacteria per ml that were present in the original dilution .For each dilution , the number of Colony Forming Units (CFUs) on the plates was conducted . Typically , numbers between 30-800 are considered to be in the range of statistical accurate data :

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

***Bacillus SP*. identification:**

Bacteria were scraped off from the agar surface and saline suspension (Nacl 0.85%)and observed under light microscope(1,000) to confirm the presence of parasporal crystals, a typical characteristic of *B.thuringiensis*.

A strain of *B. larvae* was also identified using the same methodology described for *B. thuringiensis*. The two isolates were send to Micro Analytical Center, Faculty of Science Cairo University.

Toxicity Test:

An over night pre culture was grown at 28ºC with vigorous shaking(250 rpm) in 2 ml of HCT medium complemented with 0.3% glucose. This culture was then added to 100 ml of the same medium in 500 ml flasks and grown under the same conditions.

Larvae of *C. capitata* (after five days from egg hatching) were fed on larval synthetic media(M3) treated by different concentrations of *B. thuringiensis* and *B. larvae*. (1.25 ,2.5 ,5 ,10 CFU/ml),and the Control was treated with water . 10 larvae were introduced to Petri dish (10cm diameter) .The dish was then covered. For each concentration, 10 replicates of ten larvae each were used. Number of alive and dead larvae were recorded daily till pupation .Data obtained were subjected to statistical analysis to evaluate the relative efficiency of *B.thuringiensis* and *B.larvae* isolates. Mortalities were corrected for the natural mortality according to(Abbott`s formula ,1925).

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

Culture media for isolation of *Bacillus sp*.:

3 different media were used to isolate ***Bacillus thuringiensis***

M1(PSD) peptone saline diluents

Peptone …….. 1g
Sodium chloride …… 8.5 g
Water …… 1L
PH …… 7
Temp. at …… 25Cº

For isolation of *B.th*.

M2 (HCT)

Tryptone …… 5g
Casein hydrolyzate …… 2g
K_2HPO_4 …….. .5mM
$MgSO_4$ …….. 12.5g
$MnSO_4$ ……... .05mM
$ZnSO_4$ …… .2mM
$Fe_2(SO4)_3$ …… 1.2mM
H_2SO_4 …… 0.5%
CaC_{l2} …… 25mM

Complement with 0.3% glucose. Colonies with crystal shape as observed by light microscopy.

M3 semi defined diet (larvae feed on it):

Wheat germ …… 37.5g
Yeast extract …… 37.5g
Maize flour …… 142.5g
Ascorbic acid …… 12.5g
Agar-agar …… 16.25g
Sunflower oil …… 0 .5ml
Temp. at …… 25 C º

Statistical analysis:

The obtained data were analyzed by standard statistical analysis of variance for a completely randomized block design; the least significant differences (L.S.D.) were calculated at 5 %, according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The average number of bacteria per ml of water was determined for each isolate, was (7.20 $x10^6$ bacteria / ml); and (4.3$x10^6$ bacteria / ml)for *B.thuringensis* and *B.larvae,* respectively,

Effect of *B.thuringiensis* and *B.larvae* isolates on the mortality percentages of larval stage of *C . capitata* :

Feeding of *Ceratitis capitata* larvae after five days from egg hatching on food treated with *B. thuringiensis.* and *B. larvae*. revealed adverse effect on the total percentage of larval mortality. (Tables1,2) The total larval death recorded 6% in control trials . All of the tested concentrations (1.25,2.5,5,10) CFU/ml, recorded the highest percentage of mortality occurred with the first two days after application, then the larval mortality started to decrease in. the third day until the end of indicated days highest mortality % was recorded after 2 days from application in both treatments at concentration of 10 CFU/ml.and recorded 45 %,29% mortality
in treatment with *B . thuringensis* and *B . larvae* respectively. (tables 1, 2) It is clear from data obtained that *B.thuringensis* was more efficient against *Ceratitis capitata* larvae than *B. larvae*, the corrected total mortality % were 73 % and 54 % , respectively.

Table(1) :Larval mortality of. *C. capitata*. after application of *Bacillus thuringiensis* on larvae after five days from egg hatching.

Conc. (CFU/ml)	Mortality % after indicated days												Total mortality %	
	١	2	3	4	5	6	7	8	9	10	11	12	Obs.	Corr.
0	0	0	0	0	0	2	1	1	1	1	0	0	6	0
1.25	7	16	4	0	0	0	2	1	1	0	1	1	33	28.5
2.5	8	22	2	2	4	4	2	2	0	2	1	0	49	35
5	13	29	9	0	1	1	0	0	3	1	0	1	58	54
10	13	45	5	5	0	0	0	0	0	4	3	0	75	73

***100 larvae in 10 replicates were tested**

Table(2) :Larval mortality of *C. capitata* after treatment of *Bacillus Larvae* on larvae after five days from egg hatching.

Conc. (CFU/ml)	Mortality % after indicated days												Total mortality %	
	1	2	3	4	5	6	7	8	9	10	11	12	Obs.	Corr.
0	0	0	0	0	0	2	0	1	1	2	0	0	6	0
1.25	3	5	1	1	0	0	0	3	1	0	0	0	14	7.9
2.5	7	13	0	1	1	2	0	0	0	1	1	0	26	21
5	7	23	2	2	2	0	4	0	2	0	0	0	42	37
10	9	29	7	9	0	2	0	0	1	1	0	0	58	54

***100 larvae in 10 replicates were tested**

Toxicity test (LC_{50} of *Bacillus thuringiensis* and *Bacillus larvae* isolates):

The standard bioassay procedures were calculated according to Dumlage (1971). All bioassays were carried out using larvae of *C . capitata*. The LC_{50} values of the tested potent isolate were computed from the data obtained on the percentage of larval mortality at each of the tested concentration through probit analysis within 95% confidence . The LC_{50} of the most potent isolates (*B. thuringiensis*) was 3.5, and followed by*(B. larvae*) with LC_{50} 8. (Table,3) (Figs.1and 2)

Table(3):LC_{50} at confidence limits(95%)of potent *Bacillus thuringiensis* and *Bacillus larvae* isolates obtained from soil samples against larvae of *C. capitata* .

Isolates	LC_{50}
Bacillus thuringiensis	**3.5 (2.9-4.7)**
Bacillus larvae	**8 (6.4-11)**

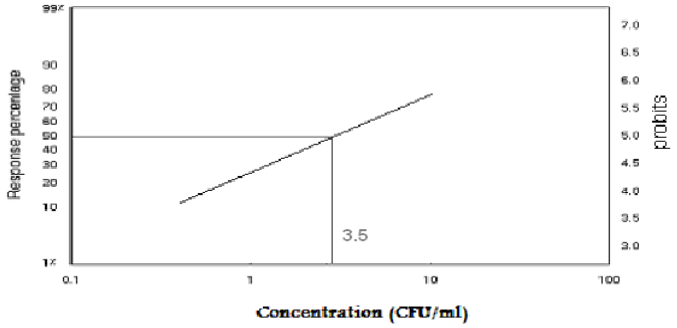

Fig. (1): Concentration / mortality regression lines for *ceratitis capitata* larvae treated with *Bacillus thuringiensis*

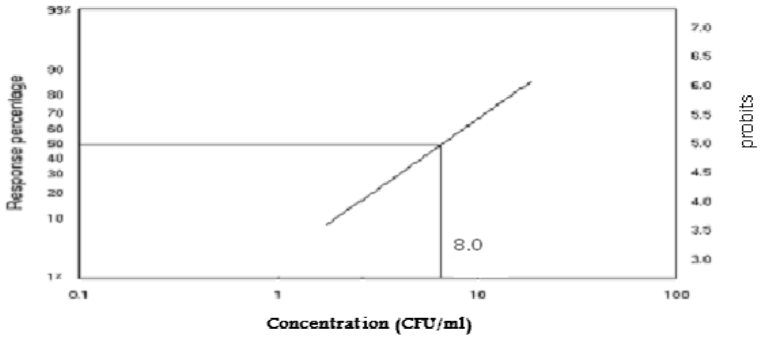

Fig. (2): Concentration / mortality regression lines for *ceratitis capitata* larvae treated with *Bacillus larvae*

The soil microorganism *Bacillus thuringiensis* (*B.t*.)used as insecticide for more than 40 years .*B . thuringiensis* produces five substances toxic to insects (Hassan,2002). Because it has no contact action and must be ingested to be effective it should be applied to sites where larvae are feeding .The mode of action of insecticidal crystal proteins (ICPS)come from its binding to different sites in the brush border membrane of mid –gut epithelial cells and causes osmotic lysis through pore formation in cell membrane (Gill *et.al*.,1992). Also , it is obvious that increasing of concentration ratio of micro-organisms resulted in increasing of mortality % of larvae population of *C. capitata* as a result of increase of toxins produced by the bacteria . Abde –Halim (1993) who found that ability *S. littoralis* larvae treated with dipel 2x (commercial preparation *B.T*) decreased with the increase in concentration or / and feeding time .Also, Mohamed *et al* (2000) reported that larval mortality of *S . littoralis* and *A. ipsilion* increased by increasing the concentration of *B. t* or the pest treatment .

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فاعلية بكتريا الباسلس ثيرونجينسيس وبكتريا الباسلس لارفا ضد يرقات ذبابة فاكهة البحر المتوسط (رتبة ذات الجناحين – عائلة تفريتدى) فى محافظة شمال سيناء

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****قسم وقاية النبات مركز بحوث الصحراء**

تم إجراء تجربة لاختبار فاعلية بكتريا الباسلس ثيرونجنسيس و الباسلس لارفا على يرقات ذبابة البحر المتوسط وقد أوضحت النتائج أنه عند تغذية اليرقات بعد خمسة أيام من فقس البيض عند أى من التركيزات المختبرة(١.٢٥ و ٢.٥ و ٥ و١٠) أن هناك علاقة طردية بين النسبة المئوية للوفيات فى اليرقات والتركيز لكل من نوعى البكتريا كما بينت النتائج أن أعلى نسبة للوفيات فى اليرقات كانت فى الثلاثة أيام الاولى ثم تناقصت بعد ذلك ، كما أن أعلى نسبة للوفيات فى اليرقات هى ٤٥% بعد يومين من المعاملة ب بكتريا الباسلس ثيرونجنسيس عند تركيز ١٠ CFM ، ٢٩ %بعد يومين من المعاملة ب الباسلس لارفا بنفس التركيز السابق وقد كانت LC_{50} لعزلات الباسلس ثيرونجينسيس والباسلس لارفا هى ٣.٥ ، ٨ على النرتيب٠

قام بتحكيم البحث

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