

MICROBIAL CONTROL OF THE COTTON SEED BUG
Oxycarenus hyalinipennis Costa ,
AFFECTING SEED GERMINATION IN OKRA
Hibiscus esculentus

BY

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Received 14/7/1992 Accepted 15/9/1992.

ABSTRACT

The microbial control of the cotton seed bug Oxycarenus hyalinipennis Costa , (Hemiptera : Lygaeidae) was studied on Okra plants under laboratory and field conditions. The results indicated that, B. thuringiensis var. kurstaki (H3a, 3b) was more efficient than B. pumilus (which is isolated and recorded for the first time in Alexandria, Egypt) against the adults of the cotton seed bug in the laboratory and against the nymphs and adults under field conditions. Also, B. thuringiensis and B. pumilus proved to be satisfactory as microbial pesticides. As to seed germination in Okra , it was increased with microbial control which decreased number of cotton seed bug in dry Okra pods.

INTRODUCTION

According to Hammad et al. (1972) , it was stated that the cotton seed bug Oxycarenus

hyalinipennis Costa , is the most widely distributed species of the genus Oxycarenus and extends from the Mediterranean subregion to the whole Ethiopian region. Also, this insect as a cotton pest has been known in Egypt from very long time, as early as 1860 (Ballou, 1919). However, O. hyalinipennis was recorded from over 57 plant species (Ram and Chopra, 1984) , especially Okra. Little is published on the economic significance for this insect pest and its control (Dimetry, 1973). In this concern , it seems that the only possible approach to reduce pest damage , by reducing pest numbers without using insecticides is the use of microbial control, which receives a considerable attention in recent years (Steinhaus , 1963; Ahalis, 1969; Goldberg and Margalit, 1977 ; Bajac, 1981 ; Armelle et al., 1988 ; Zaki, 1991 ; Pasqualini, 1991 and Salam, 1991). The present study aims to investigate the following points : (1) Isolation and identification of the bacterial type. (2) Pathogenicity of the isolated bacteria from O. hyalinipennis and B. thuringiensis var. kurstaki (H3a, 3b) isolated from Hypera burnneipennis (Coleoptera : Curculionidae). (3) Field application of the two types of bacteria at two seasons. (4) The effect of bacterial application on Okra seed germination as well as in the control.

MATERIALS AND METHODS

1- Isolation and identification of bacteria:

Bacillus pumilus was isolated from abnormal , moribund , dead nymphs and adults of the cotton seed bug Oxycarenus hyalinipennis Costa (Hemiptera : Lygaeidae) which was obtained from dry Okra pods (Hibiscus esculentus). This bacterial strain was cultured on Glycerol Agar Medium (3 gm beef extract. , 5 gm pepton , 20 gm agar , 20 ml glycerol which dissolve in water

to prepare one litre of solution). Plate count technique (Király *et al.*, 1970) was used to determine the number of viable spores of B. pumilus per ml to prepare the concentration of bacteria used for the control of the pest in laboratory and in the field. Slant cultures and prepared slides were carried out and sent to Prof. Dr. H. De Barjac of the Biological Control, Pasteur Institute, WHO, Collaborating Centre for Entomopathogenic Bacillus, Paris, for the identification of the studied bacterium.

2- Pathogenicity tests:

These tests were carried out during the season of 1990 under laboratory conditions of 28°C and 65 ± 5% R.H. for the two isolated types of Bacillus, B. pumilus isolated from O. hyalinipennis and B. thuringiensis var. kurstaki (H3a, 3b) isolated from larvae and adults of the Egyptian Alfalfa weevil, Hypera brunneipennis Boh. (Coleoptera : Curculionidae). Infested Okra pods were collected from the field and kept in glass jars, each containing a piece of cotton soaked with water. The hatching nymphs and Okra pods (as food material) were separated in other glass jars. The emerging of 3 days old adults were used in the present experiments. Six concentrations for each bacterial species were used. These concentrations were 4.23x10⁵, 6.34x 10⁵, 8.45x10⁵, 10.14x10⁵, 12.67x10⁵ and 16.90 x10⁵ v.s/ml for B. pumilus and 10.70x10⁵, 16.05 x10⁵, 21.40x 10⁵, 25.68x10⁵, 32.10x10⁵ and 42.80x10⁵ v.s/ml for B. thuringiensis. Dry pods of Okra, nearly in the same size, were dipped for 30 seconds in each concentration. After drying, adults were kept with the treated Okra pods. Each concentration was replicated 4 times with 10 adults each. Untreated Okra pods were used as control. Mortality of adults were recorded after 7 days of treatment and data were corrected according to Abbot's formula (1925). Lc-P lines

were drawn to estimate the L_{50} values by using the method of Litchfield and Wilcoxon (1949).

3- Field application:

A field experiment was carried out during the two seasons of 1990 and 1991 in Sabahia Horticultural Experimental Station, Alexandria, Egypt, to study the effect of the two isolated types Bacillus : B. pumilus and B. thuringiensis var. kurstaki (H3a, 3b) , at concentrations of 16.90×10^8 v.s/ml and 42.80×10^8 v.s/ml, respectively. These bacteria were cultivated from a pure colony on slant agar under sterile conditions of 28°C for 24 hours. Five ml from sterile buffer solution were put in each tube (60 tubes were used). The bacteria were harvested from the agar surface and 4 ml from each tube were taken and gathered in a conical jar. Ten plants of Okra in the seed stage were chosen for this study as replicates. Fifteen pods were chosen from each plant; 5 pods were sprayed with B. pumilus, whereas the other five pods were sprayed with B. thuringiensis. The adults and nymphs were counted after ten days of treatment. The two stages of insects were counted in the unsprayed five pods which were used as control. Statistical analysis was carried out according to Cochran and Cox (1957).

4- Seed germination of Okra:

Dry seeds were taken from each of the three treatments (B. pumilus, B. thuringiensis and the control), and seed germination was recorded for these seeds at 30°C (Knott, 1957) during the two successive seasons of 1990 and 1991. The recorded data were statistically analysed according to Cochran and Cox (1957).

RESULTS AND DISCUSSION

The isolated bacterium B. pumilus from nymphs and adults of the cotton seed bug O. hyalinipennis Costa, (Hemiptera : Lygaeidae) is the first record in Alexandria district, Egypt.

Laboratory experiments (Table 1 and Figure 1) showed that the mortality of adults of cotton seed bug was enhanced with increasing of bacterial concentrations of both B. pumilus and B. thuringiensis. The percentages of mortality after 7 days from treatment with B. pumilus were 25%, 30%, 40%, 45%, 50% and 60%, while the percentages of mortality of the adults with B. thuringiensis were 40%, 62.50%, 70%, 72.5%, 77.5% and 85% at the concentrations of 4.23×10^5 , 6.34×10^5 , 8.45×10^5 , 10.14×10^5 , 12.67×10^5 and 16.90×10^5 v.s/ml for B. pumilus and 10.70×10^5 , 16.05×10^5 , 21.40×10^5 , 25.68×10^5 , 32.10×10^5 and 42.80×10^5 v.s/ml for B. thuringiensis, respectively. It is clear that the susceptibility of the cotton seed bug to B. thuringiensis was more than B. pumilus. The values of LC_{50} and slope for this bug are shown in Table (1).

Field experiments indicated that B. thuringiensis at 42.80×10^5 v.s/ml and B. pumilus at 16.90×10^5 v.s/ml reduced significantly the numbers of adults and nymphs than the check control at both the seasons of 1990 and 1991. There were no significant differences between B. thuringiensis and B. pumilus in controlling either adults or nymphs of the cotton seed bug in 1990. However, B. thuringiensis reduced significantly the number of cotton seed bugs than B. pumilus in 1991 (Table 2).

B. pumilus and B. thuringiensis application to Okra pods increased seed germination significantly comparing with the control (Table 3), at the same time no significant differences were

Table (1): Parameters determined according to the statistical method of analysis adopted by Litchfield and Wilcoxon (1949) for susceptibility of the adult Oxycarenus hyalinipennis to B. pumilus and B. thuringiensis var. kurstaki (H3a, 3b).

<u>Bacillus</u> types	<u>L₅₀</u> viable spores per ml	Slope (S)	Confidence limits of <u>L₅₀</u>
<u>B. pumilus</u>	12.67x10 ⁵	4.85	16.38x10 ⁵ : 9.69x10 ⁵
<u>B. thuringiensis</u>	10.00x10 ⁵	4.28	12.80x10 ⁵ : 7.81x10 ⁵

Table (2): Average numbers of nymphs and adults of Oxycarenus hyalinipennis present in dry Okra pods treated with B. pumilus and B. thuringiensis var. kurstaki (H3a, 3b) in the field experiment during the seasons of 1990 and 1991.

Treatments	Average number of insect at each stage			
	1990		1991	
	Adults	Nymphs	Adults	Nymphs
Control	126.5	172.1	127.6	178.0
<u>B. pumilus</u>	79.1	119.1	100.5	135.0
<u>B. thuringiensis</u>	64.0	97.5	70.0	102.5
L.S.D. at 0.01	43.98	49.73	24.57	28.27

Table(3):Seed germination percentages and their corresponding angles for pods treated with B. pumilus, B. thuringiensis and in the control in 1990 and 1991 seasons.

Treatments	Seed germination			
	1990		1991	
	%	Angles	%	Angles
Control	21.65	27.71	27.18	31.39
<u>B. pumilus</u>	79.28	62.96	81.38	64.47
<u>B. thuringiensis</u>	82.70	65.44	86.10	68.22
L.S.D. at 0.01	---	10.81	---	14.45

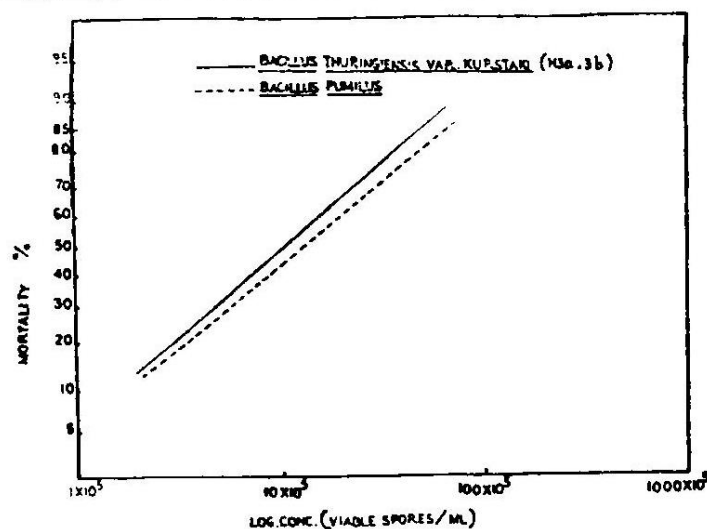


Fig. (1):Lc-P lines showing susceptibility of the cotton seed bug Oxycareus hyalinipennis to B. thuringiensis var. kurstaki (H3a, 3b) and B. pumilus.

detected regarding seed germination for the pods treated with B. pumilus and B. thuringiensis. Knott (1957) stated that the minimum seed germination percentage in Okra should not less than 50%. Therefore, it is clear that the effect of O. hyalinipennis on germination of Okra seeds must be taken in to consideration.

It appears from the above-mentioned results that the B. thuringiensis and B. pumilus were proved to be efficient control methods against the cotton seed bug in Okra plants in both laboratory and field tests.

It should be mentioned that, B. pumilus was never isolated before from hemipterous insects. On the other hand, Goldberg and Margalit (1977), found that B. thuringiensis var. israelensis was effective against five species of mosquitoes belonging to the genera Aedes, Culex, Anopheles and Uranotaenia. Also, Barjac (1978 b) mentioned the mode of action of B. t. i. on some dipterous insects. In Italy, Pasqualini (1991), stated that B. thuringiensis var. israelensis and B. thuringiensis var. kurstaki were successful in controlling several species of Lepidoptera and Diptera. Moraes (1991), stated that there are many applications of B. thuringiensis mainly in big plantations and in forested areas especially in the field of the development of exotoxins to be used against mosquitoes Aedes aegyptii.

ACKNOWLEDGEMENT

The authors wish to thank Prof. Dr. H. De Brajac of the biological control, Pasteur Institute, WHO Collaborating Centre for Entomopathogenic Bacillus, Paris, for helping in identifying the studied bacteria B. thuringiensis var. kurstaki (H3a, 3b) and B. pumilus.

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الملخص العربي

المقاومة الميكروبية لحفرة بقعة بذرة القطن المؤثرة على انبات بذور الباميا

اجريت هذه الدراسة بمحطتي بحوث وقاية النبات وبحوث البساتين بالصباحية بالاسكندرية خلال موسمي ١٩٩٠ و ١٩٩١ لدراسة تاثير المقاومة الميكروبية على حفرة بقعة بذرة القطن تحت الظروف الحقلية والمعملية .
وقد دلت النتائج على ان البكتيريا Bacillus thuringiensis var. kurstaki (H3a , 3b) اكثر فعالية من البكتيريا Bacillus pumilus (والتي عزلت وسجلت لأول مرة بالاسكندرية - مصر) على الحشرات الكاملة لبقعة بذرة القطن في المعمل وعلى الحوريات والحشرات الكاملة بالحقل . وقد اعطى كلا النوعين من البكتيريا نتائج مرضية كمبيد ميكروبي في مقاومة هذه الحشرة . وقد أدت المقاومة الميكروبية لهذه الحشرة الى انخفاض اعدادها وزيادة نسبة انبات بذور الباميا .