

Bacillus thuringiensis var . kuristaki Berliner as a biological control agent agaist lemon butterfly , Papilio demoleus L.(Lepidoptera :Papilionidae)

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Abstract :

The effects of *Bacillus thuringiensis* var . *kuristaki* were tested against lemon butterfly , *Papilio demoleus* , in laboratory . Four different concentrations of lyophilized form of the bacteria (0.25, 0.5, 1.0, and 2.0 mg/ml) were used. The results indicated that there was a direct correlation between the mortality rate and bacterial concentration. Mortality rates were: 75%, 100%, 100%, and 100% at concentrations of 0.25, 0.5, 1.0, and 2.0 mg/ml respectively. The data also revealed that the developmental period was extended due to bacterial application. Egg production was significantly reduced. Some developmental deformalities were observed in individuals that have been treated with 0.25 mg/m completed their development.

الخلاصة :

تم في هذه الدراسة تقييم تأثير البكتريا *Bacillus thuringiensis kuristaki* في فراشة الليمون *Papilio demoleus* L . مختبريا . استخدمت أربعة تراكيز من البكتريا المجففة وهي 0.25 و 0.5 و 1.0 و 2.0 ملغم / مل . أوضحت النتائج بان هنالك علاقة طردية بين نسبة الوفيات وتركيز البكتريا ، وقد كانت 75% ، 100% ، 100% ، 100% في التراكيز 0.25 ، 0.5 ، 1.0 ، 2.0 ملغم / مل على التوالي . كذلك بينت النتائج بان هنالك زيادة في فترة النمو مقارنة بالحشرات غير المعاملة بالبكتريا . بالإضافة الى ذلك فقد لوحظت تشوهات مظهرية في الأفراد التي أكملت نموها بعد معاملتها بتركيز 0.25 ملغم / مل .

INTRODUCTION

Lemon butterfly, *Papilio demoleus* L. is a serious pest of citrus trees in Iraq. Due to larval feeding, a great deal of damage affects the young leaves and buds. Some times a complete defoliation occurs due to larval feeding (Al-Rawy , 1976) , thus affecting the growth of small trees in the nurseries . In an attempt to search for efficient feasible control measure such as the use of bacteria as biological control agent (MaGhaughey , 1978; Kinsinger and MaGhaughey , 1989 , Milistead , et.al.1980 ; Salama , 1984 ; and Alford and Holmes ,1986) ,this study was conducted to evaluate the potential use of *B. thuringiensis* var . *kuristaki* as microbial control agent against lemon butterfly *P.demoleus* .

MATERIALS AND METHODS

Lemon butterfly eggs and larvae were collected from citrus trees in residential area of Baghdad, during March – May 1990. Stock culture, as well as, experimental treatments were maintained in the laboratory under incubator conditions of $25\pm 1^{\circ}$ C. and 60-75% relative humidity. Fresh young leaves of sour orange, *Citrus aurantium* L . Were used during the entire study as food source. Foliages were maintained in a turgid condition by inserting the petiole of each leaf in a vial containing distilled water , and kept in a plastic rearing container (17 cm in diameter and 8 cm height) .

Bacillus thuringiensis var . *kuristaki* which is used in this study was previously isolated from locally infested larvae of cabbage butterfly , *Pieris rapae* . (Al- Hindawi and Melconian , 1978 ; Al-Zubaidi , Melconian , Al-Kayatt , and Adnan , 1989) , which kept in lyophilized form .

Four different concentrations (0.25, 0.5, 1.0, and 2.0 mg) of the lyophilized growth material in 1 ml nutrient broth, were used as contaminants. Topical application of this suspension was done by

drenching the leaves from both surfaces by 1 ml of *B. thuringiensis* var. *kuristaki* grown in a broth culture at 30° C for 3 days under shaking conditions. During the course of this study, 10 5th- instar larvae (24 hr. old) were placed in the experimental container (8 cm in diameter and 5 cm height) / 10 replicates, supplied with young leaves contaminated with bacterial concentration under the study. The control group larvae were fed on leaves treated with nutrient broth only. All experimental containers were supplied with freshly cut young leaves on daily basis. The mortality rates were recorded after one day, 2, 3, and 4 days after bacterial application.

The experimental design was based on completely randomized plot technique. Statistical analysis was carried out by using one – way analysis of variance and correlation coefficient with confidence interval of 95% (Snedecor and Cockran, 1967).

RESULTS AND DISCUSSION

Application of different concentrations of *B. thuringiensis* var. *kuristaki*, significantly affected the survival rate of lemon butterfly, *P. demoleus* (tables 1, and 3). As indicated in tables 1 and 3, there is a direct correlation between the concentration of *B. thuringiensis* *kuristaki* and the mortality rate of lemon butterfly ($r = 0.7$). The mortality rates were: 75%, 100%, 100%, and 100%, in concentrations of 0.25, 0.5, 1.0, and 2.0 mg/ml respectively.

The data also showed that young larvae (instars 1-3) were very susceptible to *B. thuringiensis* infection, especially, with concentrations of 1.0 and 2.0 mg/ml (table 1, fig. 1). A 100% mortality was obtained within two days after application of 2.0 mg/ml of the bacterial suspension, while it took about 3-5 days with concentration of 1.0 mg/ml. Similar studies with different lepidopteran species indicated that the mortality rate were directly correlated with the concentration of the bacterial suspension (Al-Hindawi and Melconian, 1978; McGaughey, 1978; Milistead, et al., 1980; and Boroza, Sneh, Yawetz, Oron, and Hanigman, 1984). Alrubeai et al. (1997 a) found that there are differential susceptibilities among the four pyralid species tested and among the formulations of *B. thuringiensis* var. *kuristaki* tested. Whiles AL - Maadhidi, et al. (1997) found that *B. thuringiensis* var. *aizawai* have a high insecticidal activity against 10 - 2 days old larvae of waxmoth *Galleria mellonella*. Alrubeai, et al. (1997 b). Found that *B. thuringiensis* var. *kuristaki* reduce the infestation of date palm trees by *Batrachedra amydraula* to 30% after one spray, and 80% after 2-3 sprays. The above mentioned studies generally supported the present study findings.

Developmental period of various stages of lemon butterfly was also significantly extended, due to the application of *B. thuringiensis* (table 2). The developmental period of all immature stages as well as, the total developmental period of *P. demoleus* were significantly prolonged as compared with control (tables 2 and 3). The data revealed that there is a direct correlation between the prolongation of the developmental period and the concentration of the bacterial suspension. McGaughey (1978) and Kinsinger and McGaughey (1979) found that *B. thuringiensis* caused large increase in the developmental time of *Ephesia cautella* larvae, but little increase in that of *Plodia interpunctella*. Salama (1984) also, mentioned that there is a prolongation of developmental period of *Heliothis armigera*, *Spodoptera exigua*, and *S. littoralis* after application of sub lethal doses of commercial preparation of *B. thuringiensis*. While, Alford and Holmes (1986) found that the larval development of both sexes of spruce budworm, *Choristoneura fumiferana*, was significantly extended after exposure to 100 IU/ml of *B. thuringiensis*. All above mentioned findings are in agreement with present study results.

Egg production of lemon butterfly was significantly affected by the application of *B. thuringiensis*. A pronounced reduction in egg production was found when the larvae treated with 0.25 mg/ml (table 3); only one female completed its development at this concentration. Other adults were unable to emerge from the pupal case, or they were abnormal. In this respect McGaughey (1978) did not find any effect on egg production when he was studying the susceptibility of almond moth and Indian meal moth to *B. thuringiensis*; this is conflicted with our

findings . This may be due to the different effects of *B. thuringiensis* varieties on insects (Osborn , et . al .,1985). While Salama (1984) mentioned that sub lethal doses of some commercial preparations of *B. thuringiensis* caused a reduction in egg production of *S. littoralis* ; low endo toxin concentration of *B. thuringiensis* caused deformalities in *H. atmigera* , *S. exigua* , and *S. littoralis* . Mahmoud , et al (1988) in their study on the influence of *B. thuringiensis* on the survival and development of the great wax moth , *Galleria mellonella* , found that the pupal development , and female fecundity were reduction in female , generally , support our finding the reduction in female fecundity and the delayed resulted from *B. thuringiensis* infection .

The results of the present study, clearly indicate that the use of *B. thuringiensis* var *kuristaki* . as a biological control agent against lemon butterfly , *P. demoleus* is a powerful tool , especially , with the concentrations of 1.0 and 2.0 mg/ml . This can be incorporated with the findings of Halify and Al-Zubaidi (1989) to give a good control measure against this pest . We suggest that, the influence of the environmental factors, efficiency of various application rates, formulation, frequencies and economics of *B. thuringiensis* var . *kuristaki* must be defined before its utilization to control lemon butterfly , *P. demoleus* or other pest species (Al-Hindawi and , Melconian ,1978 ; Al-Zubaidi et .al .1989).

Table 1:

Cumulative mortality rate (%) of different developmental stages of lemon butterfly, *P. demoleus* as affected by different concentrations of *B. thuringiensis* var . *kuristaki* .

Developmental stages	Larval instars					Prepupa	Pupa
	1 st	2 nd	3 rd	4 th	5 th		
Concentration (mg /ml)							
0.25	30	33.3	40.0	73.3	—	75	—
0.5	30	43.3	60.0	91.4	100.0	—	—
1.0	54.0	100.0	—	—	—	—	—
2.0	100.0	—	—	—	—	—	—
control	20.0	—	—	—	—	—	40.0
L .S .D .	6.17	8.07	22.85	13.81	23.03	21.0	13.19

(—) no change in the mortality from previous rate .

Table 2:

Developmental period (in days) of lemon butterfly , *P . demoleus* as affected by different concentrations of *B . thuringiensis* var . *kurstaki* .

Developmental stages	Larval instars					Prepupa	Pupa
	1 st	2 nd	3 rd	4 th	5 th		
Concentration (mg /ml)							
0.25	4.0± 0.32	2.7±0.3	4.0±0.37	4.4±0.4	5.9±0.4	1.3±0.3	8.3±0.7
0.5	4.1±0.4	3.2±0.5	3.8±0.6	4.5	—	—	—
1.0	4.0±0.6	—	—	—	—	—	—
2.0	—	—	—	—	—	—	—
control	3.4±0.5	2.7±0.2	3.5±0.2	3.9±0.3	4.6±0.5	1.5±0.2	7.0±0.5
L .S .D .	0.2	0.2	0.2	0.25	0.33	0.22	0.42

(—)100 % mortality.

Table 3:

Developmental period, mortality rate, and egg production of lemon butterfly, *P. demoleus* as affected by different concentrations of *B. thuringiensis* var . *kurstaki* .

Concentration (mg /ml)	Developmental Period (days)	(%) mortality	Egg production (No. /female)
0.25	30.46 ± 0.43	75	20
0.5	—	100	—
1.0	—	100	—
2.0	—	100	—
Control	26.19 ± 0.31	40	35 ± 3.7
L .S .D.	1.58	15.45	2.51

(—) , individuals in this treatments did not completed their development , and no Egg production.

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