Egyptian Journal of Biological Pest Control, 13 (1&2), 115-117

Evaluation of Agerion, A Commerical Formulation of *Bacillus thuringiensis* against Certain Insect Pests of Cabbage

Fatma, A. Atallah, Mona A. Shoeb and M.S.T. Abbas
Plant Protection Research Institute, Dokki, Cairo, Egypt.
(Received, August 15, 2003; Accepted, November 21, 2003)

ABSTRACT

Agerin, a commercial formulation of *Bacillus thuringiensis*, was evaluated under laboratory and field conditions against three economic insect pests infesting cabbage; *Pieris rapae, Plutella xylostella* and *Spodoptera littoralis*. Three concentration levels (0.625, 1.25 and 2.5 g/l of water) were tested. *P.rapae* and *P. xylostella* larvae in the 3*rd* instar showed high susceptibility levels to Agerin. In all cases, over 80 and 90% mortalities were obtained 2 and 8 days post treatment, respectively. treatment of *S. littoralis* neonate larvae by Agerin at the three mentioned concentrations did not affect their viability. Also, Agerin did not have deleterious effect on two common predators, *Chrysoperla carnea* and *Coccinella undecimpunctata* when fed on aphids sprayed with the recommended dose for field application (1.25 m/L.). Spraying Agerin in two cabbage plantations at the recommended dose caused 36.5 – 48.1% in *P. rapae* larvae.

Key Words: Agerin, *Bacillus thuringiensis*, insect pests, cabbage.

INTRODUCTION

Massive application of pesticides results in adverse effects on the beneficial arthropods, beside their toxic residues in the food and environmental pollution. As a result, the chemical control of pests is under increasing pressure. Pesticide use in the world is declining, largely due to major reduction as a result of regulatory mechanisms, environmental activities and public pressure. This has necessitated the use of target specific compounds with low persistence, and an increase in emphasis on integrated pest management based on host plant resistance to insect pests as well as bio- insecticides mainly entomopathogenic bacteria.

Bacillus thuringiensis, a soil- dwelling bacterium, produces an insecticidal protein crystal within the bacterial cell during sporulations.The crystal protein, known as δ endotoxin, is the primary active ingredient of *B. thuringiensis* formulations.Ingestion of δ endotoxin by susceptible insects results in gut paralysis and feeding inhibition followed by disruption of midgut epithelial cells and, eventually,death (Fast 1981,). In the last decade, tens of commercial formulations of *B.t.* have been produced worldwide for controlling wide range of lepidopterous, coleopterous and dipterous insect pests.

Cabbage is one of the important vegetable crops in Egypt. It is subjected to infestation by different pests mainly whiteflies, aphids, the cabbage worm, *Pieris rapae*, the diamond-back moth, *Plutella xylostella* and the cotton leafworm, *Spodoptera littoralis.*

The present investigation aims to evaluate the role of Agerin as a bio-insecticide against *P. rapae, P. xylostella* and *S. littoralis*. Side effect of Agerin was tested against two common predators prevailing in cabbage fields.

MATERIALS AND METHODS

The commercial bioinsecicide utilized in this study was Agerin; a genetically ingineered *Bacillus thuringiensis* formulation produced by Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research, Centre,Egypt. According to AGERI, the product is effective against lepidopteran, dipteran and coleopteran insect pests.

Laboratory Trials

Assessment of Agerin Effects on *Pieris rapae* and *Plutella xylostella*

Pieces of cabbage leaves were sprayed with three concentrations of Agerin; 0.625, 1.25,and 2.5 g/L of water (the recommended dose for field application is 1.25g/L). The treated leaves were kept in Petri-dishes (one/dish) lined with a moistened filter paper. Five larvae of (in the 3*rd* instar) were introduced to each dish to feed for 24 hrs.. The larvae were then transferred to similar Petri – dishes and fed on untreated cabbage leaves till death or pupation. 10 replicates, 5 larvae each, were used for each concentration. Percent mortalities were calculated 2,4, 6 and 8 days post treatment. 25 larvae were fed on untreated leaves as control.

Effect of Agerin on *S. littoralis* Larvae Hatched from Treated Eggs

To confirm some reports that Agerin has an ovicidal effect, this part of study was carried out. Twenty four-hour old egg-masses of *S. littoralis* were sprayed with Agerin at concentrations of 0.625, 1.25 and 2.5 g/L of water (10 egg-masses/concentration). The treated eggs were kept in glass vials 7x2 cm (one egg-mass/vial) stoppered with pieces of cotton wool.

The eggs were inspected daily until hatching. 5 replicates of hatched larvae (10/replicate)were reared on leaves of cotton plant until pupation to estimate the effect of eggs treatment on the hatched larvae. Five egg-masses were treated with water and the hatched larvae were fed on cotton leaves as control.

Effect of Agerin on Two Common Predators

Fifteen 3*rd* larval instars of each of *C. carnea* and *C. undecimpunctata,* were confined, individually, in glass

vials, 5 X 1cm stoppered with pieces of cotton wool. The larvae were provided daily with aphids sprayed with Agerin at a concentration of 1.25 g/L of water until reaching the pupal stage. The dead larvae, if any, were removed and% mortality was estimated. 15 larvae of each predator were fed on aphids sprayed with water as control..

All trials were carried out at 25±1°C and 60-70% R.H.

Field Trials

Effect of Agerin on *P. rapae.*

A trial was carried out in 2 cabbage plantations (300 m apart from each other) at Kerdasa province (Giza city) naturally infested with the cabbage worm *P. rapae*. 50 cabbages, in each plantation, were sprayed with Agerin (complete coverage) at the recommended dose for field application (1.25 g/L of water) using a knapsack sprayer (20 L) equipped with one nozzle. 25 cabbages were treated with water as control.

The treated cabbages were inspected 5 and 7days post application and the numbers of dead and alive larvae of *P. rapae* were recorded. The dead larvae were examined to make sure that they were *B.t* infected. The prevailing temperature in this province was ranging from a minimum of 16°C and a maximum of 28°C.

RESULTS

Laboratory Trials

Effect of Agerin on the Cabbage worm, *P. rapae*

Data presented in Table (1) reveal that initial mortality (2 days post treatment) in 3rd instar larvae was high at all tested concentrations of Agerin (84, 88 and 94% at concentrations of 0.625, 1.25 and 2.5 g/L, respectively).% mortality increased by time after treatment to reach 94, 98 and 100%, respectively, on the eighth day. Mortality in control was 4%.

Effect of Agerin on *P. xylostella*

The results presented in Table (1) show that the susceptibility of *P. xylostella* larvae to Agerin is similar or slightly higher than *P. rapae* larvae.Percent mortality reached 100% at the concentrations 1.25and 2.5g/L four days post treatment. Mortality in control was 6%.

Effect of Agerin on *S. littoralis* Larvae Hatched from Treated eggs.

Agerin, at all tested concentrations, was found to have no effect on egg-masses of *S. littoralis*. All treated and untreated eggs hatched within 2-3 days post treatment. % Mortality in larvae hatched from treated eggs ranged from 12-14% at the three tested concentrations (Table 2). Mortality in the control, however, was 10%. No statistical differences were found between treated and untreated eggs (T test showed that.).

Effect of Agerin on Predators

Agerin was found to have no deleterious effect on both *C. carnea* and *C. undecimpunctata.* The 3*rd* instar larvae of both predators fed daily on Agerin-treated aphids developed normally to pupae and to the adult stage.

Table (1): Effect of Agerin on larvae of *P. rapae* and *P. xylostella*in the laboratory.

Insect pest	Conc. (g/L)	% Mortality after indicated days			
		2	4	6	8
P. rapae	0.625	84	90	92	94
	1.25	88	98	98	98
	2.5	94	94	98	100
	control	2	4	4	4
P. xylostella	6.25	88	90	92	92
	1.25	92	100	100	100
	2.5	94	100	100	100
	control	2	4	6	6

Table (2): Effect of Agerin, at 3 different concentrations on *S. littoralis* larvae hatched from treated eggs.

Conc. (g/L)	% Mortality at indicated days after hatching			
	2	4	8	12
0.625	0	10	10	14
1.25	0	10	10	12
2.5	0	8	12	14
control	0	4	6	10

Field trials

Effect of Agerin on *P. rapae* Larvae

As presented in Table (3), spraying Agerin at the recommended dose for field application, 1.25 g/L, resulted in 36.5 and 48.1% mortality in *P. rapae* larvae in the two plantations. The total number of *Pieris* larvae found in the 50 cabbages in each of the two plantations was 84 and 108, respectively. The respective total numbers of *B.t*-killed larvae were 30 and 52. No dead larvae were noticed in the control cabbages.

Table (3): % Mortality in *P. rapae* after application of Agerin in cabbage plantations (50 cabbages/plantation) at the recommended dose for field application (1.25 g/L).

Treatment	No. of dead larvae	No. of alive larvae	% Mortality
Plantation 1	30	54	36.5
Plantation 2	52	56	48.1

DISCUSSION

The present investigation revealed that the *B.t.* commercial formulation, Agerin, was virulent to 3*rd* instar larvae of *P. rapae* in the laboratory.% mortality reached 84, 88 and 94% two days post treatment at concentrations of 0.625, 1.25 and 2.5 g/L., respectively.In contrast, when Agerin was applied in cabbage fields at the recommended dose for field application, 1.25 g/L, mortality in *P. rapae* ranged between 36.5 and 48.1% 7 days post application. Kares *et al.* (1992) found that the *B.t.* formulation, Bactospeine, was virulent to 2*nd*, 3*rd* and 4*th* larval instars of *P. rapae* in the laboratory and the 2*nd* instar was the most susceptible one.

Our laboratory studies indicated also that 3*rd* instar

larvae of *P. xylostella* were highly susceptible to Agerin. At concentrations of 0.625, 1.25 and 2.5 g/L, mortality reached 88, 92 and 94%, respectively, 2 days post treatment. Other workers came to similar results with different formulations of *B.t.*; Higuchi *et al.* (2000), Justin *et al.* (2001), Pokharkar *et al.* (2002) and Singh *et al.* (2003). However, *P. xylostella* was reported to develop resistance *to B. thuringiensis;* Imai and Mori (1999); Maruyama *et al.* (1999); Sayyed et al (2000); Yu *et al.* (1999).

Agerin was found to have no deleterious effect on the larvae of *S.littoralis* hatched from treated eggs. Abdel-Hafez *et al.* (1994) found that *B. t.* formulations, Dipel and Delfin did not affect eggs of *Pectinophora gossypiella* but had adverse effect on survival and development of the hatched larvae. Similarly, Zang and Liu (1997) reported that *B.t.* applied on the eggs of *P. xylostella* did not affect egg hatching but caused substantial mortality for newly hatched larvae.

Laboratory studies showed also that Agerin did not affect larvae of the two predators, *C. carnea* and *C. undecimpunctata,* fed on aphids sprayed with Agerin. Insect predators, however, are not expected to be affected by pure bio insecticides, especially *B.t.* and Nuclear polyhedrosis virus, but they, in addition, may transmit such pathogens to target pests; Abbas and Boucias (1984), Boucias *et al.* (1987).

REFERENCES

Abbas, M.S.T. and D.G. Boucias. 1984. Interaction between nuclear polyhedrosis virus infected *Anticarsia gemmatalis* larvae and predator *Podisus maculiventris.* Environ. Entomol., 13 (2): 599-602.

Abdel-Hafez, A.; S.H. Taher and S.M. Abdel-Halim. 1994. Effect of two formulations of *Bacillus thuringiensis* on *Pectinophera gossypiella* treated in egg stage. Egypt. J. Biol. Pest Control, 4 (1): 89 -95.

Boucias, D.G.; M.S.T. Abbas; L. Rathbone, L. and N. Hostetter. 1987. Predators as potential dispersal agents of the nuclear polyhedrosis virus of *Anticarsia gemmatalis.* Entomophaga, 32 (1): 97-108.

Fast, P.G. 1981. The crestal toxin of *Bacillus thuringiensis*, pp. 223- 248. In H. d. Burges and N.W. Hussey (eds.), Microbial Control of Insects and Mites, Academic Press, New york.

Higuchi, K.; H. Saitoh; E. Mizuki; T. Ichimatsu and M. Ohba. 2000. Larval susceptibility of the diamondback moth, *Plutella xylostella* to *Bacillus thuringiensis* isolated in Japan. Microbiological Research, 155 (1): 23- 29.

Imai, K. and Y. Mori. 1999. Levels, inheritance and stability of resistance to *Bacillus thuringiensis* formulations in a field population of *Plutella xylostella* from Thailand. Applied Entomology and Zoology, 34 (1): 23-29.

Justin, C.G.L; R.P. Soundararajan.; R.J. Rabindra and M. Swamiappan. 2001. Dosage and time mortality response of the diamond- back moth, *Plutella xylostella* to *Bacillus thuringiensis* formulations Pest Management and Economic Zoology, 9 (2): 109 -113.

Kares,E.A.; A.A. El-Mursy; A.A. Abdel-Rahman and M.B.R. El-Mandarawy.1992. Efficacy of bioinsecticide (Bactospeine) on larvae of *Artogeia rapae.* Egypt. J. Biol. Pest Cont., 2 (2): 123-130.

Maruyama, T.; H. Hama and S. Asano. 1999. Establishment and maintenance of high resistance to *Bacillus thuringiensis* formulations in *Plutella xylostella.* Japanese J. Appl. Entomol. and Zool., 43 (1):7-12.

Pokharkar, D.S.; A.B. Hadapad and T.R. Puranik. 2002. Bioassay and persistence of *Bacillus thuringiensis* against *Plutella zylosteklla* on cabbage. Annals Plant. Protect. Sci., 10 (1): 1-4.

Sayyed, A.H; J. Ferre and D.J. Wright. 2000. Mode of inheritance and stability of resistance to *Bacillus thuringiensis* in *Plutella xylostella* population from Malaysia. Pest Management Science, 56 (9): 743 - 748.

Singh, M.K.; S.V.S. Raju and H.N. Singh. 2003. Laboratory bioassay of *Bacillus thuringiensis* formulations against *Plutella xylostella.* Indian J. Entomol., 65 (1): 86-93.

Yu, D.; Z. Zhan and B. Tang. 1999. Advances in studies on resistance to *Bacillus thuriengiensis* in *Plutella zylostella* and its management. Natural Enemies of Insects, 21 (1): 21-27.

Zang, G.M. and S.S. Liu. 1997. Effect of *Bacillus thuringiensis* on the egg stage of *Plutella xylostella.* Chinese J Biol. Control, 13 (2): 53-56.