ENHANCEMENT OF THE EFFICIENCY OF SOME BIOPESTICIDES AGAINST PHTHORIMAEA OPERCULELLA (ZELLER) BY ADJUVANTS UNDER LABORATORY AND GREENHOUSE CONDITIONS

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(Manuscript received 26 March 2007)

Abstract

Laboratory and greenhouse experiments were carried out to study the effect of calcium and potassium carbonate in increasing the potency of some biopesticides.

The results of laboratory study indicated that xentari gave the highest RL_{50} value of 77.91 days indicating long persistence and high toxicity against PTM followed by Verotecto and Agerin, while Dipel 2x, Ecotech and MVPII caused the lowest effects.

Also xentari gave the highest RL_{50} value of 36.99 days in greenhouse while Verotecto gave the lowest result where its RL_{50} was 9.95 days

The results provided evidence that the initial and residual effect of the tested biopesticides can be markedly increased by using calcium or potassium carbonate at 0.1% in combination with each of the candidate biopesticides. The RL_{50} values indicated that all tested biopesticides when combined with potassium carbonate at 0.1% were more effective than calcium carbonate in protecting potato plants against PTM in laboratory and greenhouse. This may be attributed to the fact that the addition of such salts change pH of the gut, being more alkaline and thus enhancing the endotoxin breakdown and release of toxic fragments.

INTRODUCTION

Potato tuber moth attacks potato plants in two ways: by mining the foliage and feeding on tubers. Larvae penetrate the leaves and weaken or break the stem while, in tubers, they deposit eggs near eye buds, causing irregular galleries deep inside the tubers (Mannickavasgar, 1953)

Chemical pesticides are still primary means of control pests, but their use is becoming more controversial. The concept of biocides is being ecofriendly as well as environmentally safe and specificity to most of the target pests (Girigs, 2003).

Baculoviruses have been registered as insecticides with the US Environmental Protection Agency (Inceoglu *et.al.* (2001).

The potency of *Bacillus thuringiensis* subsp.entomocidus and aizawai against Lepidopterous could be increased by modifying the conditions presented in the insect midgut throught the incorporation of alkaline compounds (salama *et.al,* 1984).

The activity of the delta-endotoxin increased as alkalinity increased from 8 to 10 and then declined rapidly at pH>10 (Gringorten *et.al*, 1992, Venugopal *et.al*, 1992 and Abdel Hai, (2001).

Therefore, the target of this work was to evaluate the role of the chemical additives on increasing the efficacy of these compounds.

MATERIALS AND METHODS

The tested products used were:

- The granulosis virus (GV) formulation (Verotecto 4% a.i), (300 gm/fed.): produced by the Plant Protection Research Institute, Agriculture Research Centre in Callaboration with the International Potato Centre (CIP), Min. of Agriculture, Egypt
- 2) B.t formulations used
 - a) Agerin 6.5%w/w (200 gm/fed.): based on *B.t* subsp. Serovar aegypti. Produced by Agricultural Genetic Engineering Research Institute, ARC, Min of Agric. Egypt.
 - b) Dipel 2x 6.4% a.i (200 gm/fed.): wettable powder compound, based on *B.t* subsp. Kurstaki. It contains 6.4% lepidopteran active toxin produced by abbott/Laboratories North Chicago, USA.
 - c) Ecotech Bio 10% a.i (300 gm/fed.): *B.t* subsp. Kurstaki strain EG 2371 lepidopteran active toxin 10% produced by Agrevo S.A. France Ecogen USA.
 - d) MVPII 20% a.i (1000 ml/fed.): aqueous flowable, a genetically engineered bacterium that produces delta-endotoxin derived from *Bt*. Subsp. Kurstaki the active ingredient consists of endotoxin protein crystals produced by Mycogen USA.
 - e) Xentari 10.3% (water dispersible granule) based on *Bacillus thuringiensis*, subsp.aizawai Lepidopteron active toxin. Produced by Abbott Laboratories North Chicago, USA.
- 3) Chemical additives used
 - a. potassium carbonate used at 0.1%
 - b.calcium carbonate used at 0.1%

The six treatments were combined with potassium or calcium carbonate at 0.1%.

The Laboratory experiments:

The tests were carried out using individual potato tubers var. (Diamond) Ninety potato tubers free from any infestation and about 100 gm weight for each were sprayed with the recommended concentration of each product alone, or in combination with potassium or calcium carbonate at 0.1%, and finally placed individually in plastic containers 10 cm diameter and 15 cm high.

Every treatment was replicated five times. Each potato was infested with $10 \, 1^{st}$ instar larvae at intervals of 0, 1,3,6,9,12,17,20 and 27 days after treatments and the container was sealed with nylon gauze. Untreated potato was each infested with $10 \, 1^{st}$ instar larvae. The containers were left undisturbed for 7 days after infestation. Infestation was measured by counting the number of visible holes on the tuber surface.

Greenhouse experiments:

Potato tubers var."Diamond", were planted singly in ninety pots (yocmdx socmh.) on the 1st of January 2003, under greenhouse conditions at the Central Pesticides Laboratory ARC, Min. of Agriculture, Egypt. Day time greenhouse temperature ranged from 16°c to 22°c. One month after planting, each plant was sprayed with the recommended concentrations of mentioned compounds alone or in combination with calcium or potassium carbonate at 0.1% using a Knapsack low volume sprayer. Each potato plant was infested with 10 1st instar larvae at intervals of 0, 1,3,6,9,12,17,20 and 27 days after treatment and untreated plants were each infested with 10 1st instar larvae. The plants were left for 5 days after infestation, until, the larvae had reached the second instar inside the leaves. The leaflets were examined using a binocular microscope, and the total number of larvae was recorded.

The residual half-life times (RL_{50}) were adapted according to Finney probit analysis (1971) and the mortality percentages were corrected according to Abbots formula (1925).

RESULT AND DISCUSSION

The initial and residual effects of the tested biopesticides against the 1st instar larvae of *P.operculella* infesting potato tuber in laboratory are shown in table 1. Xentari gave the highest RL₅₀ value of 77.91 days indicating long persistence and high toxicity against PTM followed by Verotecto and Agerin, which gave RL₅₀ of 60.67 and 39.53 days, respectively. The lowest results were obtained with Dipel 2x, Ecotech and MVP II, where their RL₅₀ values were 36.56, 28.65 and 22.11 days, respectively.

The data in table 2 represent the initial and residual effects of the tested of biocides against PTM infesting potato plants in greenhouse.

Also Xentari gave the highest RL_{50} values of 36.99 days while Verotecto gave the lowest results where its RL_{50} value was 9.95 days.

The initial and residual effect of the tested biopesticides combined with calcium or potassium carbonate at 0.1% against PTM under laboratory and greenhouse conditions are summarized in tables 1 and 2, respectively

The RL_{50} values indicated that all tested biopesticides when combined with potassium carbonate at 0.1% were the most efficient combinations in protecting potato tubers against PTM (table 1). Their RL_{50} values were 83.11, 95.86, 69.86, 72.88, 80.82 and 48.65 for Xentari, Verotecto, Agerin, Dipel 2x, Ecotech and MPV II respectively. The mixtures of these compounds with calcium carbonate at 0.1% were moderately active where their RL_{50} values were 79.05, 79.19, 44.62, 50.03, 46.02 and 37.70 days respectively (table 1).

The data in table 2 represent the initial and residual effects of the tested biopesticides combined with calcium or potassium carbonate at 0.1% against PTM infesting potato plants in greenhouse. The results indicate that the mixtures of biopesticides with potassium carbonate at 0.1% were also more effective in protecting potato plants against PTM. Combination with potassium carbonate gave the highest values of RL₅₀ 56.69, 34.36, 46.92, 26.12, 23.53 and 17.96 days for Xentari, Agerin, MPV II, Dipel 2x, Ecotech, and Verotecto respectively. The RL₅₀ values were only 40.47, 30.54, 41.46, 20.05, 19.24 and 15.10 days for the same compounds when combined with calcium carbonate 0.1%.

Girgis, (2003) reported that pH, conductivity and salinity for the combinations of biocides with potassium and calcium carbonate at 0.1% were higher than for the biopesticides alone. The role of potassium and calcium carbonate in increasing the toxicity and residual effect of the tested biopesticides against PTM could be explained by assuming that both inorganic ions and variation in physico-chemical properties of spray emulsions contribute to the toxicity.

Some studies has been reported about the effect of alkalinity on the activity of the protein delta-endotoxin produced by *Bt* In these studies, solubility of the delta-endotoxin was linked to the alkalinity associated with the digestive tract of the target insect. The protoxin extracted from *Bt* .kurstaki is soluble at pH>10(Tandada and Kaya, 1993).These toxins are effective against Lepidoptera larvae, which have an alkaline digestive tract (Gringorten et al, 1992).

It may be concluded that the two inorganic salts, calcium and potassium carbonate at 0.1% proved to be an extremely efficient activator of the biopesticides against PTM.

Table 1. Initial and residual effect of the tested bio-compounds alone or combined with calcium carbonate or potassium carbonate 0.1% against *P.operculella* infesting potato tubers in laboratory

Treatments	Rate Dose/fed.			ınitial effect %	RL ₅₀ days							
	•	0	1	3	6	9	12	17	20	27		
Xentari	240 gm	100	100	100	100	97.83	95.83	92.31	86.49	83.33	100	77.91
+C.C		100	100	100	100	100	100	93.24	88.12	85.10	100	79.05
+P.C		100	100	100	100	100	100	100	92.20	87.11	100	83.11
Verotecto +c.c	300 gm	100	100	97.14	96.05	89.47	86.11	76.92	75.68	72.22	100	60.67
		100	100	100	100	98.10	96.40	90.30	89.81	86.50	100	79.19
+P.C		100	100	100	100	100	100	97.30	92.25	89.11	100	95.86
Agerin	200 gm	100	97.37	90.00	86.84	83.78	72.22	71.79	59.46	55.56	100	39.53
+C.C		100	100	100	97.20	90.61	88.62	82.11	74.18	69.00	100	44.62
+P.C		100	100	100	100	100	98.20	94.15	90.22	84.00	100	69.86
Dipel 2x	200 gm	100	100	95.71	92.11	86.84	80.56	76.92	62.16	52.77	100	36.56
+c.c +P.C		100	100	100	96.12	92.81	89.22	85.50	78.17	66.77	100	50.03
		100	100	100	100	98.81	96.75	92.60	88.44	85.22	100	72.88
Ecotech	300 gm	100	97.37	85.71	84.21	81.08	72.22	66.66	51.35	44.44	100	28.65
+c.c +P.C		100	100	97.82	90.10	85.25	80.44	74.11	72.90	65.41	100	46.02
		100	100	100	100	94.61	90.36	86.50	81.11	79.20	100	80.11
MPV II	1000 ml	100	92.11	85.71	78.71	72.97	66.67	61.54	45.94	41.66	100	22.11
+C.C		100	100	92.55	86.57	80.75	77.61	71.81	66.59	51.44	100	37.70
+P.C		100	100	100	95.00	88.21	81.26	79.55	75.16	68.32	100	48.65

Table 2. Initial and residual effect of foliar spraying bio-compounds alone or combined with calcium carbonate or potassium carbonate at 0.1% against *P.operculella* infesting potato plants in green house.

Treatments	Rate Dose/fed.			Percer	initial effect %	RL ₅₀ days						
		0	1	3	_6	9	12	17	20	27		
Xentari	240 gm	100	100	100_	100	94 59	93.59	8 <u>6.84</u>	78.05	60.00	100	36.99
+C.C +P.C		100	100	100	100	160	96.45	91.59_	81.41	74.00	100	40.47
		100	100	100	100	100	100	95.72	86.00	78.33	100	56.69
Agerin +c.c +P.C	200 gm	100	100	96.25	87.18	75 <u>.68</u>	74.36	63.16	58.54	34.29	100	21.99
		100	100	100	95.46	89.86	76.11	72.60	64.00	59.00	100	30.54
		100	100	100	100_	91.66	84. <u>45</u>	79.05	68.00	60.0	100	34.36
MPV II	1000 ml	100	100_	93.75_	82.05_	97.57	66.67	60.5 <u>3</u>	53.6 <u>6</u>	28.57	100	20.30
+c.c + P.C		100	100	100_	93.31	85. <u>75</u>	81.22	79.15	70.61	61.01	100	41.46
		100	100	100	97.71	91.64	87.33	80.95	75.91	72.03	100	46.92
Dipel 2x +c.c +P.C	200 gm	100	100_	93.75	74.36	64.86_	64.10	55. <u>26</u>	51.22	20.00	100	16.88
		100_	100	97.41	82.51	71.86	67.11	61.25	56.41	44.31	100	_ 26.05
		100	100	100	90 <u>.60</u>	76.79	71.43	64.24_	59.65	49.25	100	26.12
Ecotech	300 gm	100	100	87.50	69.2 <u>3</u>	61. <u>54</u>	59. <u>46</u>	52.63_	43.90	17.14	100	12.85
+c.c + P.C		100	100	94.61	81.50	70.22	66,61	55.63	51.21	35.14	100	19.24
		100	100	99.01	85.4 <u>1</u>	75. <u>95</u>	68.50	61.75	60.25	53.00	100	23.53
Verotecto	300 gm	100	100	80.0	69.23	54.05	48.72	47.37	39.02	05.71	100	9.95
+c.c +P.C		100	100	84.21	75.0 <u>1</u>	61.11	58. <u>81</u>	4 <u>9.31</u>	44.66_	31.21	100	15.10
		100	100	89.23	79.22	68.65	60.00	52.23	49.91	36.22	100	17.96

C.C=calcium carbonate

P.C=potassium carbonate

RL₅₀=Residual half life.

REFERENCES

- Abbott,W.S. 1925. A method of computing the effectiveness of an insecticide. J.Econ.Ent.18:265-269
- 2. Abdel-Hai, N.S. 2001. Studies on some bio-insecticides against the cotton leaf worm. M.Sc.Thesis, Fac. Agric., Al-Azhar Univ., Egypt, pp. 122.
- 3. Finney, D.J. 1971. Probit analysis. Cambridge Univ. Press, London
- 4. Girgis, N.R. 2003. Studies on various control methods of the potato tuber moth *Phthorimaea opreculella* (zeller). Thesis Ph.D.Fac.Agric.Cairo Univ., Egypt.
- 5. Gringorten, J.L., R.E. Mile, P.E. Fast, S.S. Soho and K. Vanframdenhyzen. 1992. Suppression of *Bacillus thuringiensis* d-endotoxin activity by low alkaline pH. J., Inver.path, 60:47-52.
- Inceoglu,A.B., S.G.Kamita, A.C. Hinton, Q. Huang, T.E. severson, K. Kang, and B.D Hammock. 2001. Recombinant baculouiruses for insect control. Depart. Ent&Concer Res.center Univ. California. USA, Pest Management Science, 57:981-987.
- 7. Mannickavasgar, P. 1953. The potato tuber moth, *Gnorimoschema opreculella* (zell.)Trop.Agric.109 (2):118-122.
- 8. Salama, H.S., M.S. Foda and A. Sharaby. 1984. Novel biochemical avenues for enhancing *Bacillus thuringiensis* endotoxin potency against *Spodoptera littoralis*, Entomophaga, 29:171-178.
- 9. Tandada, Y. and H.K. Kaya. 1993. Insect pathology. Academic, San-Diego, C.A.
- Venugopal, M.G., M.G. Wolferaberger and B.H. Wallace. 1992. Effect of pH on conformational properties related to the toxicity of *Bacillus thuringiensis* deltaendotoxin. Biochem.Biophys.Acta.Int.J.biochem.Biophys, 1159:185-192

زيادة كفاءة بعض المبيدات الحيوية ضد فراشة درنات البطاطس بواسطة المواد المضافة تحت الظروف المعملية والصوب

نيروز رزق الله جرجس

المعمل المركزي للمبيدات-مركز البحوث الزراعية-الدقي

تم اختيار كل من كربونات البوتاسيوم وكربونات الكالسيوم لدراسة تأثيرها في زيادة كفاءة بعض المبيدات الحيوية تحت الظروف المعملية والصوب.

أظهرت النتائج ان المركب الحيوي البكتيري زنتارى ذو كفاءة عالية ضد فراشة درنات البطاطس وله اثر باق طويل يصل الى ٧٧,٩١يوم تحت الظروف المعملية يليه المركب الفيروسي فروتكتو والمركب البكتيرى اجرين. وكان مركب الديبل والايكونيك وام في بي تو اقل فاعلية.

كذلك أعطى الزنتارى كفاءة عالية ووصل الأثر الباقي له تحــت ظــروف الصــوب إلـــى ٣٦,٩٩ يوم في حين كان فروتكتو اقل المركبات فاعلية وكان اثره الباقي قليل حيث وصل إلى ٩,٩٠ يوم فقط.

عند إضافة كل من كربونات الكالسيوم وكربونات البوتاسيوم بنسبة ٠,١٪ لكل من المركبات الحيوية المختبرة. أظهرت النتائج زيادة فاعلية هذه المركبات بهذه الإضافات حيث ارتفعت نسبة الموت الأولية و زاد الأثر الباقي لهذه المركبات خاصة عند إضافة كربونات البوتاسيوم.

من هذا يتضح إن إضافة هذين الملحين قد يكون لها تأثير على زيادة قلوية الأس الهيدروجيني في معدة فراشة درنات البطاطس مما يزيد من فاعلية هذه المركبات الحيوية.