Pakistan J. Zool., vol. 45(3), pp. 629-633, 2013

Pyrethroids Synergize New Chemical Insecticides in Field Populations of *Plutella xylostella* (Lepidoptera: Plutellidae)

Muhammad Nasir, Muhammad Imran and Munir Ahmad*
Department of Entomology, Faculty of Crop and Food Sciences, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan

Abstract.- Diamondback moth, an important insect pest, causes major economic losses to cruciferous vegetables. Two field strains were tested for single as well as their mixtures at respective LC_{50} values for synergistic interaction with laboratory standard leaf-dip bioassays method. There existed no variation in response of *P. xylostella* to tested λ-cyhalothrin, profenofos, emamectin benzoate and lufenuron for both field strains. λ-cyhalothrin when mixed with emamectin benzoate and lufenuron proved synergistic for Taxila strain but other combinations were antagonistic. Same insecticide mixtures when tested on Rawalpindi strain showed synergistic effect for all the tested combinations. Variation is response might be due to possible different insecticide use. Importance of insecticide mixtures with relevance to resistance field problems has been discussed.

Key words: *Plutella xylostella*, insecticide mixtures, pyrethroid, organophosphate, new chemical insecticides.

INTRODUCTION

Mixtures of insecticides belonging to different insecticide classes are usually used in the field to enhance the spectrum of control when multiple pests attack or have developed resistance to one or to another insecticide (Ahmad *et al*., 2009; Ahmad, 2004). They are also recommended to increase the efficacy of control against a single pest or to delay the development of insecticide resistance or combat current resistance in a pest species (Attique *et al*., 2006). Ideally, the insecticides having different modes of action are mixed on the assumption that they would complement the action of each other for killing the target pest.

Cauliflower (*Brassica oleracea* var *botrytis*) having high nutritive value is attacked by several insect pests causing high yield losses (Zerkoune, 2000; Sayyed *et al*., 2001). The most important insect pest of crucifer vegetables is *Plutella xylostella* (Lepidoptera: Plutellidae) throughout the world (Talekar and Shelton, 1993), and since it feeds on leaves of vegetable from seedling to harvesting stage, it inflicts serious economic losses. Its serious attack can cause 30-100% crop failure if no insecticide is used for its management (Verkerk and Wright, 1996). Major damage is caused by fourth instar larvae and only a few can make cabbage unfit for sale (Maltais *et al*., 1998). Despite usage of all available methods and pest control technologies, it has remarkable ability to develop resistance to virtually every insecticide which make it prominent insect pest in one of the 20 resistant insect species (Shelton *et al*., 2000; Mota-Sanchez *et al*., 2002).

Previously low level of resistance to *P. xylostella* strains has suggested the possibility of involvement of multiple mechanisms of resistance (Attique *et al*., 2006). Under such circumstances, insecticide mixtures can serve better than single insecticides. Keeping in view the future possible insecticide mixtures availability and information, present study was planned using conventional insecticides mixed with newly marketed chemical insecticides and a chitin synthesis inhibitor.

MATERIALS AND METHODS

Insects

About 200 larvae of *P. xylostella* were collected randomly from cauliflower field crops of Rawalpindi and Taxila vegetable growing areas during 2010-11 and kept separately, previously tested for initial susceptibility levels. The larvae were kept at 23±2°C, 60% RH and 16 hour

* Corresponding author: munirahmad@uaar.edu.pk , maqmunir@gmail.com
0030-9923/2013/0003-0629 $ 8.00/0

photophase in plastic jars (1 kg) on cauliflower leaves with daily change. Pupae were collected daily and provided with 10% honey solution at adult stage. Two to three fresh leaves of cauliflower were provided daily for eggs. Bioassays were performed after one generation laboratory rearing to have sufficient number of larvae for bioassays.

Bioassays and insecticides

Four commercials insecticides namely λ-cyhalothrin (Karate® 2.5EC; Syngenta (Pvt) Ltd, Pakistan), profenofos (Curacron® 50EC; Syngenta (Pvt) Ltd, Pakistan), emamectin benzoate (Proclaim® 1.9EC, Syngenta (Pvt) Ltd, Pakistan) and lufenuron (Match® 05EC, Syngenta (Pvt.) Ltd, Pakistan) were used. Top Film® (Helb Pesticides (Pvt) Ltd, Pakistan) as a surfactant was used at 5 ppm for increased adhesiveness to leaf surface in preparation of insecticide solutions and also in control.

Leaf dip method was used for estimation of efficacy of insecticides singly or in mixture against second instar larvae of *P. xylostella* (Ahmad *et al*., 2009). Six 3.5cm diameter leaf discs of cauliflower were dipped in serial solutions of each insecticide for 10 seconds and surface dried on tissue papers in fume hood. Stock solution for each bioassay was prepared using ppm concentration of each insecticide either alone or in mixture. Treated leaves were then placed on the moist filter paper with adaxial surface upward. Five 2^{nd} instar larvae were released in each Petri plate comprising thirty larvae at each concentration level for control, untreated leaf discs were exposed to same number of larvae as control. These Petri plate were covered tight with lid to avoid larval escape. Mortality as end point was observed 48 hours after exposure to insecticides.

Data analysis

Mortality data was subjected to Probit analysis using Polo-PC software (Abbott, 1925; Finney, 1971). After performing bioassays for single insecticides, these insecticides were tested by mixing two insecticides at their respective LC_{50} values. To estimate synergism, antagonism or addition response of these mixtures, combination index (CI) was calculated (Ahmad *et al*., 2009).

RESULTS AND DISCUSSION

Lufenuron was more toxic to Taxila strain compared with emamectin benzoate, λ-cyhalothrin and profenofos followed by emamectin benzoate. λ-cyhalothrin with emamectin benzoate was more toxic compared when mixed with lufenuron and other mixtures (Table I). Toxicity of profenofos/lufenuron mixture was lower than all other insecticide mixtures. λ-cyhalothrin and emamectin benzoate when mixed with lufenuron were synergistic (Table II). However, profenofos with λ-cyhalothrin, emamectin benzoate and lufenuron and emamectin benzoate with lufenuron were antagonistic intimating to avoid these combinations when used for single of multiple pest situations to decrease economic cost of management.

Emamectin benzoate was more toxic to Rawalpindi strain when compared with lufenuron, profenofos and λ-cyhalothrin. λ-cyhalothrin was less toxic than all other tested insecticides (Table I). In mixtures, λ-cyhalothrin with emamectin benzoate was more toxic than all other insecticides mixture and it was least with profenofos. Mixture of λ-cyhalothrin and profenofos with emamectin benzoate and lufenuron for Rawalpindi population showed synergistic effect for all the tested combinations at their LC_{50} ratio. However, the combination index values were more close to additive effect of near to one (Table II).

Rawalpindi strain was comparatively more susceptible than Taxila strain against tested insecticides as previously observed by Attique *et al*. (2006) and Khaliq *et al*. (2007). Vegetable growers especially of cabbages and cauliflower are prominent for their application of insecticides against *P. xylostella*, *Spodoptera litura* and *Brevicoryne brassicae*. Low level of toxicity in field strains may be due to the collection time from field affecting the level of toxicity of insecticides (Sayyed *et al*., 2012). Short life period, continuous food availability and wide exposure to insecticides made *P. xylostella* resistant to almost every insecticide around the world (Khaliq *et al*., 2007; Sayyed *et al*., 2012). Multiple resistances against many insecticides having different resistance mechanisms resulted for insecticide mixtures like

Table I.- **Toxicity of four insecticides alone and in combination against Taxila (TXL) and Rawalpindi (RWP) field strains of *Plutella xylostella*.**

Strain	Insecticide tested	Ratio	LC_{50} (FL at 95%)	Slope±SE	χ^2	df	P	n
Taxila	λ-cyhalothrin	1:0	0.89 (0.46–1.35)	1.82±0.38	0.96	3	0.81	180
	Profenofos	1:0	0.94 (0.52–1.03)	2.21±0.45	2.02	3	0.57	180
	Emamectin	1:0	0.78 (0.45–1.09)	2.14±0.41	0.40	3	0.94	180
	Lufenuron	1:0	0.63 (0.24–1.02)	1.56±0.34	1.70	3	0.63	180
	λ-cyhalothrin + profenofos	1:1.0	0.89 (0.33–1.62)	1.16±0.27	0.66	4	0.96	210
	λ-cyhalothrin + emamectin	1:1.1	0.49 (0.22–0.78)	1.49±0.29	2.49	4	0.65	210
	λ-cyhalothrin + lufenuron	1:1.4	0.54 (0.23–0.93)	1.19±0.25	1.58	4	0.81	210
	Profenofos + emamectin	1:1.2	0.83 (0.34–1.47)	1.13±0.25	0.73	4	0.95	210
	Profenofos + lufenuron	1:1.5	0.90 (0.16–1.57)	2.04±0.65	2.59	4	0.63	210
	Emamectin + lufenuron	1:1.2	0.74 (0.25–1.45)	0.95±0.24	1.58	4	0.81	210
Rawalpindi	λ-cyhalothrin	1:0	1.18 (0.85–2.97)	1.44±0.36	0.71	3	0.87	180
	Profenofos	1:0	1.13 (0.48–1.94)	1.27±0.33	0.45	3	0.93	180
	Emamectin	1:0	0.84 (0.19–1.67)	1.01±0.31	0.01	3	0.99	180
	Lufenuron	1:0	1.12 (0.41–2.01)	1.16±0.32	0.98	3	0.80	180
	λ-cyhalothrin + profenofos	1:1.0	0.93 (0.39–1.48)	1.89±0.41	2.16	4	0.71	210
	λ-cyhalothrin + emamectin	1:1.4	0.46 (0.19–0.75)	1.29±0.25	0.50	4	0.97	210
	λ-cyhalothrin + lufenuron	1:1.1	0.64 (0.31–0.99)	1.54±0.29	1.42	4	0.84	210
	Profenofos + emamectin	1:1.3	0.65 (0.34–1.00)	1.57±0.29	2.76	4	0.59	210
	Profenofos + lufenuron	1:1.0	0.72 (0.33–1.15)	1.56±0.30	0.79	4	0.94	210
	Emamectin + lufenuron	1:1.3	0.78 (0.30–1.36)	1.38±0.30	0.69	4	0.95	210

χ^2, Chi square; n, number of insects tested.

Table II.- **Combination index and intrinsic and synergistic mortalities for six insecticide mixtures against Taxila (TXL) and Rawalpindi (RWP) field strains of *Plutella xylostella*.**

Strain	Active ingredient	Active ingredient ratios			At LC_{50}			
		Used ratio	Calculated value of A	Calculated value of B	CI	%M1	%M2	%M (1+2)
Taxila	λ-cyhalothrin + profenofos	1:1	0.44	0.44	1.21	60.07	59.99	57.14
	λ-cyhalothrin + emamectin	1:1.1	0.23	0.26	0.67	60.07	61.66	63.33
	λ-cyhalothrin + lufenuron	1:1.4	0.22	0.32	0.84	60.07	64.44	59.04
	Profenofos + emamectin	1:1.2	0.38	0.46	1.19	59.99	61.66	55.23
	Profenofos + lufenuron	1:1.5	0.36	0.54	1.48	59.99	64.44	66.18
	Emamectin + lufenuron	1:1.2	0.34	0.40	1.33	61.66	64.44	53.80
Rawalpindi	λ-cyhalothrin + profenofos	1:1	0.46	0.46	0.97	47.21	53.33	66.66
	λ-cyhalothrin + emamectin	1:1.4	0.19	0.27	0.51	47.21	57.77	65.71
	λ-cyhalothrin + lufenuron	1:1.1	0.30	0.34	0.63	47.21	53.33	65.71
	Profenofos + emamectin	1:1.3	0.28	0.36	0.77	53.33	57.77	60.47
	Profenofos + lufenuron	1:1	0.36	0.36	0.74	53.33	53.33	63.80
	Emamectin + lufenuron	1:1.3	0.34	0.44	0.95	57.77	53.33	61.90

CI, Combination Index; %M, per cent mortality.

cyhalothrin with emamectin and lufenuron (Attique *et al*., 2006; Ahmad *et al*., 2009).

Variation is response to tested populations proved such trends existed for the insecticide mixtures. Bifenthrin, with emamectin proved synergistic indicated the possibility of other pyrethroids to increase the toxicity of new chemistry insecticides including emamectin when used in mixture against *P. xylostella* in field crops (Attique *et al*., 2006). Present results shows similar interaction of a pyrethroid and an organophosphate with either additive or antagonistic effect that might be due to selection of resistance alleles for these mechanisms in field populations (Ahmad, 2004; Ahmad *et al*., 2009). There existed variation in response to tested insecticides which might be due to variation in exposure to insecticide dose rate, frequency, location, crop and difference in insecticide choice for its control (Mohan and Gujar, 2003). Present expression of toxicity and mixture effects proved that even populations under different insecticide use stress can have different response where Rawalpindi strain showed more synergistic response as previously observed for different populations of *H. armigera* (Ahmad, 2004, Martin *et al*., 2003).

There existed non-significant variation for the mixing of insecticides either at LC_{50} ratios or their percent mortalities (Table II). This, however, differed in previously field population where synergistic response was more when a ratio of 1:10 instead of 1:1 (Attique *et al*., 2006; Martin *et al*., 2003). This suggests the identification of target sites of these insecticides in field populations of *P. xylostella* and proper utility of the insecticide mixtures in local pest management rather than wide application (Ahmad *et al*., 2009). Rotational use of insecticides belonging to different groups has been suggested area wide pest management of these pests.

ACKNOWLEDGEMENTS

We are grateful to the cauliflower growing farmers for their help in collection of *Plutella xylostella* field populations and to the Department to provide facilities to perform laboratory studies. Two anonymous reviewers are thankful for guiding to improve the manuscript. There exists no conflict of interests among authors.

REFERENCES

ABBOTT, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent*., **18**: 265-267.

AHMAD, M., 2004. Potentiation/antagonism of deltamethrin and cypermethrin with organophosphate insecticides in the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pestic. Biochem. Physiol*., **80**: 31-42.

AHMAD, M., SALEEM, M.A. AND SAYYED, H.A., 2009. Efficacy of different insecticide mixtures against pyrethroid- and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Manag. Sci*., **65**: 266-274.

ATTIQUE, M.N.R., KHALIQ, A. AND SAYYED, A.H., 2006. Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures?. *J. appl. Ent*., **130**: 122-127.

FINNEY, J.D., 1971. *Probit aalysis* (third edition). Cambridge University Press, UK.

KHALIQ, A., ATTIQUE, M.N.R. AND SAYYED, A. H., 2007. Evidence for resistance to pyrethroids and organophosphates in *Plutella xylostella* (Lepidoptera: Plutellidae) from Pakistan. *Bull. entomol. Res*., **97**: 1-11.

MALTAIS, P. M., NUCKLE, J. R. AND LEBLANC, P. V., 1998. Economic threshold for three lepidopterous larval pests of fresh-market cabbage in southeastern New Brunswick. *J. econ. Ent*., **91**: 699–707.

MARTIN, T., OCHOU, O.G., VAISSAYRE, M. AND FOURNIER, D., 2003. Organophosphorus insecticides synergize pyrethroids in the resistant strain of cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) from West Africa. *J. econ. Ent*., **96**: 468-474.

MOHAN, M. AND GUJAR, G. T., 2003. Local variation in susceptibility of the diamondback moth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. *Crop Prot*., **22**: 495-504.

MOTA-SANCHEZ, D., BILLS, P. S., WHALON, M. E. AND WHEELER, W.B., 2002. Arthropod resistance to pesticides: status and overview. In: *Pesticides in agriculture and the environment* (eds. W. Wheeler, B. Gainesville and M. Decker), pp. 241-272.

SAYYED, A. H., DENIS, J. AND WRIGHT, D. J., 2001. Fitness costs and stability of resistance to *Bacillus thuringiensis* in a field population of the diamondback moth *Plutella xylostella* L. *Ecol. Ent*., **26**: 502–508.

SAYYED, A.H., NAVEED, M., RAFIQUE, M. AND ARIF, M.J., 2012. Detection of insecticides resistance in *Spodoptera exigua* (Lepidoptera: Nocitudae) depends upon insect collection methods. *Pak. Entomol*., **34**: 7-15.

SHELTON, A. M., SANCES, F. V., HAWLEY, J. AND TANG, J.D., 2000. Assessment of insecticide resistance after the outbreak of diamondback moth (Lepidoptera: Plutellidae) in California in 1997. *J. econ. Ent*., **93**: 931–936.

TALEKAR, N.S. AND SHELTON, A. M., 1993. Biology, ecology and management of the diamondback Moth. *Annu. Rev. Ent.*, **38**: 275-301.

VERKERK, R.H.J. AND WRIGHT, D.J., 1996. Multitrophic interactions and management of the diamondback moth: a review. *Bull. entomol. Res*., **86**: 205-216.

ZERKOUNE, M.A., 2000. *Field evaluation of cauliflower varieties grown in southwest low desert soils*. The University of Arizona College of Agriculture, Vegetable Report index at http://ag.arizona.edu/pubs/crops/az1177/.

(Received 3 November 2012, revised 12 March 2013)