PYRETHROIDS AND NEW CHEMISTRY INSECTICIDES MIXTURES AGAINST *SPODOPTERA LITURA* (NOCTUIDAE: LEPIDOPTERA) UNDER LABORATORY CONDITIONS

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ABSTRACT

Most crops face pest situations where more than one insect pest become economic pest and require mixture of insecticide application for management. Presently two pyrethroids and three new chemistry insecticides in mixtures at their lethal concentrations were tested against second instar larvae of *Spodoptera litura*, under laboratory conditions using leaf dip method. LC_{50} of deltamethrin, bifenthrin, emamectin benzoate, chlorfluazuron and flubendamide were 619 and 100, 74.2 and 65.8, 0.08 and 0.06, 73.4 and 52.5, and 0.37 and 0.31 ul/ml, respectively after 48 and 72 hour exposure. Pyrethroids were least effective due to their high LC_{50} values as compared to new chemistry insecticides. Emamectin and flubendamide were more toxic with less LC_{50} values than chlorfluazuron at observed time periods. Mixtures of deltamethrin with chlorfluazuron, emamectin-benzoate and flubendamide were antagonistic. However, bifenthrin showed potentiation with emamectin and flubendamide which revealed that some pyrethroids can result in potentiating mixtures with new chemistry insecticides for *S. litura* control under multiple pest problem scenarios.
Keywords: *Spodoptera litura*, pyrethroids, new chemistries, potentiation

INTRODUCTION

Hazards and harmful effects of insecticides as chemical control especially the wide application of conventional insecticides necessitate the new chemistry insecticides which are more effective, safer for humans and much less toxic to our ecosystem (Korrat et al., 2012). These new chemistry insecticides are, however, more specific or specialist for particular insect pest management. To enhance crop productivity with multiple pest situations, more than one insecticide in mixtures are used having different chemical groups. These may also be used to manage resistant field population of a certain pest and delay the development of insecticide resistance (Attique et al., 2006; Ahmad et al., 2009). Such insecticide mixtures are assumed to improve the toxicity of each other and improve their effectiveness against the target insect pests.
Synthetic pyrethroids and organophosphate insecticides have intensively been used for effective control of *Spodoptera litura* and other agricultural insect pests. Resistance to conventional organophosphates, carbamates, pyrethroids and chlorinated hydrocarbons has already been observed to this pest (Ahmad et al., 2007a). Wide spread *S. litura* population recorded in South Asia was due to developed resistance against high usage of the insecticides (Armes et al., 1997; Kranthi et al., 2002). Its outbreak in the last decade destroyed the cotton crop in Pakistan and almost every available insecticide was used to manage its population. Resistance against conventional as well as new chemistry insecticides was observed for quinalphos, monocrotophos, lindane and endosulfon, benzene hexachloride, avermectins, spinosad, fipronil, indoxacarb and chitin synthesis inhibitors (Ahmad et al., 2008, Shankarganesh et al., 2012). More than 34 insecticides of different groups have been applied either singly or in mixtures to control lepidopteran pests including *Spodoptera sp.* in Pakistan (Saleem et al., 2008).
Indiscriminate use and ill practice of pesticide application without pest scouting provided favorable environment for the development of insecticide resistance in the cotton belt of Pakistan (Ahmad and Arif, 2010). To control the problem of multiple pest situations or when resistance is observed to other insecticides, different insecticide mixtures belonging to different insecticide classes may helps in enhancing the spectrum of their control. Sometime increasing efficacy is also recommended to control single pest. Several workers advocated the use of insecticide mixtures for resistance management (Ishaaya et

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al., 1985; Ascher et al., 1986; Tabashnik, 1989; Dittrich et al., 1990).

Spodoptera litura (Fabricius), major leaf feeder, is polyphagous with wide host range of more than one hundred plants (Ahmad et al., 2013; Rao et al*.,* 1993; Qin et al., 2004). These flourish under warm and humid field conditions of South Asian countries causing higher economic losses to crops at their blossoming and vegetative stages with 70 to 100% yield loss (Qin et al*.,* 2004; Ahmad et al*.,* 2007a,b). Major mechanism to control insect pests in cotton crop is chemical control with long range of insecticides with different mode of actions that has already resulted in multiple type of resistance in the field (Ahmad et al., 2008, 2009). Cross-resistance mechanism developed due to previous insecticides exposure reduced the effectiveness of newly introduced insecticides. This urged to find new possible potentiating mixtures against this leaf feeder for its effective control under field situations.

MATERIALS AND METHODS

This study was conducted in the Toxicology Laboratory, Department of Entomology at Pir Mehr Ali Shah, Arid Agriculture University Rawalpindi during 2012. The efficacy of pyrethroids and new chemistry insecticides at their LC_{50} ratios alone and in mixtures was tested against a field population of *S. litura.*

Test insects

Larvae of *S. litura* were collected randomly from a farmer field of cauliflower at Rawalpindi and reared in plastic petri-plates at a semi-synthetic wheat-germ based diet (Ahmad et al., 2007a). Pupae developed were collected on daily basis and kept in a separate plastic pot lines with tissue paper. Adults emerged were reared in a 4 kg transparent plastic box covered with muslin cloth and moist cotton and provided with 15% honey solution on alternate days. Towel papers strips were provided for egg laying and collected daily. Standard rearing conditions of 25±2°C, 60±10% relative humidity with 16:8 lights to dark ratio were followed.

Insecticides

Commercial formulations of deltamethrin (Decis Super 10.5 EC, Bayer Crop Science, Pakistan), bifenthrin (Talstar 10EC, FMC Pakistan), emamectin benzoate (Proclaim 1.9EC, Syngenta Pakistan), chlorfluazuron (chlorfluazuron 5EC, 4B Pesticides, Pakistan), flubendamide (Belt 48SC, Bayer Crop Science, Pakistan) were used to study their lethal effects against *S. litura* larvae. Stock solutions of these insecticides were prepared in 50 ml of distilled water with 5-6 serial solutions. For mixtures, stock solution was prepared by adding insecticides at their respective LC_{50} and further dilutions.

Bioassays

Toxicity of insecticides was assessed against 2nd instar larvae of *S. litura* using leaf-dip method. Petri-dishes of 5cm diameter were lined with moist filter paper. Fresh and non-treated castor leaves were cut into discs and dips in the stock solution for 5-10 seconds later air-dried on paper towel in a fume hood. Treated leaves with their adaxial surface downward were placed in petri-dishes and five early second instar larvae were released per petri dish with the help of a camel hair brush and covered with lid to avoid escape. Mortality as end point was observed after 24, 48 and 72 hours for individual insecticides and after 48 hours for mixtures.

Statistical analysis

The data observed was subjected to the statistical analysis using POLO-PC for their respective LC_{50}, LC_{90}, fiducial limits and slope ± SE based on mortality data at 5% probability level. Efficacy of insecticide mixtures was calculated by using the combination index (Ahmad et al., 2009).

RESULTS AND DISCUSSION

Efficacy of five single insecticides viz., deltamethrin, bifenthrin, chlorfluazuron, emamectin-benzoate and flubendamide were observed against 2nd instar larvae of *S. litura* field population collected from Rawalpindi and LC_{50} values observed at 48 hours interval were used for mixture preparation and observe their effects.

For the tested pyrethroids, the LC_{50}s and LC_{90}s of deltamethrin after 24, 48 and 72 hours were 345, 133, 100 and 2969, 619, 416 ppm, respectively whereas bifenthrin revealed LC_{50}s of 365, 74.2, 65.8 ppm and LC_{90}s of 4879, 899, 707 ppm after 24, 48 and 72 hours, respectively (Table 1). Insect growth inhibitor, chlorfluazuron showed LC_{50} of 266, 73.4 and

52.4 ppm and LC_{90}s of 2470, 447 and 250 ppm after 24, 48 and 72 hours. Emamectin with LC_{50}s of 0.87, 0.08 and 0.06 ppm and LC_{90}s of 16.8, 0.91 and 0.33 ppm after 24, 48 and 72 hours, respectively was the most toxic among tested insecticides. Ryanoreceptor affecting insecticide, flubendamide gave the LC_{50}s of 2.81, 0.37 and 0.31 ppm while LC_{90}s were 91.3, 2.77 and 2.11 ppm after 24, 48 and 72 hours, respectively (Table 1).

Mixture of deltamethrin with chlorfluazuron showed LC_{50}s of 304 and 86.9 ppm and LC_{90}s of 3814 and 1082 ppm after 24 and 48 hours, respectively and its combination index was 1.02 which showed antagonistic effects. Mixture of deltamethrin with emamectin showed the LC_{50}s values of 414 and 57.9 ppm and LC_{90}s of 7825 and 435 ppm after 24 and 48 hours, respectively with combination index of 1.05 with antagonistic effect. Deltamethrin with flubendamide showed LC_{50}s (375 and 117 ppm) and LC_{90}s (67517 and 12867 ppm) after 24 and 48 hours, respectively with combination index of 2.52 showing antagonism against *S. litura* second instar larval stage (Table 2).

The LC_{50}s of mixture of bifenthrin and chlorfluazuron after 24 and 48 hours were 574 and 205 ppm, respectively while LC_{90}s were 7641 and 12456 ppm, respectively with combination index of 4.71 having high antagonistic effect. Mixture of bifenthrin with emamectin showed LC_{50}s of 51.3 and 10.8 ppm and LC_{90}s of 1668 and 125 ppm after 24 and 48 hours, respectively. This mixture showed synergistic effects with combination index value of 0.314. Similarly, mixture of bifenthrin with flubendamide resulted in LC_{50}s of 90.2 and 15.9 ppm and LC_{90}s of 6049 and 413 ppm after 24 and 48 hours, respectively. The combination index of bifenthrin mixed with flubendamide was 0.47 showing synergistic effects (Table 2). Overall results revealed that mixtures of deltamethrin with chlorfluazuron, emamectin benzoate and flubendamide were antagonistic and bifenthrin with chlorfluazuron also showed antagonistic effect but it was synergistic with emamectin benzoate and flubendamide.

Pyrethroids have long been used for control of different lepidopteran insect pests with different levels under field crops to enhance crop yields (Srivastava et al., 1983; Wang et al*.*, 1994; Biradar et al., 2001) as deltamethrin been used in mixture with triazophos against cotton and vegetables insect pests. Similarly the most common pyrethroid, cypermethrin is still in use with profenofos and chlorpyrifos as commercial products. These have been in use for more than twenty years against different chewing insect pests including *Helicoverpa armigera*, *S. litura* and *Pectinophora gossypiella* and *Plutella xylostella* (Martin et al., 2003; Ahmad et al., 2009, Attique et al., 2006). Continuous exposure to these mixtures may have resulted in resistance development and decrease in effectiveness for these mixtures. Bifenthrin and deltamethrin have some potentiating response to insect pests like *H. armigera* and *S. litura* field populations when mixed with ethion (Ahmad, 2008, 2009; Ahmad et al., 2009). Their responses with new chemistry insecticides, however, not tested earlier but highlight possibilities of their possible synergistic results. Presently, only bifenthrin proved productive to be mixed with emamectin benzoate and flubendamide insecticides. Emamectin benzoate has also shown synergistic effect with bifenthrin for *P. xylostella* previously and with different insect confirms its possible use in mixture for different insect pests (Attique et al., 2006). This might be due to some variation in selection of these populations with variable insecticide exposure under field conditions.

Potentiation has been observed for chlorfluazuron with fenpropathrin and a new chemistry insecticide spinoteram against *S. littoralis* but present study against *S. litura* revealed its antagonistic effect. This might be due to increased ratios tested which stress to further test these mixtures at lower doses for their possible potentiation effect as observed previously (Abdel-Hafeez and Mohamed, 2009). These will help in economic use of these insecticides and hazard problems for the environment. Emamectin proved effective singly as well as in mixture with bifenthrin for low cost treatment to manage other important insects especially *H. armigera* and *P. xylostella* (Attique et al., 2006; Razaq et al., 2005). Mixtures that proved potentiating in present study focus for wise use of their application at the recommended dose rates. Further detailed studies of these tested insecticides at different combination rates can even provide possible potentiating ratios suggesting their intelligent and long term utilization whenever mixed together for low cost application and environment protection.

Table 1: Toxicity of single pyrethroids and new chemistry insecticides against second instars larvae of leaf worm, *Spodoptera litura* under laboratory conditions

Insecticides	Time	LC_{50} (FL at 95%)	LC_{90} (FL at 95%)	Slope ± SE	df	χ^2	CR
deltamethrin	24	345 (230-705)	2969(1199-23935)	1.37±0.28	3	0.75	396
	48	133 (101-178)	619(399-1321)	1.92±0.29	3	2.42	1662
	72	100 (75-130)	416(285-776)	2.08±0.31	3	1.35	1666
bifenthrin	24	365 (226-852)	4879(1662-50280)	1.14±0.22	4	0.47	419
	48	74 (48.5-106)	899(454-3293)	1.18±0.21	4	0.47	925
	72	65.8 (43.1-95.2)	707(379-2229)	1.24±0.21	4	1.07	1096
chlorfluazuron	24	266 (190-428)	2470(1190-8999)	1.32±0.19	4	1.16	306
	48	73.4 (56.1-94.7)	447(299-818)	1.63±0.19	4	1.86	918
	72	52.0 (40.6-65.9)	250(180-403)	1.89 ±0.22	4	2.99	867
emamectin	24	0.87(0.52-2.19)	16.8(5.04-218)	0.99 ±0.19	4	0.48	1.0
	48	0.08(0.05-0.11)	0.91(0.55-2.19)	1.22 ±0.18	4	0.84	1.0
	72	0.06(0.04-0.08)	0.33(0.24-0.55)	1.72 ±0.23	4	1.94	1.0
flubendamide	24	2.81(1.31-19.8)	91.4(14.9-18964)	0.85 ±0.22	4	0.19	3.2
	48	0.37(0.26-0.52)	2.77(1.62-6.91)	1.46 ±0.22	4	0.66	4.6
	72	0.31(0.23-0.43)	2.11(1.29-4.69)	1.54 ±0.22	4	0.65	5.2

CR = comparative ratio divided by the least value at each time interval for LC_{50} values

Table 2: Toxicity of mixtures of pyrethroids and new chemistry insecticides against second instars larvae of leaf worm, *Spodoptera litura* under laboratory conditions

Insecticide mixture	Time	LC_{50} (FL at 95%)	LC_{90} (FL at 95%)	Slope±SE	df	χ^2	CI
deltamethrin +	24	304 (173-954)	3814 (1139-64438)	1.16±0.24	4	1.96	-
chlorfluazuron	48	86.9 (46.5-249)	1082 (331-50755)	1.17±0.21	4	5.07	1.02
deltamethrin +	24	414 (204-2147)	7825 (1680-431134)	1.00±0.23	4	3.96	-
emamectin	48	57.9 (33.5-110.3)	435 (190-3525)	1.46±0.22	4	5.26	1.05
deltamethrin +	24	375 (136-27610)	67517 (3550-0.00)	0.57±0.19	4	1.63	-
flubendamide	48	117 (58.6-595)	12867(1519-43222580)	0.63±0.19	4	1.82	2.52
bifenthrin +	24	574 (314-2106)	7641 (2090-183523)	1.14±0.25	4	2.72	-
chlorfluazuron	48	205 (109-749)	12456 (2083-3357830)	0.72±0.19	4	3.10	4.70
bifenthrin +	24	51.3 (24-347)	1668 (275-302114)	0.85±0.21	4	2.97	-
emamectin	48	10.9 (7.46-17.5)	125 (57-568)	1.21±0.21	4	2.98	0.31
bifenthrin +	24	90.0 (31.7-3733)	6049 (484-129609861)	0.70±0.26	4	1.15	-
flubendamide	48	15.9 (9.67-37.3)	413 (115-9056)	0.91±0.19	4	0.51	0.47

CI = combination index at 48 hours interval for insecticide mixtures

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