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Evaluation of novel insecticides and their persistency against diamondback moth, *Plutella xylostella* Linn.

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Abstract
Toxicity and persistency of different novel insecticides with diversified mode of insecticidal action was determined on the basis median lethal concentration (LC50) values against third instar larvae of diamondback moth, *Plutella xylostella* under laboratory conditions. The median lethal concentration LC50 values (%) deduced for the test insecticides *viz.*, emamectin benzoate, chlorantraniliprole, flubendiamide, fipronil and chlorfenapyr were 0.0028, 0.0328, 0.0267, 0.0172 and 0.0219, respectively with emamectin benzoate to be most lethal, followed by fipronil, chlorfenapyr, flubendiamide and chlorantriniliprole. The persistency of the test insecticides from 24 hours after treatment based on PT values revealed that chlorantraniliprole possessed highest persistence toxicity (856.52) followed by flubendiamide (649.61) > chlorfenapyr (606.6) > fipronil (549.89) > emamectin benzoate (519.90).

Keywords: *Plutella xylostella*, emamectin benzoate, chlorantraniliprole, flubendiamide, fipronil, chlorfenapyr, persistency toxicity

Introduction
India owing to wide variability in climate and soil is the leading producer of diversified vegetable crops. A total of more than 50 varieties of vegetable crops are grown in India of which cruciferous crops are utmost important both in terms of nutritional and economic significance. Among all these cruciferous crops, cabbage and cauliflower occupy prime position in terms of yield. India ranks second in production of cauliflower and broccoli (36% of world production) and cabbage (13% of world production) [1]. Cabbage, *Brassica oleracea var. capitata* Linn. being a popular crucifer crop is extensively cultivated but the production is hampered due to vast insect pests among which diamondback moth, *Plutella xylostella* (Linn.), cabbage caterpillar *Pieris brassicae* (Linn.), cabbage semilooper, *Thysanoplusia orichalcea* Fabricius, tobacco caterpillar, *Spodoptera litura* (Fab.), cabbage leaf webber, *Crocodolomia binotalis* Zeller and cabbage headborer, *Hellula undalis* Fabricius) are the pests of major importance [2].

Diamondback moth (DBM) is the most serious pest of cabbage. Diamondback moth (*Plutella xylostella* Linnaeus) has retained its status as the most destructive member of the insect pest complex infesting crucifers [3]. The yield loss attributed by this pest varies from 31-100 per cent [4, 5]. DBM larvae are voracious defoliators with a innate potential to destroy the entire crop, if left uncontrolled. Ability to migrate and establish in new exotic areas, shorter life cycle coupled with high reproductive potential, year round availability and perpetuation on host plants are the few said causes of DBM menace. The control management strategies of diamondback moth globally with conventional insecticides often failed because of indiscriminate, irrational use of insecticides at higher doses that accentuated DBM problem due to resistance rift and extermination of natural enemies of this pest with subsidiary resurgence and insecticide residue setback [6-9]. Virtually, resistance to as many as > 82 insecticidal compounds, encompassing major groups of insecticides has been documented against DBM in Arthropod Pesticide Resistance Database [10]. The tandem intervention of Bacillus thuringiensis commercial formulations used for the management of DBM has also got succumbed at field level. The present day scenario of insecticide usage has transposed to entities with unique mode of action, usage at low doses, easily degradable with no detrimental effect to the environment and biological systems. The novel insecticide molecules would be a key for designing management strategy in a much better way for sustained and

economically feasible production and would translate into attainment of higher production and productivity in cabbage and cauliflower in a cost-effective manner. Hence the present study was undertaken to deduce the median lethal concentration coupled with a persistent nature of novel insecticides possessing diversified mode of action against larvae of DBM infesting cabbage.

Material and Methods

Mass rearing of diamondback moth

The larvae and pupae of DBM were collected from infested cabbage fields in Bahadhurguda Village, Shamshabad Mandal of Ranga Reddy District. The larvae and pupae collected from the infested cabbage fields were reared on insecticidal free cabbage leaves maintained at 25±2 °C,
70-75 per cent relative humidity and D: L 14:10 hrs up to the pupal stage to establish a laboratory strain. The pupae were placed in egg laying cages (30 x 30 x 30 cm). The *P. xylostella* adults after emerging from pupa were provided with 10 per cent honey solution and mustard seedlings (4-5 cm height) for oviposition. After hatching, young larvae fed on the mustard leaves by mining and then larvae were transferred to the insecticidal free fresh cabbage leaves. Larvae after attaining third instar were used for bioassay.

Test Insecticides: Commercial formulations of five novel insecticides categorized to different classes *i.e.*, emamectin benzoate 5SG @ 0.45 g l-1 (Proclaim), Syngenta Crop Science Ltd, Mumbai; chlorantraniliprole 18.5SC @ 0.3 ml l-1 (Coragen), Du-Pont India Ltd, Gurgaon; flubendiamide 39.35EC @ 0.2 ml l-1 (Fame) Bayer Crop Science Ltd, Mumbai; fipronil 5SC @
0.2 ml l-1 (Regent) Aventis Crop Science Ltd., Mumbai and chlorfenapyr 10 SC @ 1.5 ml l-1 (Intrepid) BASF India Ltd, Mumbai were evaluated for toxicity and persistence studies against the larvae of DBM.

Larval bioassay

Leaf dip bioassay was used for assessing the toxicity of individual insecticide. Stock solutions of test insecticides were prepared based on active ingredients. Serial dilutions from stock solutions of each individual insecticide were carried out to makeup broad concentrations that yielded 20-80 per cent larval mortality. Broad range concentrations of the test insecticides were lowered to narrow range concentrations to deduce the median lethal concentration (LC50) for each test insecticide. Leaf discs of size 3-4" in diameter were used in the bioassay. Cabbage leaf discs were dipped in water (control) or test insecticide solutions for about 20 sec and then air dried prior to the exposure of leaf discs for feeding by the larvae. Each insecticidal treatment consisted of 10-15 third instar larvae and replicated four times. Control treatment consisted of leaf discs dipped in distilled water. Larval mortality was observed for 24 and 48 hours after treatment.

Persistent toxicity studies

For persistent toxicity studies cabbage plants (var. Gayathri) were sprayed with novel insecticide molecules at field recommended dosage post 45 days after transplanting while control treatment received distilled water spray. The treated leaves were tagged in order to differentiate from newly grown leaves. The insecticide treated leaves and cabbage leaves from control treatment were subjected to larval feeding till 20DAS (days after spray) in the laboratory to evaluate the most persistent insecticide among the five test insecticides.

Bioassay for persistency toxicity

The insecticidal treated and insecticidal free cabbage leaves (control) were subjected to feeding with third instar larvae of *P. xylostella*. All the treatments were replicated four times and each replication consisted of 10 larvae. The feeding of the insecticidal treated and insecticidal free cabbage leaves (control) was done with a fresh larval batch every 24hours and was extended up to 20 days to enumerate the persistency of the test insecticide. The observations pertaining to larval mortality were recorded at regular interval *i.e.,* at 24 and 48 hours after treatment.

Data analysis

The mortality data was observed at 48HAT and corrected according to the Abbott's formula [13]. LC_{50} for individual insecticides and fiducial limits were determined according to probit analysis SPSS soft by Finney formula [14] by using SPSS (v16) software. Bioassay was repeated for the insecticides wherever the control mortality exceeds 20 per cent. The values of the relative toxicity of the insecticides were calculated by the following formula

$$\text{Relative Toxicity} = \frac{LC_{50} \text{ of potent insecticide}}{LC_{50} \text{ of least toxic insecticide}}$$

Data on mortality were subjected to modified Abbott's formula for correction whenever required. Based on the larval mortality data, for determining the persistent toxicity (PT) for each test insecticide was deduced as the product of average residual toxicity (T) and the period (P) for which the toxicity persisted. The persistent (PT) values were calculated as given below [15].

$$\text{Average residual Toxicity} = \frac{\text{Sum of corrected mortalities at different intervals}}{\text{Number of observations}}$$

Persistent toxicity = Average residual toxicity x Period for which toxicity was observed.

Results and Discussion

Bioassay studies with third instar larvae of *P. xylostella* in deducing the median lethal concentration (LC50) of all the test insecticides were emamectin benzoate (0.0027%), chlorantraniliprole (0.0319%), flubendiamide (0.025%), fipronil (0.0159%) and chlorfenapyr (0.0210%) (Table1). The present findings revealed that emamectin benzoate exerted more potency in causing larval mortality. The order of toxicity in the present study was emamectin benzoate> fipronil> chlorfenapyr> flubendiamide> chlorantraniliprole. Relative toxicity of the test insecticides were, emamectin benzoate 11.81 folds-, fipronil 2.01 folds-, chlorfenapyr 1.51 folds- and flubendiamide 1.28 folds- more toxic than chlorantaniliprole.
The results of present investigation are in accordance with studies conducted in evaluating different insecticides against 3rd instar larvae of *P. xylostella* following leaf-dip bioassay and confined that emamectin benzoate (LC50 0.0002%) was the most toxic compound in comparison to other conventional insecticides evaluated [16]. Likewise, leaf-dip bioassay in deducing LC50 values of emamectin benzoate 20SC, chlorfenapyr 10SC and fipronil 5SC against third instar larvae

of *P. xylostella* were reported as 0.070, 0.111 and 0.136 µg/L, respectively [17]. The present findings of emmamectin benzoate in being more toxic has been reported elsewhere against different insect pests studied *P. xylostella, S. litura* [18, 19]. The potency of emmamectin benzoate in causing mortality with LC90 values ranging from 2 to 16 mg/ml for various lepidopteran pests has been reported by Janson and Dybas [20]. Similarly the effectiveness of chlorantraniliprole, flubendaimide and fipronil were also been reported against *P. xylostella* and *S. litura* [21, 22]. Its quiet evident from the present investigation potential of the novel insecticides against *P. xylostella* and their integration in insect pest management programme will certainly reduce the selection pressure in insect and thus help in increasing the useful life of insecticide and delaying the development of resistance.

Table 1: Toxicity of different insecticides to third instar larvae of *P. xylostella*

Insecticides	Heterogeneity	LC50 (%)	Fiducial limits (%)	Regression equation	Relative toxicity
Emamectin benzoate 5 SG	$\chi2$ = 0.998	0.0028	(0.0019-0.0039)	Y=11.221+ 2.435x	11.71
Chlorantraniliprole 18.5 SC	$\chi2$ = 1.000	0.0328	(0.0186- 0.0577)	Y= 7.220+ 1.495x	1.00
Flubendiamide 39.35 EC	$\chi2$ = 0.996	0.0267	(0.0178-0.0400)	Y= 8.260+2.072x	1.23
Fipronil 5SC	$\chi2$ = 0.998	0.0172	(0.0098-0.0303)	Y=7.642+ 1.497x	1.91
Chlorfenapyr 10 SC	$\chi2$ = 1.000	0.0219	(0.0143-0.0338)	Y= 8.220+ 1.942x	1.50

The data on persistent toxicity of different treatments against *P. xylostella* revealed significantly higher mortalities during the first few days after application of the treatments, which further declined as the period advanced. Though more than 50 per cent larval mortality was recorded 10 DAS and 7 DAS considerable larval mortality and persistency expression of chlorantraniliprole and flubendiamide lasted for 14 DAS and 13 DAS respectively. Chlorfenapyr recorded more than 50 per cent mortality at 7 DAS and showed a considerable mortality at 12 DAS. Emamectin benzoate and fipronil gave more than 50 per cent mortality at 6 DAS and showed a considerable mortality at 10 DAS and 11 DAS respectively.

The persistent toxicity values of emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide obtained in the study were 519.90, 856.52, 606.6, 549.89 and 49.61, respectively. The order of efficacy of the test insecticides in the present by taking the PT values into consideration, are chlorantraniliprole > flubendiamide > chlorfenapyr > fipronil > emamectin benzoate. The results revealed that chlorantraniliprole had a maximum period of toxicity with RPT (1.65) followed by flubendiamide (1.20), chlorfenapyr (1.17), fipronil (1.06) and emamectin benzoate (1.00) (Table 2).

The present study is in accordance with the long lasting effect of chlorantraniliprole and causing mortality against *P. xylostella* on radish up to 21 DAS [23]. The long-lasting residual efficacy of chlorantraniliprole is also been reported elsewhere against oblique banded leaf roller *Choristoneura rosaceana* (Harris) upto 21 DAT and *Chilo suppressalis* in rice upto 36 DAS [24, 25]. The persistency of chlorfenapyr and flubendiamide in the present study exerted for 12-13 DAS studies elsewhere carried out against *P. rapae* and *P. xylostella* revealed 91.96 and 95.73 per cent larval mortality at 7 DAT, respectively with chlorfenapyr 10 SC [26]. On contrary, the persistent toxicity of chlorfenapyr 10% F and emamectin benzoate 1% EC with persistence up to 5-10 days was reported against *P. xylostella* [27]. In studies pertaining to the efficacy of flubendiamide 480 SC against *S. litura* and *H. armigera* the long-lasting residual action and persistency in suppressing larval populations was reported up to 10 DAT [28]. Likewise, flubendiamide 480 SC @ 18 and 24 g a.i.ha-1 persisted up to 15 days after spraying for control of *P. xylostella* [29]. In corroboration with the present study the persistent toxicity of emamectin benzoate, chlorfenapyr and fipronil against *P. xylostella* on cabbage was found effective up to 7- 14 days [30]. On contrary spray concentration of emamectin benzoate @ 25 mg a.i.l-1 in cotton field resulted in 90% suppression of *H. armigera* larvae up to 28 days after treatment, likewise 90% mortality of the Egyptian cotton leaf worm, *S. littoralis* 3 days only [12].

Table 2: Comparision of persistent toxicity of insecticides based on field dosages against *P. xylostella* on cabbage

Insecticide	Dosage (g/L)	Corrected percentage mortality (%)															T	P	PT	RPT	ORPT
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
Emamectin benzoate 5 SG	0.45	100	83.33	76.66	60	56.61	50	43.33	33.33	13.33	3.33	0					51.99	10	519.9	1	5
Chlorantraniliprole 18.5SC	0.3	100	96.66	93.33	90	83.33	76.66	70	66.66	56.66	43.33	40	23.33	13.33	3.33	0	61.18	14	856.52	1.65	1
Chlorfenpyr 10SC	1.5	100	93.33	83.33	70	66.66	53.33	40	33.33	26.66	20	13.33	6.66	0			50.55	12	606.6	1.17	3
Fipronil 5SC	0.2	96.66	86.66	73.33	63.33	56.61	50	46.66	36.66	23.33	10	6.66	0				49.99	11	549.89	1.06	4
Flubendimide 39.35 EC	0.2	100	86.66	80	76.66	70	56.66	50	43.33	40	23.33	13.33	6.66	3	0		49.97	13	649.61	1.25	2

DAS = Days after spraying; P = Total period; ART = Average residual toxicity; PT= Index of persistent toxicity; RPT = Relative persistent toxicity; ORPT = Order of relative persistent toxicity

Conclusion

The present investigation proves the insight with regards to the potential of the novel insecticides in compromising the menace from *P. xylostella* and their durable persistency ability. The deployment of the novel insecticides in integrated pest management of cabbage and cauliflower agro ecosystem as an insecticidal rotation pattern will certainly reduce the selection pressure by the pests coupled with increasing useful life of insecticide as well as delaying the development of resistance.

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