



## Baseline Susceptibility of Diamondback Moth *Plutella xylostella* L (Lepidoptera:Plutellidae) to chlorantraniliprole 18.5 SC in Tamil Nadu

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The baseline susceptibility of diamondback moth, *Plutella xylostella* L. to chlorantraniliprole 18.5 SC was studied in the Insecticide Resistance Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. The median LC<sub>50</sub> of chlorantraniliprole against diamondback moth larvae for F<sub>1</sub> population was 20.06 ppm and LC<sub>95</sub> was 835.68 ppm; whereas, the LC<sub>50</sub> of F<sub>25</sub> population was 0.91 ppm and LC<sub>95</sub> was 23.11 ppm. The susceptibility increased up to F<sub>22</sub> population of diamondback moth without exposure to insecticides. The susceptibility index (SI) after F<sub>25</sub> generation over F<sub>1</sub> generation was 22.02 and 36.15 based on LC<sub>50</sub> and LC<sub>95</sub>, respectively. The rate of resistance decline (R) was -0.0505 and the number of generations required for a 10 fold decrease of LC<sub>50</sub> was 20. Based on LC<sub>95</sub> of F<sub>25</sub> population, a tentative discriminating dose (DD) was fixed as 23.00 ppm. *P. xylostella* resistance in three major cauliflower and cabbage growing areas i.e., Coimbatore, Oddanchatram and Udagamandalam was monitored. Insecticides tested viz., chlorantraniliprole 18.5 SC at 23 ppm, spinosad 2.5 SC at 12 ppm, emamectin benzoate 5 SG at 2 ppm and quinalphos 25 EC at 3 ppm recorded high resistance levels during fourth week of January, 2013 and third week of February, 2013. Among the insecticides tested, chlorantraniliprole 18.5 SC recorded low level of resistance with Udagamandalam (53.83 %), Coimbatore (58.83 %) and Oddanchatram (64.00 %) population, whereas it was high in the case of emamectin benzoate, spinosad and quinalphos in the three regions.

**Key words :** Chlorantraniliprole, *Plutella xylostella*, Resistance, Monitoring

Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the most destructive insect pests of cruciferous vegetables around the world. Its rapid generation time, high proliferation and particularly extensive selection pressure in field, have resulted in *P. xylostella* evolving resistance to various types of traditional insecticides. In India, it is one of the major constraints in the profitable cultivation of cole crops. Krishna Kumar *et al.* (1984) noted 52 per cent loss in marketable cabbage yield due to this pest. In the last 50 years *P. xylostella* has become one of the most difficult insects to control, primarily because of resistance evolution to every class of insecticide used extensively against it (Shelton *et al.*, 2000 ; Sarfraz and Keddie, 2005). The extensive use of number of commercial insecticides hassled the development of resistance in this insect across South East Asia (Georghiou, 1990). Zhao *et al.* (2002) reported that some populations of *P. xylostella* have developed resistance to newer active chemicals, including spinosad, avermectins (abamectin and emamectin benzoate), indoxacarb and the biopesticide *Bacillus thuringiensis* Cry toxins in the

field. To date, the pest has developed resistance to 81 insecticides (Anonymous, 2009) and has become one of the most difficult pests to control in cruciferous vegetables.

Chlorantraniliprole is the first pesticide from the anthranilic diamides and it was found to provide broad spectrum activity within Lepidoptera (Temple *et al.*, 2009). It acts as selective agonist for ryanodine receptors in Lepidopteran insects. It causes unregulated Ca<sub>2+</sub> release from intracellular calcium stores, which results in the insect's ability to regulate muscle function being impaired, generating poisoning symptoms that include: rapid feeding cessation, lethargy, muscle paralysis, and ultimately insect death (Lahm *et al.*, 2005; Sattelle *et al.*, 2008). It was relatively harmless to beneficial arthropods and has not been found to exhibit cross resistance with existing insecticides (Lahm *et al.*, 2009). These favourable characteristics was found useful as an additional management tool to control *P. xylostella* and a good fit for integrated pest management (IPM) since its introduction in India during 2008.

However, repeated applications of chlorantraniliprole at higher dose on cauliflower and cabbage

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against *P.xylostella* might result in resistance development in the high intensity production area. The present study was undertaken to elucidate information on baseline susceptibility of *P. xylostella* to chlorantraniliprole 18.5 SC and resistance status of chlorantraniliprole in cauliflower and cabbage growing areas of the Tamil Nadu.

### Materials and Methods

Laboratory studies were carried out in the Insecticide Resistance Laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. The larvae collected from cauliflower and cabbage growing areas of Coimbatore (Thondamuttur) was reared up to 25 generations without exposure of insecticides using cauliflower leaves maintained in pot culture. Mass culturing of *P. xylostella* was carried out by following the method described by Liu and Sun (1984) with little modifications. Mustard seedlings were used for egg collection in oviposition cage and same cage was used for larval rearing. Raising of mustard seedling and rearing of *P.xylostella* was done under the lab conditions at 12:12 (L:D) and at prevailing room temperature  $28 \pm 2^\circ \text{C}$ . The third instar larvae ( $0.5 \pm 0.1 \text{ cm}$ ;  $1.75 \pm 0.25 \text{ mg}$ ) were collected on tenth day after egg laying for conducting bioassay.

The dilutions required for bioassay were prepared from the formulated product of known purity of insecticide using distilled water. The insecticide formulation used was chlorantraniliprole 18.5 SC (Coragen 18.5 SC M/s E.I. DuPont India Pvt. Ltd, Gurgaon, Haryana.)

The leaf disc bioassay method originally described by Hirano (1979) further supplemented by Tabashnik and Cushing (1987) was adopted. The leaf discs of 6 cm diameter were cut covering either side of the midrib. These leaf discs were dipped in test concentrations for about a min, and then removed; excess fluid drained and air dried by hanging to a thread for about one hour and transferred to a small round plastic container of 7 cm height and 6 cm dia. over a moistened filter paper. The leaf discs were placed slantingly to rest on the sides of container so that larvae can move on either side. Ten larvae, starved for 12 h, were transferred to each disc and the container was covered with lid provided with holes for aeration which constituted one replication. Each treatment was replicated three times and observations on mortality of the larvae were taken at 24h after treatment.

For generation of baseline data, the insects were reared without exposure to insecticides and cultured continuously without selection pressure throughout  $F_1$  to  $F_{25}$  generations. Bioassays were conducted to construct LCPM lines for a susceptible population. Based on lethal concentrations obtained for the test insecticide a tentative discriminating dose was fixed

based on  $LC_{95}$  value of the  $F_{25}$  generation. The susceptible indices (SI), rate of resistance decline (R) and the number of generations required for ten-fold decrease in the  $LC_{50}$  value (G) were calculated (Regupathy and Dhamu, 2001).

Monitoring studies was done at weekly interval from January 2013 to March 2013, in major cauliflower and cabbage growing areas of Tamil Nadu viz., Coimbatore, Oddanchatram and Udagramandalam. Frequently used insecticides in cauliflower and cabbage viz., chlorantraniliprole 18.5 SC (Coragen®), spinosad 2.5 SC (Success®), emamectin benzoate 5 SG (Proclaim®), and quinalphos 25 EC (Ekalux®) were selected for resistance monitoring. Test insecticides were used at discriminating concentrations viz., chlorantraniliprole 23 ppm (obtained from base line data) spinosad 12 ppm, emamectin benzoate 2 ppm (Lavanya *et al.*, 2010) and quinalphos 3 ppm (Chandrasekaran and Regupathy, 1996). The larvae collected at weekly intervals were reared upto  $F_1$  generation and third instar ( $1.83 \pm 0.28 \text{ mg}$ ) larvae were used for the bioassay. Leaf disc method was followed for bioassay with each treatment comprising of 60 larvae. The mortality was recorded at 24 h after treatment and resistance percentage was calculated by using Abbott's formula (Abbott, 1925).

### Results and Discussion

The  $LC_{50}$  and  $LC_{95}$  values of chlorantraniliprole 18.5 SC for 25 generations against *P.xylostella* are presented in Table 1. The  $LC_{50}$  for  $F_1$  population was 20.06 ppm and  $LC_{95}$  was 835.68 ppm. The susceptibility was found increasing over generations. In terms of  $LC_{50}$ , susceptibility increased up to  $F_{22}$  generation and got stabilized from  $F_{23}$  to  $F_{25}$  generations (0.91 ppm). The susceptibility in terms of  $LC_{95}$  also increased up to  $F_{23}$  generation (23.26 ppm) and got stabilised in  $F_{24}$  and  $F_{25}$  generations (23.11 ppm).

The susceptibility indices based on  $LC_{50}$  and  $LC_{95}$  were 22.02 and 36.15, respectively after  $F_{25}$  generations. (Table.2). The number of generations required for 10 fold decrease in  $LC_{50}$  was 20 generations. The rate of resistance decline (R) was -0.0505. The computed  $LC_{50}$  and  $LC_{95}$  values indicated that the susceptibility gradually increased with succeeding generations from  $F_1$  to  $F_{25}$  (20.06 ppm to 0.91 ppm) and similarly  $LC_{95}$  values from  $F_1$  to  $F_{25}$  decreased from 835.68 ppm to 23.11 ppm. A tentative discriminating dose (DD) 23 ppm was arrived based on  $LC_{95}$  value of 23.11 ppm computed for  $F_{25}$  generation and it recorded 99-100 per cent mortality of the susceptible population. Similar studies made by Wang *et al.* (2010) reported that the variation among 16 field populations was low (fivefold) with chlorantraniliprole and  $LC_{50}$  values ranged from 0.221 to 1.104 mg/litre. The discriminating dose of 23 ppm computed in the

**Table 1. Baseline susceptibility of *Plutella xylostella* L. to chlorantraniliprole 18.5 SC by leaf disc method**

Generation	X <sup>2</sup>	Slope(Y=a+bx)	LC <sub>50</sub> Fiducial limits			LC <sub>95</sub> Fiducial limits		
			LL	UL	LL	UL	LL	UL
G1	3.1292	Y= 3.8183+ 0.9673x	20.0623	11.4859	35.0428	835.3863	156.6569	4457.9376
G2	2.5820	Y= 3.7943+ 1.0069x	15.7504	9.6827	25.6206	677.2772	142.8088	3212.0178
G3	3.6407	Y =3.9035+ 0.9835x	13.0268	8.0695	21.0297	612.7997	128.0867	2931.7900
G4	3.4771	Y =3.9226+ 1.0367x	10.9429	7.0129	17.0752	422.3502	105.1708	1696.0951
G5	3.7211	Y =4.1478+ 0.9850x	7.3301	4.6251	11.6172	342.8161	81.8475	1435.8755
G6	3.1383	Y =4.3259+ 0.9704x	4.9499	3.0518	8.0285	245.2993	62.9668	955.6102
G7	3.0694	Y =4.4657+ 0.9641x	3.6959	2.1675	6.3022	206.8826	52.1341	820.9678
G8	1.0806	Y =4.5954+ 0.9320x	2.7169	1.6713	4.4166	158.1009	35.2729	708.6411
G9	1.5183	Y =4.6266+ 0.9674x	2.4322	1.5076	3.9237	121.9875	30.3789	489.8447
G10	2.5891	Y =4.6153+ 0.0273x	2.3715	1.5035	3.7404	95.2052	26.8525	337.5485
G11	3.902	Y= 4.7045+ 1.0491x	1.9124	1.1949	3.0610	70.7185	20.3714	245.4961
G12	1.1696	Y = 4.7947+1.0204x	1.5892	1.0242	2.4659	65.0428	17.1799	246.2511
G13	1.0241	Y = 4.8274+1.1045x	1.4329	0.9499	2.1614	44.1994	14.1537	138.0273
G14	1.7806	Y = 4.8381+1.1812x	1.3732	0.8721	2.1623	33.8320	13.3661	85.6349
G15	2.4457	Y = 4.8476+1.2080x	1.3370	0.8482	2.1074	30.7434	11.8982	79.4364
G16	1.9755	Y = 4.8644+1.2139x	1.2933	0.8178	2.0450	29.2871	11.5587	74.2069
G17	4.1302	Y = 4.8885+1.2079x	1.2366	0.7784	1.9644	28.4453	11.3020	71.5921
G18	3.0674	Y = 4.9339+1.1869x	1.1366	0.6988	1.8484	27.6336	10.8996	70.0591
G19	4.379	Y = 4.9393+1.2124x	1.1220	0.6940	1.8139	25.5086	10.1038	64.4006
G20	2.7629	Y = 4.9857+1.2035x	1.0275	0.6884	1.5336	23.9045	8.9006	64.2009
G21	2.6611	Y = 5.0042+1.9469x	0.9918	0.6660	1.4891	23.6205	8.7614	63.6798
G22	2.2102	Y = 5.0296+1.1795x	0.9438	0.6256	1.4239	23.4132	8.6318	63.5068
G23	4.8641	Y = 5.0447+1.1708x	0.9158	0.6019	1.3935	23.2656	8.2367	65.7166
G24	1.7261	Y = 5.0466+1.1718x	0.9125	0.6011	1.3852	23.1170	8.4991	62.8769
G25	2.0801	Y = 5.04736+1.1709x	0.9110	0.6018	1.3719	23.1151	8.5246	62.7978

present investigation was slightly higher than the 15 ppm reported by Wang *et al.* (2010) which would be due to the variation in the susceptibility of population tested. Silva *et al.* (2012) reported that *P. xylostella* populations of Brazil were highly susceptible to chlorantraniliprole (LC<sub>50</sub> values from 0.015 to 0.056 mg a.i./l of water) and a discriminating

concentration of 0.3 mg a.i./l was obtained and used for evaluating other populations causing 100 per cent mortality. The discriminating dose of 23 ppm obtained from the present base line data was used for detection of chlorantraniliprole resistance in field populations of Coimbatore, Oddanchatram and Udhagamandalam of Tamil Nadu.

**Table 2. Susceptibility index of *P. xylostella* to chlorantraniliprole 18.5 SC**

Generation	LC <sub>50</sub>	LC <sub>95</sub>	Susceptibility index		Rate of resistance decline		Slope	increase/ decrease in slope function (%)
			LC <sub>50</sub>	LC <sub>95</sub>	R	G		
1	20.0623	835.6863	22.0223	36.1533	-0.0505	19.8213	0.9676	21.0107
5	7.3301	342.8161	8.0462	14.8308	-0.0452	22.1023	0.9850	18.8731
10	2.3715	95.2052	2.0992	4.1187	-0.0277	36.1616	1.0273	13.9784
15	1.3370	30.7434	1.4196	1.3300	-0.0166	60.2784	1.2080	-3.0712
20	1.0275	23.9045	1.0887	1.0341	-0.0103	96.9514	1.2035	-2.7088
25	0.9110	23.1151	1.0000	1.0000	-	-	1.1709	-

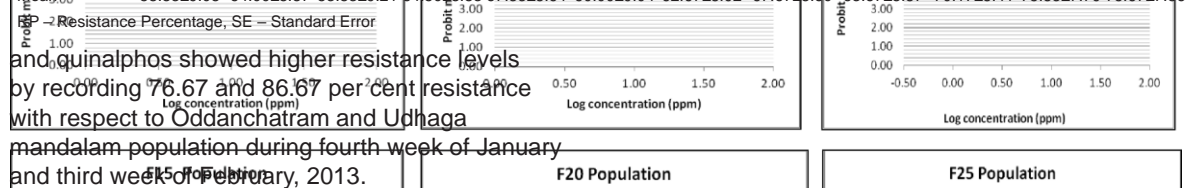
Results of weekly resistance monitoring of diamondback moth populations in three major cauliflower and cabbage growing areas Coimbatore, Oddanchatram and Udhagamandalam of Tamil Nadu, from January, 2013 to March, 2013 are presented in Table 3.

The per cent resistance recorded with chlorantraniliprole 18.5 SC ranged from 50.00 to 66.67, 56.67 to 70.00 and 46.67 to 63.33 with Coimbatore, Oddanchatram and Udhagamandalam populations respectively. Among the three regions,

higher percent resistance was recorded (70.00) with Oddanchatram population followed by Coimbatore (66.67) and Udhagamandalam (63.33) during fourth week of January, 2013. Spinosad 2.5 SC recorded 56.67 to 73.33, 60.00 to 76.67 and 53.33 to 70.00 per cent resistance with Coimbatore, Oddanchatram and Udhagamandalam populations, respectively. Oddanchatram population recorded 76.67 per cent resistance followed by Coimbatore and Udhagamandalam (73.33 and 70.00) during fourth week of January, 2013. Emamectin benzoate

**Table 3. Resistance in *P. xylostella* to insecticides in Tamil Nadu (RP±SE)**

Month / Week	Chlorantraniliprole			Spinosad			Emamectin benzoate			Quinalphos		
	CBE	ODJ	OTY	CBE	ODJ	OTY	CBE	ODJ	OTY	CBE	ODJ	OTY
I Jan 1-5	50.00±9.28	56.67±9.20	50.00±9.28	56.67±9.20	60.00±9.09	56.67±9.20	60.00±9.09	63.33±8.94	61.67±9.02	63.33±8.94	70.00±8.51	68.33±8.64
II Jan 6-12	56.67±9.20	60.00±9.09	51.67±9.28	60.00±9.09	60.00±9.09	58.33±9.16	63.33±8.95	66.67±8.75	61.67±9.03	70.00±8.50	73.33±8.21	70.00±8.50
III Jan 13-19	60.00±9.09	66.67±8.75	56.67±9.20	66.67±8.75	63.33±8.94	63.33±8.95	70.00±8.51	73.33±8.21	70.00±8.51	73.33±8.21	80.00±7.43	73.33±8.21
IV Jan 20-26	66.67±8.75	70.00±8.51	63.33±8.95	73.33±8.21	76.67±7.85	70.00±8.51	73.33±8.21	76.67±7.85	73.33±8.21	80.00±7.43	83.33±6.9	76.67±7.85
V Jan 27-Feb 2	53.33±9.26	63.33±8.94	50.00±9.28	70.00±8.51	66.67±8.75	61.67±9.02	56.67±9.20	66.67±8.75	66.67±8.75	63.33±8.94	66.67±8.75	70.00±8.50
VI Feb 3-9	60.00±9.10	63.33±8.94	53.33±9.26	61.67±9.03	66.67±8.75	53.33±9.26	58.33±9.15	63.33±8.94	56.67±9.20	71.67±8.37	75.00±8.04	76.67±7.85
VII Feb 10-16	66.67±8.75	70.00±8.50	53.33±9.26	63.33±8.64	73.33±8.21	66.67±8.75	65.00±8.86	71.67±8.34	66.67±8.75	76.67±7.85	83.33±6.92	86.67±6.31
VIII Feb 17-23	63.33±8.95	63.33±8.63	53.33±9.26	66.67±8.75	71.67±8.36	60.00±9.10	63.33±8.95	70.00±8.51	63.33±8.94	73.33±8.21	73.33±7.65	83.33±6.92
IX Feb 24-Mar 2	58.33±9.14	63.33±8.94	50.00±9.28	63.33±8.95	68.33±8.64	56.67±9.20	60.00±9.10	65.00±8.86	60.00±9.10	68.33±8.64	73.33±8.21	78.33±7.65
X Mar 3-9	53.33±9.26	58.33±9.15	46.67±9.26	58.33±9.15	63.33±8.94	53.33±9.26	56.67±9.20	60.00±9.10	56.67±9.20	61.67±9.02	65.00±8.86	73.33±8.21
Mean	58.83±9.08	64.00±8.87	53.83±9.21	64.50±8.83	67.33±8.64	60.00±9.04	62.67±8.92	67.67±8.60	63.67±8.87	70.17±8.41	75.83±7.79	75.67±7.86



and quinalphos showed higher resistance levels by recording 76.67 and 86.67 per cent resistance with respect to Oddanchatram and Udhagamandalam population during fourth week of January and third week of February, 2013.

**Table 4. Comparative levels of resistance in *P. xylostella* to insecticides Coimbatore, Oddanchatram and Udhagamandalam**

Insecticide	Coimbatore RP ±SE	Oddanchatram RP ±SE	Udhagamandalam RP ±SE
Chlorantraniliprole 18.5 SC	58.83±9.08	64.00±8.87	53.83±9.21
Spinosad 2.5 SC	64.50±8.83	67.33±8.64	60.00±9.04
Emamectin benzoate 5 SG	62.67±8.92	67.67±8.60	63.67±8.87
Quinalphos 25 EC	70.17±8.41	75.83±7.79	75.67±7.86

RP – Resistance Percentage, SE – Standard Error

test insecticides, chlorantraniliprole recorded the least level of resistance with Coimbatore and Udhagamandalam populations (58.83 and 53.83 %). Higher mean per cent resistance (64.00) observed in Oddanchatram might be due to use of chlorantraniliprole three times in a season at higher doses (0.15 to 0.20 ml/litre) starting from vegetative to harvesting stage of the crop. The present study fall in line with Wang and Wu (2012), who stated that six field populations from southern China (Guangdong Province) showed higher LC<sub>50</sub> values 2.6, 12, 18, 81, 140, and 2,000 fold than LC<sub>50</sub> of Roth

(Susceptible strain), which would be due to intensive use and misuse of chlorantraniliprole. The present line of work is also in agreement with Lavanya *et al.*(2010), who had reported that emamectin benzoate (2 ppm) and spinosad (12 ppm) recorded 5.81 to 15.6 and 34 to 15.73 per cent resistance with all the three diamondback moth populations of Coimbatore, Ooty and Oddanchatram. Similarly Zhao *et al.*(2006) reported that, population collected from collards from Oxnard CA showed low level of resistance to spinosad in 2002 (TR, 27.3 fold with resistance to spinosad in 2002 (TR, 27.3 fold with 20% survival), but it increased rapidly during 2004

**Fig. 1. Log concentration probit mortality lines for *P.xylostella* L populations from F1, F5, F10, F15, F20 and F25 generations**



(Toxicity Ratio >15,000 fold and 93% survival). Patil (2011) reported that LC<sub>50</sub> of emamectin benzoate was in the range of 9.16 to 39.07 ppm with maximum in Naski strain and minimum in Wadegoan (Akola) indicating that emamectin benzoate showed higher degree of resistance. Present findings from monitoring studies indicate that resistance in *P.xylostella* to chlorantraniliprole is in the initial stage, whereas high levels of resistance to spinosad and emamectin benzoate recorded might due to long time and repeated use in the test regions.

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