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Baseline toxicity of diamide group of insecticides against diamondback moth, *Plutella xylostella* L.

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Abstract
A study was carried out to assess the susceptibility of *Plutella xylostella* in different locations of Tamil Nadu against the diamide group of insecticides. The LC_{50} values were checked for all the populations. The LC_{50} values of the susceptible population for flubendiamide 20WG, chlorantraniliprole 18.5SC and cyantraniliprole 10.26OD were 0.021 mg ml^{-1}, 0.048 mg ml^{-1} and 0.007 mg ml^{-1}, respectively. Cyantraniliprole 10.26OD proved to be the best in toxicity to all the geographic population *viz.*, Coimbatore, Ooty, Oddanchatram, Krishnagiri and Theni compared to chlorantraniliprole 18.5SC and cyantraniliprole 10.26OD. The resistant ratio was also significantly low compared to chlorantraniliprole and flubendiamide (1.43, 1.71, 2.00, 2.43, 1.57) in Coimbatore, Ooty, Oddanchatram, Krishnagri and Theni, respectively). The resistant ratio was higher in flubendiamide 20WG (5.143 to 8.619) and chlorantraniliprole 18.5SC (2.167 to 3.562) than cyantraniliprole 10.26OD in all five different locations.

Keywords: Base line susceptibility, Diamide insecticides, *Plutella xylostella*, Tamil Nadu

1. Introduction
Diamondback moth is the most serious pest in causing economic losses in most of the cruciferous vegetables currently accounting for 2.7 billion US dollars of annual worldwide crop losses (Zalucki *et al.*, 2012) [13]. Typically, control of this pest depends solely on the use of synthetic insecticides. Though, the moth originated in the Mediterranean area, it has surpassed all the natural barriers and is believed to have become a cosmopolitan pest. The Diamondback moth (DBM), *Plutella xylostella* (L.), is one of the major hurdles for the cultivation of cabbage all over the world. The pest has developed insecticide resistance to almost all the chemical insecticides including *Bacillus thuringiensis* both under field and laboratory condition.

Diamide group of insecticides are an active class and novel modes of insect control chemistry that selectively activates insect ryanodine receptors causing mortality from uncontrolled release of calcium ion stores in muscle cells (Selby *et al.* 2013) [11]. Calcium channels are an attractive biological target for insect control due to the important role they play in multiple cell functions such as muscle contraction, neurotransmitter release and fertilization. The ryanodine receptor (RyR) is a non-voltage gated calcium channel located in the sarcoplasmic reticulum of muscle cells that regulates the release of intracellular calcium stores critical for muscle function.

Flubendiamide is an extremely effective insecticide against *P. xylostella*, especially when used as a larvicide. The parent compound structure was discovered during their pyrazine di carboxamide herbicide development program conducted in the early 1990s. The discovery of more potent substituents led to the synthesis, in 1998, of a phthalic acid diamide insecticide, later named flubendiamide. The first registration was secured in the Philippines in 2006 and was followed a year later by successful registrations in Japan, Pakistan, Chile, India and Thailand (Hirooka *et al.*, 2007) [2]. Flubendiamide was classified as the first member of the new group 28 (ryanodine receptor modulator) insecticides within the IRAC (Insecticide Resistance Action Committee) mode of action classification scheme. Chlorantraniliprole is another insecticide in the IRAC Mode of Action Group 28 family. Chlorantraniliprole was the first member of the anthranilic diamides that is relatively harmless to beneficial arthropods and was not found to exhibit cross resistance with existing insecticides (Lahm *et al.*, 2009) [5]. Products containing this active ingredient were launched on the world market in 2007.

Chlorantraniliprole binds to a site on the RyR distinct from that of ryanodine and its low toxicity to mammals is attributed to the high selectivity for insect versus mammalian RyRs and act as an insect control agent with outstanding activity against a wide range of lepidopteran pests and other 'plant-chewing' insects.

Cyantraniliprole was active against a wide range of insects on a variety of crops in worldwide field evaluations coupled with a favorable environmental-fate profile and remarkable selectivity for insect over mammalian forms of RyRs. It represents the first anthranilic diamide to target sap-feeding aphid pests with no evidence to date suggesting cross-resistance with other commercial aphid insecticides having a different mode of action. The broad spectrum of this anthranilic diamide is thought to be due to its physical properties, i.e., a lower log P and higher water solubility, in comparison to the other diamide insecticides, making it more suitable for systemic applications (Selby *et al.,* 2013) [11]. Hence, the present study was undertaken to obtain the baseline toxicity to diamide chemicals viz., chlorantraniliprole, flubendiamide and cyantraniliprole.

2. Materials and Methods

Insecticides and chemicals

Formulated insecticides namely flubendiamide 20WG (Takumi, Tata Rallis Limited), chlorantraniliprole 18.5SC (Coragen, Dupont India) and cyantraniliprole 10.26OD (Benevia, Dupont India) were purchased from market and used for bioassays to assess the toxicity against *P. xylostella.*

Collection of larva from various localities of Tamil Nadu to assess resistance

The study area included five major cauliflower and cabbage growing areas of Tamil Nadu (Fig. 1) *viz.*, Coimbatore (11.0168°N, 76.9558°E), Ooty (11.41°N 76.70°E), Oddanchatram (10.480°N 77.750°E), Krishnagiri (12.53°N 78.23°E) and Theni (10.009°N 77.47°E). In each region, the diamondback moth larvae (200 Nos.) were collected from fields. The farmers were interviewed and information on number of sprays, method of spraying, insecticides used and frequency of spray were collected.

The collected larvae were reared on cauliflower leaves and allowed for pupation. After pupation they were transferred to adult emergence cages (30x30x30cm). Sugar solution (10%) fortified with multi-vitamin drops were provided as adult feed to the moths. A day after the emergence of adults, mustard seedlings were provided for oviposition. The moths laid eggs on both the surfaces of leaves as well as on petioles. Fresh seedlings were provided once in two days until all the adults died. The method suggested by Liu and Sun (1984) [6] and Hou (1986) [3] was modified for rearing of test insects. The test insects required for various experiments were obtained from the stock culture maintained on mustard and cauliflower at insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

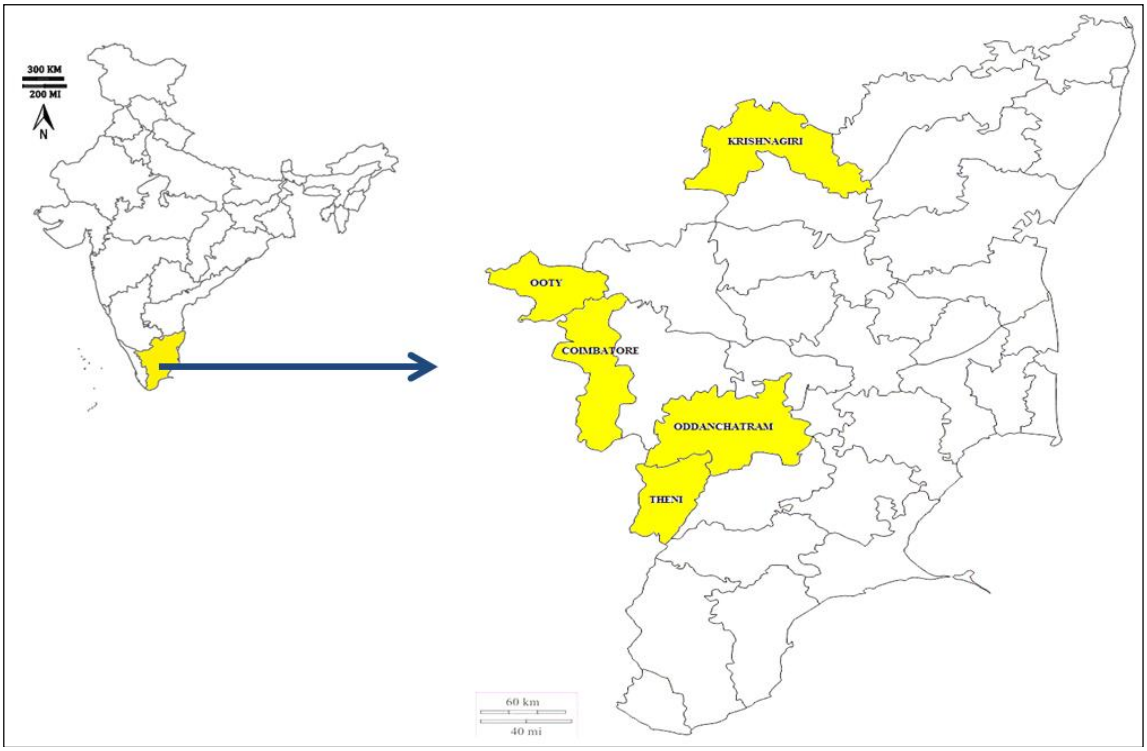

Fig 1: Survey sites of Plutella xylostella in Tamil Nadu

Larval rearing was carried out in cages with the size of 30x30x30cm. The first instar larvae hatched in about 3 to 4 days were allowed to mine into the mustard leaves and later to feed on the entire leaves. For second instar larvae, tender cauliflower leaves were provided as feed. For transferring the larvae from mustard seedlings to cauliflower leaf, a cauliflower leaf with its petiole wrapped with wet cotton was placed over mustard seedlings. Most of the larvae migrated to cauliflower leaf within a day. Then these larvae were transferred to fresh cauliflower leaves kept in conical flask. The larvae were provided with fresh leaves every day. The larval stage lasted for 12 to 14 days and the pupation mostly occurred on the lower surfaces of the leaves. The larvae pupated during different dates were collected by using a camel hair brush. To synchronies the emergence of moths, the collected pupae were stored in a refrigerator. When all the larvae were pupated, they were taken out from the refrigerator

and kept in the adult emergence cage. The pupal period lasted for 5 to 6 days.

Bioassay method

Leaf-dip bioassay method was used. The cauliflower leaves were first washed with distilled water containing 0.1% Triton X-100 thoroughly and air-dried. Leaf disc of 6-8 cm diameter were cut and dipped in different concentrations of the insecticides (Flubendiamide 20% WG, Chlorantraniliprole 18.5% SC and Cyantraniliprole 10.26 OD). Each disc was dipped for 5-10 seconds and allowed to air-dry for a period of one hour. After complete evaporation, the leaves were transferred to clean bioassay containers over a moistened filter paper. The leaf discs were placed slantingly to rest on side of the container so that larvae can move on either side. Eight to ten 2rd instar larvae (~0.4 mg) were released in each dish and four to five replicates were maintained per treatment. A treatment without insecticide served as control. Larval mortality was recorded every 24 h, consecutively for seven days. All the experiments were carried out in a room with a photoperiod of 12:12 (L: D) and experiments with control mortality more than 20% were discarded and repeated (Plate 3). The LC_{50} of different geographical population was obtained through probit regression analysis by Finney (1971) [1].

3. Results & Discussion

The median LC_{50} values of the susceptible population of *P. xylostella* for flubendiamide, chlorantraniliprole and cyantraniliprole were 0.021 mg ml^{-1}, 0.048 mg ml^{-1} and 0.007 mg ml^{-1}, respectively. The LC_{50} values of the different population in Coimbatore, Ooty, Oddanchatram, Krishnagiri and Theni for flubendiamide were 0.113 mg ml^{-1}, 0.181 mg ml^{-1}, 0.172 mg ml^{-1}, 0.129 mg ml^{-1} and 0.108 mg ml^{-1}, respectively. Similarly, the LC_{50} values for chlorantraniliprole were lower in Coimbatore (0.104 mg ml^{-1}), followed by Oddanchatram (0.128 mg ml^{-1}), Ooty (0.137 mg ml^{-1}) and Krishnagiri (0.171 mg ml^{-1}). The slope value of the susceptible population for chlorantraniliprole was 0.963. Among the different populations, Krishnagiri showed higher LC 50 values (0.017 mg ml-1) followed by Oddanchatram (0.014 mg ml^{-1}), Ooty (0.012 mg ml^{-1}), Theni (0.011 mg ml^{-1}) and Coimbatore (0.010 mg ml^{-1}).

The results of the present studies were in agreement with the findings of Selby *et al.*, (2013) [11] which reported that the EC_{50} value of cyantraniliprole was 0.07 ppm for *Plutella xylostella* (L.,), 0.21, 1.10, 0.08 and <0.1 ppm for *Heliothis virescens* (Fab.), *Myzus persicae* (Sulzer), *Bemisia tabaci* (Gennadius) and *Leptinotarsa decemlineata* (Say), respectively. Silva *et al.*, (2012) [12] reported that *P. xylostella* was highly susceptible to chlorantraniliprole which showed LC_{99} of 0.065 – 0.281 mg a.i/l and LC_{50} of 0.015 – 0.056 mg a.i/l of water by immersion or by spraying in a Potter tower. A discriminating concentration of 0.3 mg a.i./l was obtained from their baseline data and proved to be effective for evaluating other populations, causing per cent mortality.

The resistant ratio of flubendiamide and chlorantraniliprole were ranged from 5.14 to 8.62 and 2.17 to 3.56, respectively in all 5 different locations, whereas the resistant ratio of cyantraniliprole was very low ranged from 1.43 to 2.43 (Table 1 to 3). Flubendiamide resistant ratio was high in Ooty (8.62) and low in Theni (5.14). Chlorantraniliprole resistant ratio was higher in Krishnagiri (3.56) and the least in Coimbatore (2.17). Similarly, Cyantraniliprole resistant ratio was higher in Krishnagiri (2.43) and least in Coimbatore (1.429).

The results of the present study are innagreement with the findings of Samchelladurai (2015) [10] found that the population from Ooty (1.83) showed higher resistant ratio followed by Oddanchatram (1.73), Coimbatore (1.70), Theni (1.36) and Krishnagiri population (1.35) to flubendiamide. Liu *et al.* (2015) [7] reported that DBM has developed low to moderate levels of resistance to abamectin (3.3-fold), flubendiamide (14.1 fold) and chlorantraniliprole (24.3 fold). The resistance of *P. xylostella* increased to 30.6 to 326 fold for the newer chemical cyantraniliprole after 26 generations of selection, compared with the field population and susceptible population, respectively. Similarly, Roditakis *et al.* (2014) [9] reported high resistance levels for the tomato borer (*Tuta absoluta*) in Italian populations up to 2,414 fold for chlorantraniliprole and 1742 fold for flubendiamide. Resistant ratios for Greek populations were found up to 14-fold for chlorantraniliprole and 11-fold for flubendiamide.

Table 1: Susceptibility of *Plutella xylostella* to Flubendiamide 20WG

Location	LC_{50} (mgml^{-1}) 72 h	95% Confidence Limit		Slope	Chi square	Resistant ratio
Coimbatore	0.113	0.054	0.235	0.657	0.604	5.38
Ooty	0.181	0.084	0.391	0.635	0.416	8.62
Oddanchatram	0.172	0.076	0.391	0.591	0.361	8.19
Krishnagiri	0.129	0.063	0.262	0.678	0.804	6.14
Theni	0.108	0.055	0.211	0.723	0.456	5.14
Susceptible population	0.021	0.008	0.052	0.543	0.738	---

Table 2: Susceptibility of *Plutella xylostella* to Chlorantraniliprole 18.5 SC

Location	LC_{50} (mgml^{-1}) 72 h	95% Confidence Limit		Slope	Chi square	Resistant ratio
Coimbatore	0.104	0.079	0.138	1.863	0.691	2.17
Ooty	0.137	0.103	0.183	1.668	1.070	2.857
Oddanchatram	0.128	0.097	0.169	1.733	0.753	2.67
Krishnagiri	0.171	0.120	0.243	1.686	0.763	3.567
Theni	0.118	0.087	0.160	1.580	0.476	2.46
Susceptible population	0.048	0.027	0.084	0.963	1.216	---

Table 3: Susceptibility of *Plutella xylostella* to Cyantraniliprole 10.26 OD

Location	LC_{50} (mgml^{-1}) 72 h	95% Confidence Limit		Slope	Chi square	Resistant ratio
Coimbatore	0.010	0.003	0.035	0.377	1.388	1.43
Ooty	0.012	0.003	0.047	0.355	2.678	1.71
Oddanchatram	0.014	0.004	0.049	0.377	2.592	2.00

Krishnagiri	0.017	0.005	0.060	0.383	2.049	2.43
Theni	0.011	0.003	0.040	0.365	1.614	1.57
Susceptible population	0.007	0.002	0.026	0.384	1.882	---

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