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# Cross-resistance pattern in cypermethrin resistant *Amsacta albistriga* (Lepidoptera: Arctiidae) to various classes of insecticides

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**Abstract**
Cross resistance to insecticides has become a major problem in insecticide resistant insects. This has threatened the safe implementation of integrated pest management programme. To prevent development of cross resistance, a choice of suitable alternative insecticide should be made in the resistance population. In the present study, we have investigated the cross resistance pattern of several insecticides in cypermethrin resistant *Amsacta albistriga* (CypRes). The CypRes strain show high cross-resistance to permethrin, deltamethrin (58.1 and 70.2-fold) followed by dichlorvos, monocrotophos and profenofos (50.0, 47.01 and 27.3-fold), but moderate cross resistance to imidacloprid (3.8) and very low cross resistance to emamectin and NeemAzal insecticides (1.1 and 2.4-fold) respectively. This study provides important information for understanding cross-resistance pattern and facilitating a better strategy for the management of pyrethroid resistance in *A. albistriga*.

**Keywords:** *Amsacta albistriga*, Resistance selection, Pyrethroid resistance, Cross-resistance.

## 1. Introduction
The red hairy caterpillar, *Amsacta albistriga* is a lepidopteran insect pest of oilseed crops [1]. Apart from oilseed crops, *A. albistriga* can also cause damages in black gram and green gram [2]. It is estimated that an average of 2.5 to 3.0 million tonnes of pulses is lost annually owing to pest problems [3]. Chemical insecticides have long been used as an important tool for pest control that can have favored the development of insecticide resistance [4, 5]. Recently our research found that *A. albistriga* has the ability to develop resistance to cypermethrin under laboratory condition [1].
Emphasis is given to managing insecticide resistance using resistant management strategies that includes the rotation of insecticides with combination of different mode of action [6]. Rotating insecticides with different modes of action is one of the commonly recommended approaches to delay insecticide resistance. Nevertheless, successful implementation of this technique depends on a good understanding of resistance and cross-resistance patterns in population of target pests [7]. The cross-resistance pattern exhibited by a species is a useful tool in detecting the mechanism involved in resistance of a pest to a specific pesticide [8].
Many studies have been reported that insects have evolved cross resistance to conventional and newer insecticides [9-11]. Ahmad *et al.* [12] found that *Helicoverpa armigera* from field population has developed cross resistance to organophosphates and pyrethroid insecticides. Mu *et al.* [13] reported different levels of cross resistance to pyrethroids, newer insecticide and organophosphates in resistant strains of *Spodoptera exigua*. Cahill *et al.* [14] suggest that in *Bemisia tabaci* resistance to monocrotophos was not correlated with pyrethroid resistance, but, was with profenofos, showing different patterns within the same chemical group. Therefore, investigation of cross resistance in a resistant population is a useful strategy for the sustainable development of resistant management in agricultural pest. In this study we have investigated the cypermethrin resistance and cross resistance pattern to other group of insecticides in *A. albistriga* populations under laboratory conditions.

## 2. Materials and methods
### 2.1. Insects
A laboratory susceptible (Sus) and cypermethrin resistant strain (CypRes) of *A. albistriga* was maintained in our laboratory for twenty generations during the period of July 2012- January 2015. Both strains were originally collected from groundnut fields in Dharmapuri, TamilNadu, India respectively. Cultures were maintained under controlled conditions of 25 ±1 °C, 60 ± 5% relative humidity and 16 L: 8 day photoperiod. Adults were fed a 10% sugar solution on cotton swab and allowed to lay eggs on castor leaf (*Ricinus communis* L.).

**2.2. Insecticides used for bioassay**
For bioassays insecticides such as cypermethrin (10% EC, SIDCO, India), Emamectin benzoate (Dispersible granule WG, Syngenta, Turkey), imidacloprid (17.8% SL, Bayer Gujarat), NeemAzal (1% EC, Parry India Ltd), profenofos (50% EC, Hyderabad, India), dichlorvos (76% EC, Nuvan, Syngenta, Mumbai), Deltamethrin (11% EC, Bayer, Gujarat), Monocrotophos (36% SL, Hycrophos, Hyderabad) were purchased from commercial suppliers.

**2.3. Resistance selection with cypermethrin (CypRes)**
The field collected population was divided into two groups, one of which was artificially selected with cypermethrin for twenty consecutive generations under controlled conditions. The concentrations used for selection included i.e., 10, 20, 40, 50, 60, 80, 110, 140, 170 and 200ppm for the $1^{st}$ to $20^{th}$ generation ($G_1$-$G_{20}$). The second portion of the *A. albistriga* was reared on castor leaf diet without any insecticide exposure. Approximately 750-1500 larvae were exposed to cypermethrin at each generation during selection. Cypermethrin was thoroughly mixed in distilled water and castor leaves were dipped in to this solution for 10-15 seconds and then larvae were exposed to cypermethrin dipped air dried leafs (IRAC, 2010). After 24h of exposure, the living larvae were separated and then fed on normal castor leaf. The concentrations were increased to maintain the 50% mortality in each generation of selection.

**2.4. Cross resistance to other insecticides**
The cross-resistance of the cypermethrin susceptible and resistant Topical bioassay was used to determine the cross resistance to insecticides. For this study insecticide namely emamectin, imidacloprid, azadirachtin, λ-cyhalothrin, profenofos, dichlorvos, deltamethrin and monocrotophos were used. Early $3^{rd}$ instar cypermethrin resistant and susceptible *A. albistriga* larvae were used to test the cross resistance pattern against this pest. Insecticides were dissolved in deionized water to get the required dosage. 2µl of insecticide was applied on dorsal thoracic region of larvae using fine micropipette 0.2-2µl and susceptible larvae were treated with acetone alone. Six different concentrations per insecticide and three replicates per concentration and approximately thirty larvae per replicates were used. The mortality was recorded after 24h post treatment.

**2.5. Statistical analysis**
The concentration-mortality data was analyzed by probit analysis (Finney, 1971), to determined the $LC_{50}$ values, their standard errors, slopes and 95% fiducial limits (FL). Mortality was corrected by Abbott [15] formula. Resistance ratio (RR) and cross resistance ratio were calculated by dividing the $LC_{50}$ value of CypRes strain divided by the $LC_{50}$ of the Sus strain.

## 3. Results and discussion
**3.1. Selection of *A.albistriga* to cypermethrin**
Field collected *A. albistriga* population was selected with cypermethrin for up to twenty generations under control conditions which showed increased $LC_{50}$ values of cypermethrin from $G_1$ (17.89ppm) to $G_{20}$ (120.32ppm), showing a 21.5 fold increase in resistance ratio (Table 1). Avella *et al.* [16] found that a ten-fold increase in resistance developed from a seven-time selection with deltamethrin in *P. persimilis* in the laboratory. Markwick [17] reported that a ten-fold increase in resistance developed in *P. persimilis* with cypermethrin after a twelve-month selection. Abbas *et al.* [18] also reported that after 11 successive generations of profenofos selection in *S. litura* revealed 35.6 fold resistances.

**3.2. Cross-resistance pattern**
In the present study the resistance of cypermethrin resistant (CypRes) *A. albistriga* to other groups of insecticides including pyrethroids, organophosphates (OPs), neonicotinoid, avermectin and azadirachtin was determined. The results revealed that CypRes strain showed high cross resistance to permethrin, deltamethrin (58.1, 70.2-fold) and also showed high resistance to OPs followed by dichlorvos, monocrotophos and profenofos (50.0, 47.01 and 27.3-fold) compared to the susceptible strain. Suggest that selection of *A. albistriga* to cypermethrin under laboratory, its resistance to both pyrethroid and OPs was obviously enhanced. It seems that multiple resistance mechanism in this pest may caused led to pyrethroid cross-resistance. Moreover AChE and esterase was mainly involved in the organophosphate and pyrethroid resistance in *A. albistriga* populations [19].
Huang and Han [20] reported that selection of *S. litura* to deltamethrin for six generations its resistance to both pyrethroid and AChE targeted insecticide was enhanced. Obviously in India, some field populations of *S. litura* were found with multiple resistance to pyrethroid, organophosphate and other insecticides [21, 22]. The development of cross resistance in a pyrethroid resistant insect may correlate with the enhanced activity of esterase and mixed function oxidase [23, 20]. Next CypRes *A. albistriga* showed moderate resistance to imidacloprid insecticide (3.8-fold). Springate and Colvin [24] reported that a selection of pyrethroid resistance in cabbage whitefly, *Aleyrodes proletella* doesn't show any significant cross-resistance to neonicotinoid insecticide, followed by imidacloprid, emamectin and NeemAzal showed very low cross resistance (1.1 and 1.4-fold) respectively. Tong *et al.* [25] suggested that emamectin benzoate was found to be an effective tool for management of *S. litura*. Mandal *et al.* [26] also reported that the combination of neem oil with other insecticides could reduce the number of jassids and their damage in Okra. Hence, from the study we conclude that the combination of insecticides with distinct mode of actions such as emamectin and NeemAzal could become a prominent source for management of cypermethrin resistant *A. albistriga*.

**3.3. Conflict of interest**
We declare that we have no conflict of interest.

**Table 1:** Resistance ratio and $LC_{50}$ levels determined after selection with cypermethrin from the *Amsacta albistriga* population larvae at every fifth generations.

| Generations | $LC_{50}$ (ppm) | FL 95% | Slope (±SE) | $\chi^2$ | RR* |
|---|---|---|---|---|---|
| Susceptible | 5.56 | 4.53-6.24 | 1.34 (0.08) | 1.43 | - |
| $G_1$ | 17.89 | 16.54-0.21 | 1.13 (0.33) | 0.71 | 3.2 |
| $G_5$ | 26.30 | 25.27-8.08 | 2.21 (0.22) | 0.60 | 4.7 |
| $G_{10}$ | 37.83 | 34.71-0.32 | 0.78(1.47) | 0.31 | 6.8 |
| $G_{15}$ | 82.16 | 78.25-0.21 | 1.67(0.76) | 0.57 | 14.7 |
| $G_{20}$ | 120.32 | 118.23-122.7 | 1.82(0.04) | 1.31 | 21.5 |

*RR = resistance ratio, determined by dividing the $LC_{50}$ of resistant strain by $LC_{50}$ of susceptible strain. $LC_{50}$ lethal concentration that kills 50% of the exposed larvae, FL fiducial limits, $\chi^2$ chi-square.

**Table 2:** Toxicity of some conventional and newer insecticides to the susceptible (Sus) and cypermethrin selected (CypRes) *A. albistriga*.

| Insecticides | Strains | $LC_{50}$ (ppm) | FL 95% | Slope (±SE) | $\chi^2$ | RR* |
|---|---|---|---|---|---|---|
| Cypermethrin | Cyp-Res | 120.32 | 118.23-122.7 | 1.82(0.04) | 1.31 | 21.5 |
| | Sus | 5.56 | 4.53-6.24 | 1.34 (0.08) | 1.43 | - |
| Imidacloprid | Cyp-Res | 5.002 | 2.993 - 8.618 | 1.95 (0.38) | 1.21 | 3.8 |
| | Sus | 1.291 | 0.435- 5.245 | 0.21 (0.20) | 0.13 | |
| Permethrin | Cyp-Res | 57.02 | 53.129- 59.417 | 2.32 (0.87) | 1.36 | 58.1 |
| | Sus | 0.982 | 0.735-2.543 | 0.21 (0.11) | 2.54 | |
| Deltamethrin | Cyp-Res | 87.314 | 85.898- 9.759 | 2.29 (1.09) | 0.31 | 70.2 |
| | Sus | 1.243 | 0.856-3.965 | 1.65 (0.76) | 2.17 | |
| Emamectin benzoate | Cyp-Res | 0.763 | 0.440 -3.619 | 1.43 (0.19) | 1.72 | 1.1 |
| | Sus | 0.644 | 0.142-1.132 | 0.23 (0.32) | 0.20 | |
| NeemAzal | Cyp-Res | 4.056 | 0.238- 7.598 | 0.64 (0.21) | 3.52 | 1.4 |
| | Sus | 2.842 | 1.823- 3.434 | 1.12 (0.34) | 1.86 | |
| Profenofos | Cyp-Res | 43.818 | 37.40 -51.23 | 0.88 (0.28) | 3.47 | 27.3 |
| | Sus | 1.632 | 0.843-2.948 | 0.32 (0.12) | 0.56 | |
| Dichlorvos | Cyp-Res | 96.463 | 49.984- 20.40 | 2.07 (0.42) | 1.73 | 50.0 |
| | Sus | 1.923 | 1.230- 2.329 | 1.32 (0.64) | 0.26 | |
| Monocrotophos | Cyp-Res | 61.298 | 49.984-92.20 | 1.80 (0.44) | 3.17 | 47.0 |
| | Sus | 1.392 | 1.120-2.921 | 0.71 (0.23) | 1.27 | |

*RR = resistance ratio, determined by dividing the $LC_{50}$ of resistant strain by $LC_{50}$ of susceptible strain. $LC_{50}$ lethal concentration that kills 50% of the exposed larvae, FL fiducial limits.

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