

# Resistance to emamectin benzoate in *Plutella xylostella* collected from different geographic locations

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## SUMMARY

The baseline susceptibility study to emamectin benzoate in *P.xylostella* was undertaken at Insect Biotech Laboratory, Department of Entomology, Dr.P.D.K.V., Akola (M.S.) India. The 3<sup>rd</sup> instar larvae of *P. xylostella* collected from different locations of Maharashtra and tested with new molecule emamectin benzoate. Log dose probit analysis indicated LC<sub>50</sub> of emamectin benzoate in the range of 9.16 ppm to 39.07 ppm with maximum in Nasik strain (39.07 ppm) and minimum (9.16 ppm) was reported from Wadegaon (Dist-Akola). These results indicated that diamondback moth under selection pressure of emamectin benzoate showed higher degree of resistance. Indiscriminate use of insecticides, multiple generations of diamondback moth per annum and year round availability of host crop have contributed to the development of resistance.

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The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) is considered the most damaging insect pest of cruciferous crops worldwide. Larvae can devastate crops rapidly during major outbreaks and even mild infestation can reduce quality and marketability (Talekar and Shelton, 1993). In the past 50 yr, *Plutella xylostella* has become one of the most difficult insects in the world to control, primarily because of resistance evolution to every class of insecticide used extensively against it (Shelton *et al.*, 2000 and Sarfraz and Keddie, 2005).

Emamectin benzoate is one of the most important avermectin insecticides which is isolated from *Streptomyces avermitilis*. It is a semi-synthetic second generation avermectin insecticide, highly potent, unique foliar insecticide that controls lepidopteran pests (caterpillars and worms) in cole crops, turnip, leafy and fruiting vegetables. Extensive studies on the resistance of diamondback moth to conventional insecticides have revealed that this pest has an ability to develop high level of resistance in a short time when a new insecticide is introduced (Fahmy and Miyata, 1991). Insecticide resistance in the

diamondback moth, *Plutella xylostella* is unique because the development of resistance can take place quickly, at the same time the insect can lose resistance fair quickly if the population is freed from the insecticidal pressure.

In need of the present study, a new field population of *P.xylostella* collected from different geographical locations of Maharashtra was examined with the aim of investigating the genetics emamectin benzoate resistance in *P.xylostella*.

## MATERIALS AND METHODS

### Insect:

A field population of *P.xylostella* was collected from the cabbage field from the different geographical locations of Maharashtra, in May, 2008 of which cabbage is major crop in these regions. Diamond back moth population had been maintained in the laboratory for ten generations without exposure to insecticide. Continuous colonies of *P.xylostella* from different geographical locations were reared in the Insect Biotech. laboratory of Department of Entomology, Dr.

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Panjrabrao Deshmukh Krishi Vidyapeeth, Akola under controlled climatic conditions on mustard seedlings.

#### Maintenance of susceptible strain:

Insects collected from various locations of Maharashtra were tested for the toxicity of emamectin benzoate. The strain which was found most susceptible to emamectin benzoate having lowest  $LC_{50}$  was maintained in the laboratory for several generations without exposure to insecticides. The rearing was done under controlled laboratory conditions. This susceptible population was used to compare with field collected population.

#### Mass rearing of *P. xylostella*:

Continuous colonies of *Plutella xylostella* from different geographical locations were reared in the laboratory under controlled conditions of temperature  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ,  $75 \pm 5$  per cent relative humidity and photo period of 13 hrs light: 11 hrs dark. The larvae were reared on mustard seedlings.

#### Adult rearing:

The pupae were kept in oviposition chamber, keeping mustard seedlings so that adult emerged can utilize the seedlings as oviposition substrate. The adults were provided with adult diet. The mustard seedlings were kept inside the rearing chamber and were replaced by new seedling on alternate days.

#### Larval rearing:

After hatching, the larvae mine into the mustard seedlings, come out and feed on mustard seedlings. The third instar larvae were selected for bioassays. The rearing was carried out at temperature  $27 \pm 2^{\circ}\text{C}$  and RH  $75(\pm) 2 \%$  in dark-light regime of 13–11 hrs.

#### Insecticide tested

Commercial formulation of emamectin benzoate (Proclaim 5 SG, Syngenta Crop Protection Greenshoro, NC). was used for treatment.

#### Preparation of insecticide solutions:

The insecticide solutions required for the bioassays were prepared using the commercial formulation which were diluted with distilled water. Fresh solution was prepared as and when required by using the formulation.

Concentrations of insecticides were prepared by the formula:

$$V = \frac{C \times A}{a.i.}$$

where V - volume of water to be added.

C - Required concentration of insecticide.

A - Required quantity of solution

a.i. – active ingredient in insecticide formulation.

#### Log dose probit (LDP) bioassays:

The Leaf-dip bioassay as described by Tabashnik and Cushing (1987), was adopted in the present studies. Leaf discs of 5 cm diameter were cut from fully expanded untreated cabbage leaves. These discs were treated individually with various prepared test concentrations of emamectin benzoate, and also with distilled water containing acetone, which was treated as control or check treatment. After air drying, each leaf-disc was placed in 9 cm diameter Petriplates with moistened filter paper at the bottom. Ten third instar larvae were released on the leaf disc. The observations were recorded at 24 hours, upto 3 days. The corrected mortality was calculated using Abott (1925) formula. The mortality data were further subjected to probit analysis as described by Finney (1977).

$$Y = Y + b (X - X)$$

where,

Y = Probit equation

X = Log concentration

b = Slope of regression line

Surviving individuals were reared for next generation and exposed to insecticide at higher concentrations. The selection was continued up to ten generations,  $LC_{50}$  values were calculated for each generation.

#### Resistance ratio:

The resistance intensity of a population or a strain of insects to a particular insecticide is frequently quoted as the Resistance Ratio (RR), sometimes also called as the Resistance Factor (RF), which was calculated by the formula:

$$RR \text{ at } LC_{50} = \frac{LC_{50} \text{ of field collected strain}}{LC_{50} \text{ of susceptible strain}}$$

## RESULTS AND DISCUSSION

Log dose probit assays were carried out to determine the median lethal concentration of emamectin benzoate against *P. xylostella* strains, collected from different geographical locations of Maharashtra (Table 1). The

**Table 1 : Baseline toxicity of emamectin benzoate to *P. xylostella* collected from various locations of Maharashtra**

Sr. No.	Strain	LC <sub>50</sub> ppm (95 % FL)	LC <sub>95</sub> ppm	Slope (±SE)	Chi square	RR
1.	Ahemad Nagar	23.80 (20.33-27.85)	70.44	3.49(±0.55)	2.35	2.99
2.	Nasik	39.07 (28.82-42.31)	219.80	1.96(±0.34)	4.95	4.92
3.	Mancher(Pune)	24.77 (22.52-28.95)	103.26	2.57(±0.47)	2.90	3.11
4.	Patur (Akola)	10.20 (6.99-14.89)	104.20	1.43(±0.34)	1.59	1.28
5.	Wadegaon (Akola)	9.16 (5.66-11.76)	55.26	1.70(±0.36)	1.29	1.15
6.	Dongergaon (Akola)	12.72 (8.69-15.80)	142.17	1.73(±0.34)	1.69	1.60
7.	Susceptible	7.94 (5.10-12.38)	257.86	1.08(±0.24)	2.61	-

LDP assays of emamectin benzoate have indicated significant levels of resistance in the field collected populations especially from Nasik and Mancher (Pune).

LC<sub>50</sub> for emamectin benzoate was recorded in the range of 9.16 to 39.07 ppm. The maximum LC<sub>50</sub> was recorded in Nasik population (39.07 ppm). In Nasik population significantly high level of resistance over all the other strains was observed. The LC<sub>50</sub> of Nasik strain was 39.07 ppm, while its LC<sub>95</sub> was 219.80 ppm, the LDP slope for Nasik strain was 1.96(±0.34) as compared to other strains. The Wadegaon (Akola) strain was found most susceptible amongst the strains tested. LC<sub>50</sub> for Wadegaon (Akola) was 9.16 ppm where as LC<sub>95</sub> was 55.26 ppm. The fiducial limits at 95 per cent of LC<sub>50</sub> were 5.66 and 11.76 ppm. (Table 1).

Nasik population was followed by Mancher (Pune) population which was at par with Nasik and resistance was found to be 24.77 ppm and 3.11 fold resistance was observed. Data revealed from Table 1 showed that *P. xylostella* collected from Mancher (Pune) was near to Ahemadnagar strain. The LC<sub>50</sub> of emamectin benzoate in Mancher strain was 24.77 ppm having resistance ratio of 3.11 fold. The Mancher was followed by Dongergaon and Patur (Dist- Akola) having LC<sub>50</sub> 12.72 ppm and 10.20 ppm, respectively as compared to susceptible population (LC<sub>50</sub> -7.94 and slope 1.08 ±0.24).

Thus, the above results indicated that diamondback moth under selection pressure of emamectin benzoate showed higher degree of resistance. Indiscriminate use of insecticides, multiple generations of diamondback moth per annum and year round availability of host crop have contributed to the development of resistance in diamondback moth against almost all groups of insecticides (Mohan and Gujar, 2003). Ahmad *et al.* (2006), reported similar kind of trend who found out that the time related toxicity of different conventional and new chemistry insecticides including emamectin benzoate and abamectin which were used against *S. litura*.

In southern India the resistance levels were reported that LC<sub>50</sub> ranging from 0.310 to 19.96 µg/ml against diamondback moth by Muthusamy (2003). Sudhakar (2005) reported the population from Guntur strain to be 13.76 fold resistant to indoxacarb followed by strains collected from Akola (10.55 fold), West Bengal (4.5), laboratory reared indoxacarb resistant strain (3.7), Tamil Nadu strain (3.1) and Munnar strain (2.3).

Nehare (2007) reported that the Nasik strain recorded highest resistance to indoxacarb (RR-3.86) followed by Ahemadnagar (RR-3.84) as compared to laboratory susceptible population of *P. xylostella*. These results similar to present findings, which indicated that the Nasik population strain of *P. xylostella* was having comparatively high levels of resistance to all available insecticides. The strains from other locations have developed low resistance. The possible reason might be due to low exposure to chemical groups on the cruciferous crops, which leads to low selection pressure on insects resulting in low development of insecticide resistance.

Zhao *et al.* (2006) and Talekar and Shelton (1993) were collected annually six to nine population of diamondback moth from fields of United States and Mexico for base line susceptibility tests and resistance monitoring to indoxacarb, emamectin benzoate and spinosad. The toxicity ratio or resistance ratio to indoxacarb and emamectin benzoate relative to susceptible strain were 1.4 – 140, and 2.1 – 60.5 fold, respectively indicating large geographic variations in different population. It is well documented that *P. xylostella* has the potential to develop resistance in the field to most insecticides sooner or later after extensive applications.

Emamectin benzoate should maintain its apex position in coming years. Considering its importance, possible mechanisms of resistance development in *P. xylostella* against emamectin benzoate was studied. Present investigation along with further molecular studies

will be helpful in developing future strategies for effective management of emamectin benzoate resistance in *P. xylostella*.

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