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Ravi Biradar
Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka, India

M Bheemanna
Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka, India

A Hosamani
Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka, India

Harischandra Naik
Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka, India

Nagaraj Naik
Department of agriculture microbiology, University of Agricultural Sciences, Raichur, Karnataka, India

Kavita Kandpal
Department of Horticulture, University of Agricultural Sciences, Bengaluru, Karnataka, India

Corresponding Author:
M Bheemanna
Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka, India

Emamectin benzoate resistance in diamondback moth in different locations of Karnataka

Ravi Biradar, M Bheemanna, A Hosamani, Harischandra Naik, Nagaraj Naik and Kavita Kandpal

Abstract
Diamondback moth (*Plutella xylostella* L.) is one of the few insect species that has developed field resistance to all major classes of insecticides. In this view, the present investigation was carried out to study the levels of insecticidal resistance in the larvae of diamondback moth collected from major cabbage growing districts of Karnataka at Department of Agricultural Entomology, College of Agriculture, UAS, and Raichur during 2017-19. Log dose probit analysis indicated LC_{50} of emamectin benzoate in the range of 4.01 ppm to 31.77 ppm with maximum in Mysuru strain (39.07 ppm) and minimum (9.16 ppm) was reported from Raichur. The relative resistance ratio was found to be highest in the diamondback moth population of Mysuru (7.98-fold) followed by Haveri (7.02-fold), Chamrajnagar (6.84-fold), Bengaluru (5.69-fold), Chikmagalur (5.03 -fold) and Bidar (4.02-fold) over the susceptible Raichur strain. These results indicated that diamondback moth under selection pressure of emamectin benzoate showed higher degree of resistance.

Keywords: Cabbage, diamondback moth, emamectin benzoate, resistance

Introduction
India is the largest producer of vegetables in the world after China with an annual production of 184.39 million tons from 10.2 million ha [1]. Amongst vegetables cole crops are important one and most cultivated and abundantly consumed all over the world. Among crucifer, cabbage crop stands top in India with an annual production of 8970.53 thousands MT from 406.86 thousand ha and productivity of 22.05 MT/ha [2]. Productivity of cabbage in India is much lower attributing to many causes and among them insect pests are the major constraints. It has been anticipated that insect pests alone causes 40% to 100% yield loss annually [3]. Diamondback moth (DBM), *Plutella xylostella* (Linnaeus), is the major destructive pest of cruciferous vegetables in the world and was first recorded in 1914 [4] and which has now spread all over India. In the past 50 years, *P. xylostella* has become one of the most difficult insects in the world to control. All over the world, management of DBM is exclusively with the use of insecticide [5]. The reliance on this single approach has led to ever increasing application rates, decreased effectiveness and eventual breakdown of control efficiency [6]. The usage pattern of various insecticides belonging to different groups varies across the geographic locations primarily based on the intensity of pests and diseases, dealer recommendations, influence of peer groups, efficacy and availability of a particular insecticide knowledge level and socioeconomic condition of the farmer [7]. The present study therefore reports the local variation in susceptibility of nine field populations of *P. xylostella.*

Emamectin benzoate is most commonly used avermectin insecticides which are derived from *Streptomyces avermitilis*. It is a semi-synthetic second generation avermectins insecticide, highly potent, exceptional foliar insecticide that pedals lepidopteran pests (larva and worms) in cole crops, turnip, leafy and fruiting vegetables. All-embracing studies on the resistance of diamondback moth to conventional insecticides have shown that this pest has an ability to gain high level of resistance in a short period when an expose to recent molecules [8]. Resistance in the diamondback moth against insecticide is exceptional as the development of resistance can occur rapidly, simultaneously the insect can become susceptible so quickly if the population is untied from the insecticidal stress. Impoverished of the present study, a new field strain of *P. xylostella* collected from diverse geographical locations of Karnataka was examined with intend of investigating the susceptibility and resistance development to emamectin benzoate.

Materials and Methods

The population of DBM larvae were collected from farmers' fields at Raichur, Bidar, Belagavi, Haveri, Chikmagalur, Hassan, Chamrajnagar, Mysuru and Bengaluru districts during early morning hours or evening hours in bucket along with leaves, brought to the laboratory of Department of Agricultural Entomology, UAS, Raichur, immediately and were used for bioassay. For bioassay, each district collection of test insect constituted the composite collection of 4-6 cabbage ecosystems.

Mass rearing of Diamondback moth

70-75 per cent relative humidity and D: L 12:12 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 4 days mustard seedlings for oviposition. After hatching, young larvae fed on the mustard leaves by mining and then larvae were transferred to the insecticidal free fresh cauliflower leaves. Larvae after attaining third instar were used for bioassay.

Larval bioassay

F1 larval culture (II instar – Six-day old larvae) was subjected to bioassay for resistance profile assessment to insecticide. The bioassay was conducted by standard leaf dip method (IRAC-7) using 5 cm diameter leaf disk. Three sets were subjected to bioassays for insecticidal concentration of emamectin benzoate with at least 10 larvae in each replication. In general five concentrations of emamectin benzoate insecticide were used to generate dose mortality response for *Plutella xylostella*. The mortality caused by insecticide treatments was recorded at 48 hrs after treatment. The mortality obtained after 48hrs was treated as a final mortality. The leaf disk without any treatments would serve as a control. The mortality in control was also recorded. If the mortality in control exceeded 10 per cent, the set of experiments was discarded. The final mortality was corrected using Abbots formula and same was used for final analysis. The dose mortality response data was used for probit analysis.

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:

$$\textbf{RR at } LC_{50} = \frac{\textbf{LC}_{50}\textbf{ of field collected strain}}{\textbf{LC}_{50}\textbf{ of susceptible strain}}$$

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 Karnataka (Table 1). The LDP assays of emamectin benzoate have indicated significant levels of resistance in the field collected populations especially from Mysuru and Haveri. LC_{50} for emamectin benzoate was recorded in the range of 4.01 ppm to 31.77 ppm. The maximum LC_{50} was recorded in Mysuru population (31.77 ppm). In Mysuru population significantly high level of resistance (7.98-fold) over the susceptible strain was observed. The LC_{50} of Mysuru strain was 31.77 ppm, while its LC_{90} was 335.51ppm. The Raichur strain was found most susceptible amongst the strains tested and it was considered as susceptible strain to found relative resistance ratio value for other strain. LC_{50} for Raichur 4.01 ppm where as LC_{90} was 26.82 ppm. The fiducial limits at 90 per cent of LC_{50} were 1.29 and 12.46 ppm (Table 1). Mysuru population was followed by Haveri population which was at par with Mysuru and resistance was found to be 28.16 ppm and 7.02-fold resistances was observed. Data revealed from Table 1 showed that *P. xylostella* collected from Haveri was near to Chamrajnagar strain. The Haveri was followed by Chamrajnagar (27.42 ppm), Bengaluru (22.81 ppm), Chikmagalur (20.18 ppm), Bidar (16.11 ppm), Hassan (11.48 ppm) and Belagavi (5.76 ppm) during 2017-19. It was confirmed from the latest publications that DBM has the potentiality to develop resistance to microbial insecticides like spinosad, abamectin and emamectin benzoate [9]. Also reported the same kind of resistance ratio of DBM against emamectin benzoate 5 SG from 1.00 to 1.37 folds for three cabbage growing regions of Karnataka. Resistance to the semisynthetic toxin, emamectin benzoate (150 to 300 folds) was recorded in Taiwan [10].

Higher levels of resistance in Mysuru and Haveri populations might be due to its regular usage in those areas. Similar results were reported that LC_{50} of emamectin benzoate was in the range of 9.16 to 39.07 ppm with minimum in Wadegoan (Akola) and maximum in Nasik strain indicating that emamectin benzoate recorded higher degree of resistance [11]. Accordingly, the above outcome indicated that diamondback moth under selection pressure of emamectin benzoate showed higher degree of resistance. Indiscriminate use of insecticides, several generations of diamondback moth per annum and continuous cultivation of cruciferous crop have contributed to the advance of resistance in diamondback moth in opposition to majority insecticides belongs various groups [12]. The reason attributed to low development of insecticide resistance is because of less exposure to chemical groups on the cruciferous crops, which results in low selection pressure on insects [13, 14]. Six to nine population of diamondback moth were from fields of United States and Mexico collected annually for base line susceptibility tests and resistance monitoring to indoxacarb, spinosad and emamectin benzoate. The toxicity ratio or resistance ratio to emamectin benzoate and indoxacarb relative to susceptible strain were 2.1 - 60.5-fold and 1.4 - 140, respectively indicating large geographic variations in different population. Many literatures reported that *P. xylostella* has the ability to gain resistance in the field to the majority insecticides more rapidly or later after wide applications. Emamectin benzoate should preserve its apex position in future. In view of its magnitude, a probable mechanism of resistance development in *P. xylostella* against emamectin benzoate was deliberated. Present examination along with additional molecular studies will be useful on the rise future strategies for effectual management of emamectin benzoate resistance in *P. xylostella*.

Table 1: Insecticide resistance of emamectin benzoate 5 per cent SG against diamondback moth of different locations

Sl. No	Location	LC_{50} (ppm)	Fiducial limit		LC_{90} (ppm)	Slope	χ^2	Relative Resistance Ratio
			LL	UL				
1	Bidar	16.11	10.40	24.95	327.99	0.98 ± 0.17	2.00	4.02
2	Belagavi	5.76	2.23	14.84	53.46	1.43 ± 0.47	0.69	1.44
3	Haveri	28.16	19.06	41.59	335.51	1.18 ± 0.31	1.11	7.02
4	Chikmagalur	20.18	14.49	28.10	198.77	1.29 ± 0.14	1.59	5.03
5	Hassan	11.48	6.86	19.20	93.20	1.43 ± 0.22	0.49	2.86
6	Mysuru	31.77	24.38	41.40	155.81	1.85 ± 0.26	0.40	7.92
7	Bengaluru	22.81	16.86	30.86	176.21	1.45 ± 0.34	0.74	5.69
8	Chamrajnagar	27.42	22.36	33.63	110.48	2.14 ± 0.45	1.68	6.84
9	Raichur	4.01	1.29	12.46	26.82	1.60 ± 0.38	0.08	-

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