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# Effect of variable temperature on the toxicity of novel insecticides against diamondback moth, *Plutella xylostella* (Linn.)

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**Abstract**
Ecotoxicity at four different temperature levels were studied with diversified novel insecticides viz., emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide against diamondback moth, *Plutella xylostella*. The toxicity of emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide were found to be positively correlated with the temperature range tested. Based on $LC_{50}$ values deduced, the toxicity of emamectin benzoate increased significantly by -1.29 and -1.23 folds at temperatures of 30 ºC and 35 ºC as compared with toxicity at 20 and 25 ºC. Similarly, toxicity of chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide increased by -1.71, -1.48, -1.74 and -1.50 folds respectively at 30 ºC as compared with toxicity at 20 ºC and 25 ºC. All the insecticides showed overall positive temperature coefficients of 1.50, 2.35, 1.58, 2.45 and 2.17 fold respectively for the temperature range tested.

**Keywords:** Cabbage, *Plutella xylostella*, insecticides, ecotoxicity and temperature

## Introduction
India with its wide variability in climate and soil produces a large number of vegetable crops. These are the important source of proteins, vitamins, carbohydrates and minerals contributing a major role in nutritionally balanced diet of vegetarian population of our country. More than 50 varieties of vegetable crops are grown in India of which cruciferous group of vegetable crops are most important in terms of nutritional and economic significance. Among all these cruciferous crops, cabbage and cauliflower occupy the first position in terms of yield. Cabbage, *Brassica oleracea var. capitata* Linn. is a popular and extensively cultivated crop because of its nutritional and economic importance. The cabbage crop is infested and devastated by a number of insect pests namely; diamondback moth *(Plutella xylostella* Linnaeus*)*, cabbage caterpillar *(Pieris brassicae* Linnaeus), cabbage semilooper (*Thysanoplusia orichalcea* Fabricius and *Autographa nigrisigna* Walker), tobacco caterpillar (*Spodoptera litura* Fabricius), cabbage leaf webber (*Crocodolomia binotalis* Zeller) and cabbage borer (*Hellula undalis* Fabricius) are the pests of major importance [1].

Diamondback moth (DBM), (*P. xylostella* Linn.) is the most serious pest of cabbage which causes low productivity of this crop. The loss in yield caused by this pest varies from 31-100 per cent [2]. It is estimated that 53-80 per cent loss in marketable yield in cabbage crop is due to DBM infestation alone and loss could be more if the attack is grievous [3]. Indiscriminate coupled with irrational use of insecticides at higher doses for management of DBM resulted not only in resistance but also caused elimination of natural enemies which in turn has accentuated the infestation many folds. DBM has the credential of developing resistance to almost all insecticides within a span of 2-3 years post the release in the market including *Bacillus thuringiensis* commercial formulations.

In field conditions, temperature plays a pivotal role on insecticide effectiveness. Temperature affects different biological traits of insects such as fertility, fecundity, survival, adult life-span [4, 5] and sex-ratio [6]. Significant variation on the efficacy of insecticides used in field condition under different temperature conditions has been studied elsewhere [7]. Temperature as an extrinsic abiotic factor has definite effect on the toxicity of any insecticide which specifically depends on the species concerned and the extent of prevailing instant temperature. The scenario of temperature resulting in breakdown of a particular insecticide to more or less toxic metabolites within an insect species may vary among different insecticides classified within

the same chemical group. However, studies conducted elsewhere revealed variation in the toxicity within a given insecticide class [8, 9] between insect species and temperature range tested [8, 10]. This study focuses on to establish the temperature – toxicity relationship on different classes of novel insecticides against diamondback moth of cabbage which will enable the growers to select the insecticide for diamondback moth management in a specific temperature condition.

## Materials and methods

### Insect culture

The larvae and pupae of DBM were collected from the infested cabbage fields in Bahadhurguda Village, Shamshabad Mandal of Ranga Reddy District. Larvae were reared on insecticide free cabbage leaves in the glass jars (20x10cm) up to the pupal stage. Rearing jars were maintained at 25±2°C and 70 ± 5 per cent relative humidity with 14 hrs light and 10 hrs dark conditions to initiate the laboratory strain. The larvae after attaining pupal stage were placed in egg laying cage (30 x 30 x 30 cm). The adults after emerging from the pupa were provided with 10 per cent honey solution on a cotton swab. Mustard seedlings (4-5 cm height) were kept in the ovipositional cage for egg laying by *P. xylostella.* After hatching, young larvae fed on the leaves by mining and larvae after attaining second instar stage were transferred to the fresh insecticidal free cabbage leaves. Leaves were changed daily to avoid contamination. Larvae after attaining the third instar stage were used for bioassay studies.

### Test Insecticides

Commercial formulations of five novel insecticides with diversified mode of action and classes *i.e.*, emamectin benzoate 5 SG (Proclaim), Syngenta Crop Science Ltd, Mumbai; chlorantraniliprole 18.5 SC (Coragen), Du-Pont India Ltd, Gurgaon; flubendiamide 39.35 EC (Fame) Bayer Crop Science Ltd, Mumbai; fipronil 5 SC (Regent) Aventis Crop Science Ltd., Mumbai and chlorfenapyr 10 SC (Intrepid) BASF India Ltd, Mumbai were selected for assessing the toxicity at different temperatures of 20 $^{o}$C, 25 $^{o}$C, 30$^{o}$C and 35 $^{o}$C and 70 ± 5% RH with a 14: 10 ( L/D) photoperiod in BOD incubators.

### Insecticide bioassay

Test concentrations for each insecticide were prepared in distilled water by using commercially formulated products on the day of bioassay. Leaf dip bioassay was conducted in 90 mm-diameter plastic disposable petri dishes, with round moistened filter paper on the lower surface to maintain moisture and turgidity of cabbage leaves in the incubators. Insecticide-free cabbage leaves were collected from cabbage plants which were grown on the earthen pots in insecticide free condition. At each temperature, five concentrations of each insecticide were evaluated to cause wider range (20 to 80 %) of mortality based on preliminary studies. The leaves were treated through leaf dip bioassay method with the test insecticide solutions for 20 seconds and were allowed to air dry. Leaves treated with distilled water alone served as control treatment. The insecticide treated leaves were placed on the moistened filter papers in the petri dishes and ten third instar larvae were transferred to each dish using a camel hair brush. Each concentration of every insecticide was replicated four times (n=10 larvae per concentration). Petri dishes with larvae were kept in incubators at 20 $^{o}$C, 25 $^{o}$C, 30 $^{o}$C and 35 $^{o}$C temperatures with 70± 5% RH.

### Observations

The mortality of insects was recorded at every 24, 48 and 72 hours after treatment and the moribund insects were considered as dead. The mortality at 72 HAT was considered as end point for the assessment of toxicity of test insecticide [11]. Mortality data were subjected to modified Abbott's formula for correction as required. Pooled mortality for each concentration was subjected to regression probit analysis to calculate the concentration required for mortality in 50% of the population ($LC_{50}$) for each insecticide at each exposure temperature, with corresponding confidence limits and slopes of regression lines by using Biostat 2009 (5.8.3.0 version) software programme. Temperature co-efficient for each insecticide was calculated as the ratio of higher to lower $LC_{50}$ values [12-14]. The co-efficient was considered positive when the $LC_{50}$ value was lower and was considered as negative when the $LC_{50}$ value was higher at a temperature [12]. Temperature coefficients of each insecticide tested at different temperatures were calculated as the ratio of higher to the lower $LC_{50}$ and called negative when the lower $LC_{50}$ was at a lower temperature and positive when the lower $LC_{50}$ at a higher temperature [13].

## Results and Discussion

The influence of four different temperatures on the toxicity of newer insecticide molecules was determined and the results revealed the definite role of temperature on the toxicity of novel insecticides on *P. xylostella*. The toxicity of emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide were found to be positively correlated with the temperature range tested. Based on $LC_{50}$ values the toxicity of emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide were found to be positively correlated with the temperature range tested. The toxicity of emamectin benzoate increased significantly to 1.29 and 1.23 fold at temperatures of 30 and 35$^{o}$C when compared with toxicity at 20 and 25 $^{o}$C (Table 1). Similarly for chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide the toxicity increased to 1.71, 1.48, 1.74 and 1.50 fold respectively at 30 $^{o}$C as compared with toxicity at 20 and 27 $^{o}$C. All the five novel insecticides showed an overall positive temperature coefficient of 1.50, 2.35, 1.58, 2.45 and 2.17 fold respectively for the temperature range tested. Emamectin benzoate, chlorantraniliprole, chlorfenapyr, fipronil and flubendiamide showed temperature dependent toxicity, but it is quite evident from the results that effect of temperature on toxicity of emamectin benzoate was not significant as illustrated by being more toxic than other insecticides at higher temperature range (35 °C) tested.

In case of emamectin benzoate contact toxicity was increased by about 10 fold and the ingestion toxicity by about 1000 fold when the temperature was enhanced from 16 °C to 31 °C [15] similarly the activation of chlorfenapyr and its mechanism of toxicity in disrupting cellular respiration, and being metabolic by virtue, are both presumed to be temperature dependent. This would explain the stronger correlation and affinity between temperature and insecticide [16]. Fipronil and emamectin benzoate showed positive correlation with temperature because of reduction in biotransformation results of the original compounds to lesser toxic intermediary metabolites at elevated temperature level than the compounds

formed through regular biotransformation [17]. Cognizance pertaining to the biological process of biotransformation lessening the toxic effect of insecticides at lower temperature cannot be expunged as be revealed in case of *Chironomus dilutus* [18]. Hence it is quite evident to from the present study that, among the five test insecticides emamectin benzoate may be a good option to create drastic effect on the DBM under warmer climates.

Studies conducted elsewhere revealed that the toxicity of novel insecticides and avermectins increased with the increase in temperature and positive temperature coefficient in neonicotinoids and avermectin [10, 16]. On the contrary deltamethrin and spinosad showed a negative temperature coefficient [19]. The toxicity of pyrethroids such as deltamethrin decreased significantly with increase in temperature against *Phenacoccus solenopsis* (Tinsley). It was attributed to the fact that pyrethroid insecticides possessed a property of showing reduced efficacy at high temperature [9]. Enhanced neuron sensitivity caused frequent nerve firing at temperature between 15-20°C in case of pyrethroid exposed insects. At some stage of action mechanism the sodium ion channels and the sodium ions in the nerve impulse movement are controlled by pyrethroid insecticides [20]. However, reverse relationship at high temperature has been observed in *P. solenopsis* [21]. Increased biotransformation and decreased sodium influx [21, 18] are some mentioned reasons exacerbating the pyrethroid toxicity and causing the mortality at high temperature.

Overall results from the present study indicated that there was a direct significant relationship between temperature and toxicity of all test insecticides. Comparison of $LC_{50}$ values at higher temperature were lesser than $LC_{50}$ values at other lower temperature in all test insecticides in present study. Therefore all insecticides had positive temperature coefficient for DBM. On contrary negative interaction between temperature and efficacy of spinosad was observed in case of studies conducted on *O. nibulalis* from 24 °C to 35 °C [13] and grass hopper *Melanoplus differentialis* (Thomas) [22] were spinosad provided different levels of mortality at each temperature and mortality of was lower at 15 °C but increased as temperature decreased.

Emamectin benzoate showed positive temperature coefficient in our studies as observed. The results are in accordance with that of earlier studies [14] where the toxicity of emamectin to DBM larvae at different temperatures 16 °C to 31°C evaluated and revealed that toxicity of emamectin against third instar larvae of DBM increased as the temperature increased. Similarly results were observed in evaluating the effect of post treatment temperature on insecticide toxicity of emamectin benzoate to *P. solenopsis* and revealed that toxicity of emamectin benzoate enhanced 1.64 folds for the temperature range of 20 °C-32 °C [18]. Earlier studies of evaluating abamectin (avermectin) toxicity exhibited increased toxicity with increasing temperature from 17 °C-37 °C against *D. citri* adults [10].

Fipronil toxicity was also directly proportional to the temperatures assessed in the present study and showed positive temperature coefficient. Similar results were observed in the toxicity assessment of butene-fipronil to *P. xylostella* which showed toxicity increased by 15.49 folds as temperature escalated from 15 °C to 32 °C [23].

**Table 1:** Effect of temperature on the toxicity of insecticides to the larvae of *Plutella xylostella*

| Insecticides | Temp. (°C) | $LC_{50}$ (%) | 95% CL | Slope | $\chi^2$ (df) | Temperature coefficient | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 5 °C | 10 °C | 15 °C |
| Emamectin benzoate | 20 | 0.0031 | (0.0021-0.0048) | 1.971 | 0.994 | - | - | - |
| | 25 | 0.0027 | (0.0018-0.0042) | 2.019 | 0.994 | 1.15 | - | - |
| | 30 | 0.0024 | (0.0015-0.0039) | 1.889 | 0.997 | 1.13 | 1.29 | - |
| | 35 | 0.0022 | (0.0013-0.0037) | 2.009 | 0.993 | 1.10 | 1.23 | 1.40 |
| Chlorantraniliprole | 20 | 0.0845 | (0.0460-0.1566) | 1.410 | 0.991 | - | - | - |
| | 25 | 0.0687 | (0.0358 - 0.1318 | 1.294 | 0.986 | 1.23 | - | - |
| | 30 | 0.0494 | (0.0239 - 0.1022) | 1.136 | 0.998 | 1.39 | 1.71 | - |
| | 35 | 0.0359 | (0.0190 - 0.0678) | 1.309 | 0.993 | 1.38 | 1.91 | 2.35 |
| Chlorfenapyr | 20 | 0.0277 | (0.0193 - 0.0398) | 2.338 | 1.000 | - | - | - |
| | 25 | 0.0206 | (0.0136 - 0.0312) | 2.038 | 0.997 | 1.34 | - | - |
| | 30 | 0.0187 | (0.0125 - 0.0283) | 2.074 | 0.993 | 1.10 | 1.48 | - |
| | 35 | 0.0175 | (0.0121 - 0.0253) | 2.381 | 0.992 | 1.07 | 1.18 | 1.58 |
| Fipronil | 20 | 0.0313 | (0.0141 - 0.0695) | 1.058 | 0.999 | - | - | - |
| | 25 | 0.0245 | (0.0124 - 0.0486) | 1.236 | 0.999 | 1.28 | - | - |
| | 30 | 0.0180 | (0.0085 - 0.0381) | 1.108 | 0.998 | 1.36 | 1.74 | - |
| | 35 | 0.0128 | (0.0064 - 0.0256) | 1.195 | 1.000 | 1.41 | 1.91 | 2.45 |
| Flubendiamide | 20 | 0.0441 | (0.0265-0.0734) | 1.654 | 0.998 | - | - | - |
| | 25 | 0.0331 | (0.0170-0.0536) | 1.729 | 0.987 | 1.33 | - | - |
| | 30 | 0.0294 | (0.0224-0.0497) | 1.565 | 0.987 | 1.13 | 1.50 | - |
| | 35 | 0.0203 | (0.0131-0.0315) | 1.904 | 0.977 | 1.45 | 1.63 | 2.17 |

## Conclusions

Knowledge pertaining to insecticidal and its product temperature coefficient will enable pest managers to select a product that is efficacious under given environmental condition. This information will provide insight in designing proper chemical management strategy for control of *P. xylostella* in the present variable prevailing temperatures of changing climatic condition resulting into global warming.

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