



Eco-toxicological risk and impact of pesticides on important parasitoids of cabbage butterflies in cruciferous ecosystem



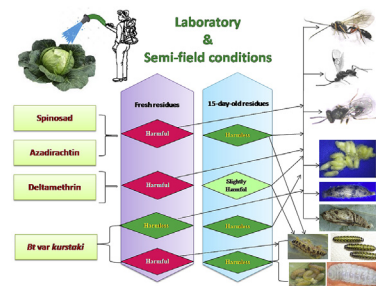
D.M. Firake*, D.P. Thubru, G.T. Behere

Division of Crop Protection (Entomology), ICAR Research Complex for North Eastern Hill Region, Umroi Road, Umiam, Meghalaya 793103, India

HIGHLIGHTS

- Impact of pesticides assessed on *Hyposoter ebeninus*, *Cotesia glomerata* and *Pteromalus puparum*.
- Fresh residues of deltamethrin, spinosad and azadirachtin were harmful to parasitoid females, grubs and cocoons.
- *Bacillus thuringiensis* var *kurstaki* was harmless to parasitoid females and cocoons.
- Deltamethrin spraying should be discouraged on cabbage.
- Spinosad and azadirachtin may cautiously be used considering peak period of natural enemies.

GRAPHICAL ABSTRACT



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ABSTRACT

Eco-toxicological risk and impact of pesticides was estimated on three important parasitoids of butterflies viz., *Hyposoter ebeninus*, *Cotesia glomerata* and *Pteromalus puparum*. Four commonly used pesticides were evaluated using standard protocol (of IOBC/WPRS-group). In laboratory tests, the survival of the female wasps decreased significantly on fresh contact and ingestion of deltamethrin, spinosad and azadirachtin; whereas *Bacillus thuringiensis* var *kurstaki* (*Btk*) was found harmless pesticide. Under semi-field conditions, parasitoid mortality decreased significantly on fresh contact with the pesticides. Although, at 72 h after treatment, spinosad and deltamethrin were found harmful (Class-IV) and azadirachtin was moderately harmful (Class-III), whereas *Btk* was harmless (Class-I). Furthermore, 15-day-old residues of pesticides (except deltamethrin) were harmless to all parasitoid species under semi-field conditions. Notably, adult emergence and pupal duration in pesticide-treated cocoons were not significantly affected; however, their survival decreased after emergence except in *Btk*. The contact and oral toxicity trends of the pesticides were almost similar for three species of parasitoid females and pupae; however little variability was observed in toxicity to the host caterpillars parasitized by *H. ebeninus* (HCPHE) and *C. glomerata* (HCPCG). In semi-field tests, fresh residues of all the pesticides were harmful to HCPHE and HCPCG. However, action of *Btk* was slightly delayed and toxicity was rather low for HCPCG. In 15-day-old residues, deltamethrin and azadirachtin were slightly harmful to the parasitized caterpillars, whereas those of *Btk* and spinosad were harmless. Since, *Btk* appeared to be safe for parasitoids; it could be used for managing cabbage butterflies in brassicaceous crops.

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* Corresponding author.

E-mail addresses: dfirake@gmail.com (D.M. Firake), d.thubru@gmail.com (D.P. Thubru), ganeshbehere@gmail.com (G.T. Behere).

1. Introduction

Cabbage butterflies of the genus *Pieris* (Lepidoptera: Pieridae) are the most destructive pests of brassicaceous crops throughout the World (Harvey et al., 2010; Lytan and Firake, 2012). The large white cabbage butterfly, *Pieris brassicae* and Indian cabbage white, *Pieris canidia* are two important defoliators of cruciferous vegetables and brassica crops in India (Azad Thakur et al., 2012). The *P. brassicae* is, however the widely distributed and most destructive species, attacking on a variety of cruciferous vegetables, including the cabbage, cauliflower, knol-khol and broccoli. The gregarious caterpillars of *P. brassicae* feed voraciously on the leaves; defoliate the plants and reducing the market value of the resultant vegetables (Firake et al., 2012). *Hyposoter ebeninus* (Hymenoptera: Ichneumonidae) and *Cotesia glomerata* (Hymenoptera: Braconidae) are major solitary and gregarious endo-larval koinobiont parasitoids, respectively, of cabbage butterflies in different parts of the World (Harvey et al., 2010; Firake et al., 2012). The former species feeds on the internal tissues of growing caterpillars leaving only the head capsules and pupate inside the host carcass (Firake et al., 2012); while the latter species feeds on the haemolymph and fat bodies of the grown up caterpillars and pupate outside the host body (Harvey and Malcicka, 2016). The *Pteromalus puparum* (Hymenoptera: Pteromalidae) is an idiobiont gregarious, ectoparasitic wasp that, parasitizes the pupae of pierids and considerably reduces their damage in a field, especially when the population of the larval parasitoids is low (Firake D M, unpublished data). Accordingly, these three parasitoids play a significant role in natural management of cabbage butterflies; therefore, their conservation is extremely important in a field.

Because of high fecundity of pierids and a low market tolerance of cole crops to pest damage, intervention using pesticides becomes essential for enhancing the marketable crop yield. Several pesticides, including synthetic inorganics, botanicals and those of microbial origin are being used in cruciferous ecosystems to manage the defoliators. The application of pesticides to a field with natural enemies of crop pests can result in serious consequences for these bio-control agents. Under field conditions, parasitoid wasps are frequently affected by direct contact with the pesticide-treated plant parts during foraging, while finding mates or grooming, and when feeding on contaminated water or honeydew available on plant parts in the sprayed field (Croft, 1990; Desneux et al., 2007; Biondi et al., 2012). Parasitoids are also indirectly affected as during their immature stages, they live and feed inside the host body (Thubru et al., 2016). In many cases, parasitoid grubs die along with their hosts (Ebrahimi et al., 2012).

Being larval endo-parasitoids, female *H. ebeninus* and *C. glomerata* stays for a longer time on plant parts for host finding and foraging (Plate 1). Parasitoid wasp, *P. puparum* also habitually sits on the pre-pupae to feed on host haemolymph and stays nearby for an additional 2–3 days until they lay eggs on the host pupa (Plate 2). To initiate the oogenesis, it is essential for the *P. puparum* female to feed on the host haemolymph by creating a wound in the host cuticle by repeatedly inserting and removing her ovipositor over the course of several minutes (Harvey et al., 2007). Consequently, these parasitoids make contact with the pesticide treated areas for a longer time, leading to toxicity. As the parasitism changes with the host population, therefore managing the menace of the cabbage butterflies with pesticides during peak season may pose serious risks to these parasitoids.

Pesticides, besides having lethal effects, also affect the demographic traits of a parasitoid wasp and its biocontrol services through sublethal effects, e.g. reduces parasitism potential, longevity, adult emergence and alter the sex ratio, etc (Croft, 1990; Desneux et al., 2007; Firake and Khan, 2010; Biondi et al., 2012;

Thubru et al., 2016). Some pesticides act on functioning of nervous system of insect (e.g. deltamethrin, spinosad); few act on mid-gut membrane (e.g. *Bacillus thuringiensis*); while pesticides like neem products have unknown or uncertain mode of actions (<http://www.irac-online.org/modes-of-action/>). Accordingly, detailed ecotoxicological studies can give useful insight into the possible adverse effects of pesticides on parasitoid performance and survival. This information can also assist in identifying safest pesticides with appropriate timing of applications, which can manage the pests without adversely affecting their natural enemies (Hafez et al., 1999). Very limited information is so far available on potential effects of pesticides on natural enemies, especially parasitoids of the cabbage butterfly. We studied the lethal and sub-lethal effects of fresh and 15 day old residues of widely used pesticides on three important parasitoids of cabbage butterflies under the laboratory and semi-field conditions.

2. Materials and methods

A series of experiments was carried out at the Division of Crop Protection, ICAR Research Complex for NEH region, Umiam, Meghalaya during 2012–2015. The spinosad, *Bacillus thuringiensis* var *kurstaki* (*Btk*), neem based pesticides, fenvalerate, carbaryl, deltamethrin, chlorpyrifos and fipronil are commonly used for the management of defoliators in brassicaceous crops. Considering the distinct mode of actions, four pesticides viz., spinosad 2.5 SC @ 1.25 ml/l, azadirachtin 0.15 EC @ 2 ml/l, *Bt* var *kurstaki* SP (contain 8% delta endotoxin and active spores each, W/W%) @ 2 gm/l and deltamethrin 2.8 EC @ 1 ml/l water were evaluated at their field doses against three major parasitoids of *P. brassicae*.

2.1. Source and mass rearing of parasitoids

Pupae of *H. ebeninus*, *C. glomerata* and *P. puparum* were collected from the entomology farm of the institute, Umiam (25°41'01.91"N, 91°54'46.24"E) and mass multiplied in a laboratory using the host, *P. brassicae* at ambient conditions (19 ± 2 °C temperature, 70 ± 5% relative humidity and 14:10, light: dark period).

2.1.1. Mass rearing of the host, *P. brassicae*

Larvae of *P. brassicae* were collected from the entomology farm of the institute and mass reared on cabbage plants in the laboratory. After emergence, adult butterflies were released inside the net cage (3 m length, 3 m width and 2 m height) for egg laying on potted cabbage plants. Adults were fed with water and honey *ad libitum*. Besides, large numbers of egg masses of *P. brassicae* were collected from the field. Egg masses obtained from oviposition cages and the fields were kept separately in plastic tubs until hatching. Newly hatched larvae of *P. brassicae* were released gently on fresh tender cabbage leaves with the help of a camel hair brush. Caterpillars of *P. brassicae* were allowed to feed on cabbage leaves until pupation. Hygienic conditions were maintained throughout the rearing and fresh cabbage leaves were provided as and when necessary. *P. brassicae* completes the larval growth with five instars and takes 35–38 days to complete the development period (Lytan and Firake, 2012). Butterfly chrysalis were collected after 3 days of formation and kept separately in the cages for adult emergence. Newly emerged adults were allowed to mate and 3 days old emerged butterflies were released in the oviposition cages with potted cabbage plants for egg laying. Field collected egg masses of *P. brassicae* were also supplemented to the laboratory culture.

2.1.2. Mass rearing of the larval parasitoids, *H. ebeninus* and *C. glomerata*

The cocoons of *H. ebeninus* and *C. glomerata* were collected from

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