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RESIDUAL TOXICITY OF NEEM-BASED INSECTICIDES AGAINST EGG PARASITOID *TRICHOGRAMMATOIDEA BACTRAE* NAGARAJA

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ABSTRACT

A study was carried out with neem-based insecticides, viz., azadirachtin (0.03%EC, 0.15%EC, 0.3%EC, 1%EC), neem seed oil (1, 2 and 3%), neem seed extract 5% and dashparni ark (ten plant leaf extract) 4%, on *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) adults using the thin film residue method under laboratory conditions. After coating glass vials with neem-based insecticides, the adults of *T. bactrae* were released on the first, fifth, and tenth days of coating and the mortality was assessed. Among all neem-based insecticides, azadirachtin 1%EC was observed to be harmful to adults of *T. bactrae* on the first, fifth and tenth days. In contrast, neem seed extract 5% and dashparni ark 4% were found safe.

Key words: *Trichogrammatoidea bactrae*, neem-based insecticides, coating, residue, contact toxicity, azadirachtin, EC, neem seed oil, need seed extract, adult mortality

Egg parasitoid *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) is used in cotton against the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). This egg parasitoid was native to Australia and introduced into cotton in California and Arizona in 1993 (Naranjo, 1993). The natural enemies are constantly exposed to insecticides in the field, which reduces their efficacy in biological control programmes. The neem-based insecticides are an important component of pest management and are economically and ecologically safe. From the first introduction, the usage of neem-based insecticides or botanicals dramatically increased in the cotton crop due to the off-target effect of pesticides on natural enemies and often limit productivity instead of improving and causing insecticide resistance, resurgence, and associated hazards to the environment. The neem-based botanical insecticides and their products are the alternatives to synthetic chemical insecticides since botanical insecticides cause low mammalian toxicity, highly safer to beneficial, non-target organisms and the environment (Miresmailli and Isman, 2014). The triterpenoid (azadirachtin) found in seeds of the neem tree has insecticidal properties and is expressed in the form of feeding and oviposition deterrence, growth inhibition, fecundity and fitness reductions (Schmutterer, 1990) and exhibits good efficacy against key insect pests in different crops. Neem products are highly photosensitive and natural sunlight degrades the insecticidal properties; hence, neem has a low residual effect under field conditions, which affects the reproducibility of the insecticide effect (Isman, 2006). However, neem-based insecticides also impart an effect on natural enemies (parasitoids and predators) and beneficial insects. Farmers usually follow a faulty procedure of non-judiciously using *Trichogramma bactrae* before or after sprayings of insecticides or neem-based botanicals without considering safe waiting periods for the use of natural enemies. Further, in day-to-day life, the number of farmers is increasing towards natural farming and several botanicals are being developed and recommended against several pests. Therefore, laboratory evaluation of the reduction in the beneficial capacity of the biocontrol agents due to biopesticides can serve as a relevant parameter for evaluation of their safety and determine the suitability of botanicals that can be integrated along with *Trichogramma* applications and to find out safe waiting periods for other such botanicals, and hence this study.

MATERIALS AND METHODS

The laboratory host, rice moth *Corcyra cephalonica* Stainton was reared in the Biocontrol Laboratory, Department of Entomology Dr P D K V Akola. Nucleus culture of *T. bactrae* Nagaraja was procured from the ICAR-NBAIR, Bengaluru and was maintained in the laboratory on the eggs of *C. cephalonica* at ambient temperature. Plastic container of 3 kg capacity was

taken and filled with ingredients of 2.5 kg crushed sorghum grains, 100 g crushed groundnut kernels, 5 g yeast powder, 5 g sulphur powder and sprayed with 0.05% streptomycin sulphate. Then 0.5 cc *Corcyra* eggs were sprinkled on the surface of mixture in the plastic container and covered it with lid. Plastic containers were kept in iron rack for emergence of *Corcyra* moths for 35-40 days. After 40 days, the *Corcyra* adults were collected in test tube, and released in the mating chamber for oviposition and these chambers were kept on the plastic tray for the collection of eggs, with 50% honey provided by soaking cotton as a diet. The eggs were collected from the base of mating chamber on every day and used for *Trichogramma* rearing as a factitious host. *Trichogrammatoidea bactrae* Nagaraja procured was used; glue was applied on the trichocard uniformly and one-c.c *Corcyra* eggs were sprinkled evenly on the trichocards with the help of mesh and cards were allowed to dry under the blower. Trichocards were kept under the U V light for 1 to 1.5 hr to kill the embryo. After U.V, treatment the cards were exposed to *Trichogrammatoidea bactrae* adults for oviposition for up to 24 hr and then cards were removed and placed separately.

On the 4th or 5th day after oviposition the eggs were turn to black, which indicates parasitization. These cards were ready for the field release in cotton ecosystem against pink bollworm. Then 3 to 4 days after parasitization the adults were released from the eggs. Commercial formulations of nine botanical insecticides were obtained from the market, selected on the basis of current and potential use for use in cotton. These include the neem formulations- azadirachtin 0.03%EC, 0.15%EC, 0.3, 1%EC (CIBRC recommendations) and neem seed oil (1, 2, 3%) and need seed extract 5% and dashparni ark (ten-leaf extract). These were tested at their field recommended concentration for their contact toxicity with residual dry-film bioassay method. Stock solutions for the dry-film method were prepared by mixing botanicals with acetone and the test solutions were prepared by diluting the stock solutions with distilled water just before use. Test concentrations were selected according to the recommended field application dosages. The treatments were fixed based on the recommendations given by Central Insecticide Board and Registration committee (CIBRC) for cotton. The dry-film method reported by Li et al. (1986) was used. Stock solutions were prepared by dissolving technical botanicals in acetone, with 1 ml of each added, respectively, into flat-bottomed glass vial (7.5 cm x 1.0 cm) and dried thoroughly by rotating the glass tubes in the fly wheel so that a uniform insecticide film is formed on the inner walls of the glass vial. After the solvent evaporated, 16 adult *T. bactrae* aged at 4-6 hr after emergence were released into each tube. The *T. bactrae* were transferred into botanical coated tubes after 1st, 5th and 10th days of treatment to the glass vials and fed with 10% honey. The botanical coated tubes were sealed with black cloths and put into a growth chamber. Acetone was used as solvent control; each treatment was repeated three times. The number of the live and dead adults was checked after 24 hr and percent mortality was calculated. An adult was deemed dead if it exhibited no movement when its body was touched with a fine brush. The mean adult mortality of *T. bactrae* were subjected to ANOVA, and mean values were compared by Tukey's Test ($p = 0.05$), using SPSS program, version 23 (SPSS 2015) and treatment means were separated at $p = 0.05$.

RESULTS AND DISCUSSION

The mortality values of *T. bactrae* adults released on first, 5th and 10th day after treatment are given in Table 1; these reveal significant differences among the treatments. Among the botanicals tested on 1st day after treatment, treatment T8 (NSE 5%) was found significantly safer recording 50% mortality and 100% mortality was recorded with T4 (azadirachtin 1%EC) indicating its harmfulness at higher dose. On 5th day treatment, the data revealed that treatment T8 (NSE 5%) recorded significantly less adult mortality of 33.33% followed by T9 (*Dashparni Ark*) with 37.5%, followed by others; treatment T4 (azadirachtin 1%EC) led to 91.67% adult mortality. On 10th-day of treatment, significantly less adult mortality of 29.17% was observed with treatments T8 and T9. The results derive support from Narendra Kumar et al. (2015) on azadirachtin 0.03% or 300 ppm that it was harmful to *T. chilonis* adults. Raguraman and Kannan (2014) reported that the plant derived insecticides show slight to moderate ill effects on parasitoids, predators and honey bees. Parasitoids are also susceptible, when they come in direct contact with plant origin insecticides including neem products. Sakthivel and Qadri (2010) observed that botanicals were slight or least toxic towards the population buildup of predatory coccinellids. The present findings revealed that botanicals were also have a significant effect on *T. bactrae;* but after 5-10 days of exposure, there was no significant effect on natural enemies. The safest botanical for *T. bactrae* adults was NSE 5% and *Dashparni ark* 4%, and azadirachtin 10000 ppm was found harmful.

Table 1. Persistence of botanicals against adults of *T. bactrae* (exposed after 1st, 5th, 10th day of treatment)

Treatments details	Concentrations (g or ml/ l)	% adult mortality/ 16 adults* after Ist, 5th and 10th day of treatment		
		1st day	5th day	10th day
T1 Azadirachtin 0.03%EC	10 ml/ l	87.50±7.21^{abc}	62.50±7.21^{bcd}	41.66±4.16^{bc}
T2 Azadirachtin 0.15%EC	10 ml/ l	83.33±11.02^{abc}	79.16±4.16^{ab}	54.16±4.16^{abc}
T3 Azadirachtin 0.3%EC	4 ml/ l	87.50±7.21^{abc}	75.00±7.21^{abc}	66.66±11.02^{ab}
T4 Azadirachtin 1%EC	3 ml/ l	100.00±0.00^{a}	91.66±4.16^{a}	75.00±7.21^{a}
T5 Neem seed oil 1%	10 ml/ l	58.33±11.02^{bc}	41.66±4.16^{de}	33.33±4.16^{c}
T6 Neem seed oil 2%	20 ml/ l	70.83±4.16^{abc}	50.00±7.21^{cde}	45.83±4.16^{abc}
T7 Neem seed oil 3%	30 ml/ l	91.66±8.33^{ab}	70.83±4.16^{abc}	50.00±7.21^{abc}
T8 Neem seed extract 5%	5 ml/ l	50.00±7.21^{c}	33.33±4.16^{e}	29.16±4.16^{c}
T9 Dashparni ark 4%	4 ml/ l	54.16±8.33^{bc}	37.50±7.21^{de}	29.16±4.16^{c}
f value		5.32	12.85	7.10
P value		0.02	<0.00	<0.00

Mean mortality (%)± standard error; Treatment columns bearing different letters significantly different- Tukey's test (p = 0.05); *Values represent means of 3 replicates.

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AUTHOR CONTRIBUTION STATEMENT

JSVM conducted the experiments, gathered and examined the data, and authored the manuscript. NSS oversaw the experiments, offered technical assistance, and performed a thorough review of the manuscript. DBU and SKB made significant revisions to it. All authors reviewed and endorsed the final version of the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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