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BIOSAFETY OF BOTANICALS TO *TRICHOGRAMMATOIDEA BACTRAE* NAGARAJA

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ABSTRACT

Bioassay was conducted with nine botanical extracts at various concentrations viz., azadirachtin 300 ppm, 1500 ppm, 3000 ppm, 10000 ppm, neem seed oil 1%, 2%, 3%, neem seed extract 5% and dashparniark 4% on *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) in completely randomized design. Assessment of these on % parasitization, reduction in parasitism and adult emergence of *T. bactrae* on *Corcyra* eggs was done. The results revealed that dashparniark (ten plant leaves extract) 4% was found safer with maximum parasitization (53.3%) and minimum reduction in parasitization (27.9%). Azadirachtin 10000 ppm was found harmful resulting in reduced parasitization (12.6%) and adult emergence (32.6%); and maximum reduction in parasitism (82.9%) on *Corcyra* eggs was observed. Maximum % adult emergence was recorded when parasitized eggs were treated with neem seed oil 1% (64.3%).

Keywords: *Trichogrammatoidea bactrae*, parasitization, *Corcyra cephalonica*, azadirachtin, neem seed oil, adult emergence, dashparni ark, neem seed extract, biosafety, bioassay.

Trichogrammatoidea bactrae Nagaraja (Hymenoptera: Trichogrammatidae) is the most important and effective egg parasitiod of pink bollworm, *Pectinophora gossypiella* (Saunders). In 1993, *T. bactrae* was introduced from Australia into California and Arizona for control of the pink bollworm in cotton (Naranjo, 1993). This parasitoid is widely distributed in the Oriental region, adapted to terrestrial humid habitats and parasitise various insect pests of cotton, sugarcane, fruits and vegetables (Nagaraja, 1978). This parasitoid was found to attack eggs of many lepidopteran insect pests such as *Helicoverpa armigera* Hubner and *Atherigona soccata* Rondani (Rao et al., 1987), *Earias vitella* F., *Achanthodelta janata* L. (Rao et al., 1980), and *Pectinophera gossypiella* (Saunders) (Hutchinson et al., 1990). In cotton, against the pink bollworm *T. bactrae* is recommended @ 60000/acre (Nagrare et al., 2018). Twelve sequential releases of *T. bactrae* showed a significant decrease in pink bollworm incidence (Kumara et al., 2022) and thus the release was as effective as insecticide (Naik et al., 2019). Due to the increased awareness regarding the deleterious effect of pesticide cotton cultivation is slowly shifting to organic agriculture. According to latest reports, the organic cotton production has increased (PIB, 2022). Hence, for the management of pests and diseases the botanicals and bioagents play prominent role, mostly dominated by neem-based botanicals (Mageshwaran et al., 2019). The active ingredient of neem formulations is azadirachtin and it is used as antifeedant. Apart from these several plant products have been utilized for the management of insect pests. The botanicals used in such ecosystem can affect parasitization or activity of natural enemies. The exposure of *T. bactrae* to different botanicals used in cotton ecosystem is possible which might affect the parasitizing behaviour and emergence of the parasitoid. Hence, the present study the focusing on the biosafety aspects of botanicals on *T. bactrae.*

MATERIALS AND METHODS

The experiments were conducted at the Biological Control Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Dr PDKV, Akola, Maharashtra during 2020-2021. For the rearing of factitious *Corcyra cephalonica* standard procedure was follwed and reared in plastic containers, and oviposition chambers. The eggs were collected from the base of oviposition chamber daily and used for rearing of *Trichogramma*. For the rearing of the parasitoid *T. bactrae*, the nucleus culture was procured from the National Bureau of Agricultural Insect Resources (NBAIR) Bangalore, India. The gum arabic was smeared on the trichocards uniformly. Then, one cc of *Corcyra* eggs was sprinkled evenly on the trichocards with mesh and cards were allowed to dry under the blower. Cards were kept under the UV light for 1 to

1.5 hr to kill the embryo. After UV treatment, the cards were exposed to *T. bactrae* adults for oviposition up to 24 hr. Then cards were removed and placed separately. On 4th or 5th day after oviposition the eggs turn black indicating parasitization. Then 3 to 4 days after parasitization the adults emerged from the eggs. The host insect *C. cephalonica* and the parasitiod *T. bactrae* reared (25±2ºC) 65-75% RH. The commercial formulations of botanical insecticides were obtained from market and their concentrations were fixed based on recommendation of the Central Insecticide Board and Registration Committee (CIBRC, 2021).

The card dip bioassay methodology was followed as given in Consoli et al. (2000), Bastos et al. (2006) and Chen et al. (2013). Irradiated fresh eggs of *C. cephalonica* were glued to the egg cards separately (@50 eggs per card strip). Each card strip dipped in test botanicals for 5 sec., and for control water was used. These treated card strips were dried under shades and was kept in glass vials (15.0×2.5 cm) @ 1 card strip/ vial. Each treatment was then labelled properly with details such as name of treatment, concentration of botanicals, date and time of application. The treated strips were exposed to adults of *T. bactrae* (@ 5:1 host: parasitoid ratio) for 24 hr for parasitization. The eggs cards were examined for parasitization after four to five days and the parasitized eggs were counted under the stereozoom microscope. For adult emergence, the already parasitized eggs (@50 eggs/ card strip) of *Corcyra* by *T. bactrae* were treated with test botanicals by same procedure as mentioned above. Such treated strips were then shade dried and placed individually in plastic vials (2.5×6 cm). Each treatment was labelled properly and observations on number of adults emerged made. The data ($\bar{x}$ ± SE) were subjected to ANOVA in completely randomized design and mean values were compared by Tukey's post-hoc test, using SPSS program, version 23 (SPSS 2015) and treatment means were separated at p= 0.05.

RESULTS AND DISCUSSION

The results given in Table 1 reveal significant differences among the treatments in parasitization, reduction in parasitization and adult emergence. Significantly maximum parasitization and adult emergence of 74 and 86.33% was in control; among the treatments, T_9 (dashparniark-ten plant leaves extract) was the safest (53.33% parasitization). It was followed by treatment T_8 (NSE 5%). Minimum % parasitization was recorded in T_4 (azadirachtin 10000 ppm) with 12.67% parasitization. The treatment T_9 (dashparniark) was thus found significantly safer followed by treatment T_8 (NSE 5%). Among the botanicals tested, treatments T_5 (neem seed oil 1%), T_8 (NSE 5%), T_9 (dashparniark) and T_6(neem seed oil 2%) were found significantly superior with 64.33, 62.33, 60.33 and 55% adult emergence, respectively and were at par.

Rao and Raguraman (2005), the aqueous neem seed extract 5% was safer to natural enemies. Observed that Singh (2015) has also reported that neem seed extract 5% and neem gold 5% were safer to *T. chilonis.* However, Narendra et al. (2013) found that azadirachtin

Table 1. Effect of botanicals on *T. bactrae* reared on *C. cephalonica* eggs

Treatment details	Dose ha^{-1} (gm. or ml/ l)	% parasitization	% reduction in parasitization	% adult emergence	Score
T_1 Azadirachtin 300 ppm	10 ml/ l	19± 2.30^{de}	74.40± 2.77^{ab}	54.33± 0.88^{bc}	Slightly harmful
T_2 Azadirachtin 1500 ppm	10 ml/ l	15± 1.2^{de}	79.32± 1.27^{a}	45± 2.30^{cd}	Moderately harmful
T_3 Azadirachtin 3000 ppm	4 ml/ l	17.33± 0.33^{de}	76.56± 0.39^{ab}	31.67± 1.85^{d}	Slightly harmful
T_4 Azadirachtin 10000 ppm	3 ml/ l	12.67± 1.45^{e}	82.93± 1.70^{a}	32.67± 2.72^{d}	Moderately harmful
T_5 Neem seed oil 1%	10 ml/ l	30± 2.08^{c}	59.42± 2.83^{c}	64.33± 2.02^{b}	Slightly harmful
T_6 Neem seed oil 2%	20 ml/ l	25.33± 4.33^{cd}	65.69± 5.88^{bc}	55± 1.00^{bc}	Slightly harmful
T_7 Neem seed oil 3%	30 ml/ l	13.33± 1.20^{e}	81.90±1.94^{a}	34±2.51^{d}	Moderately harmful
T_8 Neem seed extract 5%	5 ml/ l	46± 2.00^{b}	37.89± 1.49^{d}	62.33± 1.45^{b}	Slightly harmful
T_9 Dashparni ark 4%	4 ml/ l	53.33± 1.33^{b}	27.88± 1.88^{d}	60.33± 8.00^{bc}	Harmless
T_{10} Control		74± 1.52^{a}	---	86.33± 2.7^{a}	
F value		F= 102.10* df= 9,20	F=55.39* df= 8,18	F= 29.14* df= 9,20	

Treatment columns bearing different letters significantly different from other treatments according to Tukey's Post-hoc test* (p = 0.05); Means values represents 3 replicates; Scores given according to % reduction in parasitization as suggested by IOBC classification which is harmless <30%, slightly harmful 30-79% moderately harmful 79-99% harmful >99%. (Sterk et al*.,* 1999, Pawar et al*.,* 2020)

1 ml/ l has significantly reduced parasitization by *T. chilonis*. Azadirachtin formulations were found to affect the parasitization adversely. Rao and Raguraman (2005) reported that neem oil 3.0% was relatively toxic when compared to other botanicals against *T. chilonis* Ishii and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), which corroborates present findings. Neem seed oil 3% was found harmful for parasitization by *T. bactrae*. Raguraman and Singh (1999) also reported that the neem seed oil at 0.3% deterred oviposition (parasitization) by *T. chilonis*. Babasaheb et al. (2009) stated that neem seed oil @ 1% reduced the parasitization but it did not affect the development and emergence. Parreira et al. (2018) revealed that all oils reduced the parasitism rate. Rampelotti-Ferreira et al. (2017) found that the neem oil treatment showed 67% reduction in parasitism by *T. pretiosum.* Earlier Selvamuthukumaran and Jayakumar (2017) reported 76.29% adult emergence of *T. chilonis* when treated with NSE 5%, which is in line with present findings. It was also revealed that five leaf extracts at 10% had 69.32% adult emergence of *T. chilonis*. In the present study, dashparni showed 60.33% adult emergence of *T. bactrae.* Singh (2015) revealed that neem seed extract 5% and neem gold 5% were safer for adult emergence of *T. chilonis.* In the present study, neem seed oil 1% (T_5) recorded maximum % adult emergence (64.33%) followed by T_6, neem seed oil 2% (55%) and T_7, neem seed oil 3% (34%). These findings derive support from Selvamuthukumaran and Jayakumar (2017), on the neem seed oil. Narendra et al. (2013) revealed 78.95% adult emergence with *T. chilonis,* when parasitized eggs were treated with azadirachtin 1 ml/ l. Saber et al. (2004), reported that the effect of neemazal (azadirachtin 10000 ppm) on the egg parasitoid was tested by dipping parasitized *Sitotroga cerealella* (Olivier) and *Cydia pomonella* (L.) eggs at the field recommended concentration. These revealed that adult parasitoids are adversely affected. Sharma and Agarwal (2019) observed the effect of azadirachtin 10000 ppm on adult emergence of *T. chilonis* and *T. japonicum*. These studies revealed that two-fold doses significantly disrupted the adult emergence of *T. chilonis* and *T. japonicum*. Alcántara-de la Cruz et al. (2021) stated that essential of *A. indica* affected at-least one biological attribute of *Trichogramma galloi*. Asrar et al. (2022) concluded that neem seed oil is relatively safer to *T. chilonis*. Gladenucci et al. (2020) reported that botanical extracts initially reduced total number of parasitized eggs (*T. pretiosum*) and female longevity. Parreira et al. (2019) revealed that essentials oils are harmful to parasitism.

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AUTHOR CONTRIBUTION STATEMENT

JSVM carried out the experiments, collected and analysed the data, and wrote the manuscript. NSS supervised the experiments, provided technical support, and critically revised the manuscript. DBU and SKB substantively revised it. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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