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Biosafety Evaluation of *Tephrosia purpurea* Stem-based Formulation (Telp 3% EC) Against Three *Rhynocoris* species

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ABSTRACT

To utilize a plant-based insecticide, the study of biosafety of botanical insecticide to polyphagous natural enemies are imperative. A laboratory trial was conducted to investigate the biosafety of *Tephrosia purpurea* stem-based formulation (Telp 3% EC) against three reduviid predators such as, *Rhynocoris fuscipes*, *Rhynocoris marginatus* and *Rhynocoris longifrons* adults using Y-shaped olfactometer considering olfactory response as a tool. Telp 3% EC was impregnated in Whatman No. 1 filter paper, Bt cotton leaves (BT bunny) and groundnut leaves (TMV 4). The Access Proportion Index (API) was calculated in different time intervals like 20, 40 and 60 min. Olfactory response results revealed that impregnation of Telp 3% EC in Bt cotton leaves does not deter *Rhynocoris longifrons* olfactory response whereas, groundnut leaves deter both *Rhynocoris fuscipes* and *Rhynocoris marginatus* olfactory response. It has been concluded from the results that Telp 3% EC can be incorporated along with reduviid predators in BT cotton pest management. However, detailed studies are necessary to confirm the observation.

Key words: Biosafety, *Rhynocoris* spp., olfactory response, Telp 3% EC

INTRODUCTION

Reduviidae constitute an important group of predatory insects in various parts of the world. *Rhynocoris fuscipes* (Fabricius), *Rhynocoris marginatus* Fab. and *Rhynocoris longifrons* (Stal) (Hemiptera: Reduviidae) are brightly coloured, entomophagous, harpactorine reduviids, found in the agroecosystems, semi-arid zones, scrub jungles and reported to be preying on insect pests of Lepidoptera, Hemiptera, Isoptera, Orthoptera etc. (Ambrose, 1999; Sahayaraj, 2007).

Generally, biopesticides are considered safe to natural enemies of the target pest (Schmutterer, 1990; Ascher, 1993). Earlier studies demonstrated that application of biopesticides does not reduce of the biological control efficacy of *R. marginatus* (Sahayaraj, 2001). Sahayaraj and Paulraj (1999), Sahayaraj and Karthikraja (2003) and Sahayaraj *et al.* (2003) reworded the impact of different plant extracts against *R. marginatus* life stages.

Tephrosia purpurea (Dil.) Pers, (Fabaceae), is used traditionally for digestible, anthelmintic, alexiteric, leprosy, ulcers, antipyretic, alternative, cures diseases of liver, spleen, heart, blood, tumours, asthma, dyspepsia, diarrhoea, rheumatism, asthma and urinary disorders. Species of the Genus *Tephrosia* has been used as insecticide for instance *T. candida* DC., *T. purpurea* Pers., *T. vogelii* and *T. noctiflora* Bojer ex. Baker (Klocke, 1989). In addition, the bark of *Tephrosia*

purpurea has insecticidal activity against the third instar larvae of *Plutella xylostella* (You-Zhi *et al.*, 2011) and *Corcyra cephalonica* (Jadhav, 2009). The roots and seeds of this plant reported to have insecticidal, piscicidal and vermifugal properties (Hegazy *et al.*, 2009). However, no information has been available about the impact of this plant extracts or its formulation on any natural enemies including reduviid predators. In view of this lacuna, effect of *Tephrosia purpurea* stem based formulation (Telp 3% EC) was investigated under laboratory conditions against three important reduviids such as *R. fuscipes*, *R. marginatus* and *R. longifrons*.

MATERIALS AND METHODS

Insect rearing: Life stages of reduviids like *R. fuscipes*, *R. marginatus* and *R. longifrons* were collected from the agroecosystems (groundnut and cotton) in Tamil Nadu, India from January 2012 to March 2012. They were maintained in the laboratory conditions ($28\pm 2^\circ\text{C}$, L:D, (13:11 h) photoperiod and relative humidity of $73\pm 4\%$) using the methodology of Sahayaraj (2002). Laboratory emerged adults were selected randomly from the stock culture and used for the study.

Preparation of botanicals and bioassay: A 3% Emulsifiable Concentration (EC) of *Tephrosia purpurea* stem extract was prepared by mixing 3 mL of Telp 3% EC in 70 mL of distilled water. Biosafety evaluations were conducted in a customized Y-shaped glass olfactometer (2.5 cm internal diameter, 20 cm stem length, 20 cm arms length). The olfactometer was clamped on to a tripod in a horizontal position. Activated charcoal (Sigma), filtered air stream (Universal Lab Product) (200 mL min^{-1}) was supplied to each arm of the olfactometer by using an electric pump (Boy U, U-9900, China). Each air stream then passed through a glass chamber ($4\times 8\text{ cm}$) containing test material (a piece of 7.0 cm^2 filter paper dosed with $100\text{ }\mu\text{L}$ of EC formulation).

The $100\text{ }\mu\text{L}$ of 3% Telp was impregnated with Whatmann No. 7 filter paper (3 cm diameter), dried at room temperature and placed into the test chamber of Y-shape Olfactometer and at the other end a filter paper (3 cm diameter) with distilled water was placed. Six adult (sex is not considered) were released one after other into the release chamber of the Y-shaped olfactometer of the stem. Number of insects found on the treated and untreated filter paper was recorded after 20, 40 and 60 min continuously. The experiment was replicated six times with different uniform sized individuals of the same species. Same procedure has been followed for the emulsifiable concentration impregnated with cotton leaf (BT bunny) and groundnut leaf (TMV 4). The insects preferred either control or treated filter paper or neither. If the insects chose neither of the chambers then it was considered that insect made no choice. From the observation recorded the access proportion index API was performed (Yasuda and Wakamura, 1996). Same procedure was followed for other two reduviids.

RESULTS AND DISCUSSION

Reduviids are the dominant invertebrate predators in a variety of agroecosystems including cotton and groundnut where varieties of insecticides (synthetic, botanical, microbial) have been practiced by the farmers. In this study we studied the biosafety of a botanical formulation, Telp 3% EC against three common reduviids like *R. marginatus*, *R. fuscipes* and *R. longifrons*.

***Rhynocoris marginatus*:** Access behaviour of *R. marginatus* against Telp 3% EC is represented in Table 1. Results revealed that *Rhynocoris marginatus* oriented towards the Telp impregnated filter paper, cotton leaf and groundnut leaf without changing its usual olfactory response. However,

Table 1: Access behaviour of *R. marginatus*, *R. fuscipes* and *R. longifrons* adults against *T. purpurea* stem-based formulation (Telp 3% EC) impregnated filter paper, cotton leaf and groundnut leaf

Reduviid	Telp impregnated material	Exposure time (min)		
		20	40	60
<i>R. marginatus</i>	Filter paper	0.12	-0.05	0.31
	Cotton leaf	-0.07	-0.31	-0.08
	Groundnut leaf	-0.175	0.125	0.40
<i>R. fuscipes</i>	Filter paper	0.48	-0.015	0.93
	Cotton leaf	0.00	-0.18	0.07
	Groundnut leaf	0.13	0.02	0.03
<i>R. longifrons</i>	Filter paper	0.33	0.38	0.44
	Cotton leaf	0.10	0.93	1.00
	Groundnut leaf	0.80	0.60	0.60

at 0.8%, *R. marginatus* does not showed any positive or negative (move opposite to the test material) response during the experiment time. Olfactory response of *R. marginatus* purely depends up on leaf which impregnated with botanical formulation, Telp 3% EC. For instance at 20, 40 and 60 min observations, Telp 3% EC slightly deter (-0.08 to -0.31) the reduviid predator. At the same time, at 60 min, Telp 3% EC affect the reduviid behaviour, while the reduviid, while the botanical impregnated in filter paper (API = 0.31) or groundnut leaf (API = 0.4).

***Rhynocoris fuscipes*:** As observed in *R. marginatus*, *R. fuscipes* also showed positive chemotaxis activity against Telp 3% EC. Results showed that all the subjected *R. fuscipes* adults did not show either repellent or attraction activity while Telp 3% EC impregnated in cotton leaf. However, more than 13 and 18% of predator attracted towards groundnut leaf and filter paper, respectively impregnated with Telp 3% EC at 20 min observation. These responses had been changed with at 40 min observation. For instance, 3.0, 7.0, 93.0% of *R. fuscipes* was attracted towards groundnut leaf, cotton leaf and filter paper indicated that Telp 3% EC can be integrated along with reduviid predator in Biointensive Integrated Pest Management (BIPM) Programme.

***Rhynocoris longifrons*:** The olfactory response of *R. longifrons* seems to be the same, as showed by other reduviid predators i.e. *R. fuscipes* and *R. marginatus*. Table 1 shows the olfactory response of *R. longifrons* against Telp 3% EC impregnated filter paper, cotton leaf and groundnut leaf. This predator at any observation periods does not show any deterrent activity against Telp 3% EC of *Tephrosia purpurea*.

Previous studies by Jhansilakshmi *et al.* (1998) and Sahayaraj and Ravi (2007) also reported that botanicals can be integrated along with natural enemies. Botanical insecticides cause the death of the natural enemies (lethal effects) or change several other features of their biology and ethology without killing the individuals (sub-lethal effects). For instance, neem insecticides were found to be only slightly harmful to *Rhynocoris marginatus* (Sahayaraj *et al.*, 2003), *Geocoris punctipes* (Hemiptera) (Myers *et al.*, 2006), *Harmonia conformis* (Boisduval) (Coleoptera) and *Mallada signata* (Schneider) (Neuroptera) (Qi *et al.*, 2001), *Harmonia axyridis* (Kraiss and Cullen, 2008), coccinellids (Swaminathan *et al.*, 2010), *Philodromus cespitum* (Walckenaer) (Rezac *et al.*, 2010); *Phytoseiulus persimilis* and *Amblyseius cucumeris* (Spollen and Isman, 1996) (Araneae) and natural enemies in general (Lowery and Isman, 1995; Isman, 2006; Sakthivel *et al.*, 2012).

Podisus maculiventris (Hemiptera) had, however, slightly reduced survival and reproduction (Vinuela *et al.*, 2000). Similarly, a commercial formulation of azadirachtin (Align) affects the fertility of *Chrysoperla carnea* (Stephens) (Neuroptera) (Medina *et al.*, 2004).

CONCLUSION

The study concluded that when *Tephrosia purpurea* stem-based formulation (Telp 3% EC) sprayed in cotton and groundnut, *R. longifrons* did not show any repellent activity than *R. fuscipes* and *R. marginatus*. However, the repellency of *R. fuscipes* and *R. marginatus* were insignificant, indicates these predators can be integrated along with Telp 3% EC formulation in cotton and groundnut pest management.

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