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ORIGINAL ARTICLE **OPEN ACCESS**

Biosafety evaluation of indigenous plant extracts against grubs and adults of *Bracon hebetor* (Braconidae: Hymenoptera)

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ABSTRACT
Studies were carried out to evaluate the biosafety of various extracts of Acorus calamus, Azadirachta indica, Strychnus nuxvomica and Vitex negundo were tested against the larval parasitoid Bracon hebetor by topical application method. The hexane, methanol and aqueous extracts of indigenous plant extracts were prepared and evaluated against grubs and adults of B.hebetor. All the indigenous plant extracts were proven to be safer to adults of B. hebetor where only maximum of 6.67 per cent mortality was observed at the higher concentration tested and at all the lower concentrations the mortality was nil. However, the grub mortality was lowest of 3.33 per cent at 0.05 and 0.50 per cent concentrations of A. indica and S. nuxvomica aqueous extracts.
Keywords: *Acorus calamus, Azadirachta indica, Strychnus nuxvomica, Vitex negundo, Bracon hebetor, biosafety*

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INTRODUCTION

Pesticides derived from plants have the potential to play a major role in pest management for sustainable agriculture production [1]. Seven important families of bioactive botanicals have been classified into Meliaceae, Rutaceae, Asteraceae, Annonaceae, Malvaceae, Labiateae and Canellaceae [2].Plants produce a range of chemical substances to protect themselves from insect pests. Such chemicals are secondary metabolites and include alkaloids, terpenoids, flavonoids and acetogenins [3]. It is difficult for insects to develop resistance to these pesticides [1]. Amongst the botanicals, neem (*Azadirachta indica* A Juss.) has been the focus for large number of studies over the past five decades. Neem contains terpenoids that are phagodeterrent growth inhibitors and oviposition suppressant [4,5].

In the pursuit of naturally occurring phytochemicals in botanicals other than neem, the rhizomes of Sweetflag, *Acorus calamus* contain β-asarone, cis-asarone, tran-asarone, acoramone and a bitter glycoside, acorine along with eugenol, pinene and camphene which possess insecticidal and antifeedant activity [6,7]. Poison nut tree, *Strychnos nuxvomica* seeds contain a mixture of 13 alkaloids but the main alkaloids are strychnine and brucine which are involved in insecticidal activity (8,9). The alkaloid vitricin, flavonoid penducularisin and negundoside of nirgundi, *Vitex negundo* have strong insecticidal properties (10).

Nonetheless, detailed investigations needs to be carried out to study the biological effects of these indigenous plant extracts. With this background, in the present study, it was proposed to extract the biological active compounds from the rhizomes, seeds, and leaves of indigenous plants and safety against larval parasitoid, *Bracon hebetor*.

MATERIAL AND METHODS

Plant material collection and processing

The rhizomes of *A. calamus*, leaves of *V. negundo* and seeds of *A. indica* and *S. nuxvomica* were collected from tribal areas *viz*., Rampachodavaram of East Godavari and Buttayyagudem, Julugumilli of West Godvari districts of Andhra Pradesh. The rhizomes, seeds and leaves of selected plants were shade dried and later were crushed in grinder to powder. The grounded powder obtained was thus used for solvent extraction [11].

Hexane extract

Finely grounded plant materials (500.00g) were extracted with 2l hexane, using mechanical stirrer for half an hour and the blend was kept overnight for 24 hours. The supernatant was filtered and the plant materials were extracted two more times with hexane. The pooled extract was subjected to vacuum distillation at 40 ºC temperature to obtain hexane concentrate.

Methanol extract

The plant materials were further extracted with 2l methanol using the mechanical stirrer. The blend was thoroughly stirred for half an hour and left overnight for 24 hours. The supernatant was then filtered and subjected to vacuum distillation at 40ºC temperature to obtain methanol concentrate.

Aqueous extract (fresh plant material)

In a separate container, freshly grinded plant materials were suspended in 2l water and kept aside for 24 hours as such. It was filtered and concentrated under vaccum at 50ºC to obtain viscous material.

METHOD OF PREPARATION OF TEST SOLUTIONS

The concentrated extracts of various indigenous plants, which were explained above were first dissolved in their respective solvents and then diluted with water containing Triton X– 100, emulsifier (0.5 g in 100 ml of distilled water).

***B. hebetor* rearing**

B. hebetor was reared at Entomology laboratory, College of Horticulture, Venkataramannagudem. *B. hebetor* was mass multiplied in the laboratory on the alternate host *Corcyra cephalonica*. Grown up caterpillars of *C. cephalonica* were spread on stretched cloth over the mouth of jar and a glass plate is placed over the larvae to immobilize. Adult parasitoids were released in the jar. The female parasitoid paralyses the host before depositing its eggs on the body of the caterpillar. The eggs hatch out into grubs and feed on the body of the caterpillar. The grubs before spinning cocoons were collected on to a clean piece of paper (2"x1"). This piece of paper is sufficient for grubs to spin cocoons and attach themselves to the paper. Emergence of parasitoids starts from 7th day of parasitisation [12].

Bioassay studies of *B. hebetor* grubs

Two day old grubs of *B. hebetor* were taken in a sterilized glass petriplate (8 cm diameter) and then sprayed with 5.0, 3.0, 1.0, 0.5, 0.1, 0.05 % concentrations of various plant extracts with potter's tower at 340.00 g / cm^2. The sprayed grubs were allowed to dry under shade for 10-15 minutes. The honey- water solution was placed inside each petriplate after the spray as food source for the wasp once they emerge out. Each treatment was replicated thrice and the untreated checks sprayed with emulsified water. The influence of the extracts on the survivability of grubs was monitored at 24, 48, 72, 96 and 120 hours after spraying.

Bioassay studies of *B. hebetor* adults

Adults of *B. hebetor* were taken in glass veils to test the biosafety, which were previously treated with 5.0, 3.0, 1.0, 0.5, 0.1, 0.05 % concentrations of indigenous plant extracts. The honey- water solution was placed inside each veils after the adult introduction as food source for the wasp. Each treatment was replicated thrice and the untreated checks are treated with emulsified water. The influence of the extracts on the survivability of adults was observed at 12 and 24 hours after spraying.

RESULTS

The hexane, methanol and aqueous extracts of indigenous plants were evaluated against grubs and adults of *Bracon hebetor* in the laboratory to know the biosafety.

Effect of hexane extracts

The hexane extracts of *A. calamus* and *A. indica* at 5.0 and 3.0% concentrations caused only 3.33 per cent of *B. hebetor* adult mortality whereas in all the concentrations, *S. nuxvomica* and *V. negundo* yielded no adult mortality, while in lower concentrations (0.05 to 1.0% concentrations) the adult survivability was 100.00 per cent.

However, *A. calamus* and *A. indica* hexane extract caused more than 50.00 per cent mortality of *B. hebetor* grubs at 5.0% (66.67 and 70.00 per cent) and 3.0% (53.33 and 60.00 per cent) concentrations, respectively. However all the other concentrations of *A. calamus*, *A. indica* caused less than 50.00 per cent mortality in contrast to *V. negundo* and *S. nuxvomica* at all the concentrations.

Effect of methanol extract

The results of bioassay studies on effect of rhizomes, seeds and leaves of *A. calamus*, *A. indica* and *S. nuxvomica* and *V. negundo* extracts against adults of *B. hebetor*, revealed that *A. calamus* methanol extract caused survivability of 93.33 per cent at 5.0% concentration. However, in *A. indica*, *S. nuxvomica* and *V. negundo* the adult survivability was 96.67 per cent when compared to 100.00 per cent in control, while in lower concentrations (0.05 to 1.0% concentrations) it was 100.00 per cent (Table 1).

At the highest concentration of 5.0% the grub mortality was 76.67 per cent was recorded in *A. indica* methanol extract, followed by *A. calamus* (70.00 per cent). Lowest grub mortality of 6.67 per cent was observed at 0.5% concentration of *S. nuxvomica* and *V. negundo* which are statistically at par with each other. Interestingly, 100.00 per cent grub survivability was observed at 0.05% concentration.

Effect of aqueous extract

Highest adult mortality of 3.33 per cent was identified at 5.0% concentration of *A. calamus* and *A. indica*, whereas in all other concentrations 100.00 per cent adult survivability was recorded. However, aqueous extracts of *S. nuxvomica* and *V. negundo* induced 100.00 per cent adult survivability (Table 1). Aqueous extracts of *A. indica* and *A. calamus* caused maximum *B. hebetor* grub mortality percentage of 63.33 and 60.00 per cent at 5.0% concentration in contrast to 3.33 per cent at 0.05 and 0.5% of *A. indica* and *S. nuxvomica*.

Table 1: Effect of hexane, methanol and aqueous extracts of seeds, leaves and rhizomes of *A. calamus*, *A. indica*, *V. negundo* and *S. nuxvomica* against grubs and adults of *B. hebetor* for its toxicity.

Natural enemy	Plant extracts		5.0%	3.0%	1.0%	0.5%	0.1%	0.05%
B. hebetor Adult	Hexane	*A. calamus*	3.33	3.33	0.00	0.00	0.00	0.00
		A. indica	3.33	3.33	0.00	0.00	0.00	0.00
		S. nuxvomica	0.00	0.00	0.00	0.00	0.00	0.00
		V. negundo	3.33	3.33	0.00	0.00	0.00	0.00
B. hebetor Grub	Hexane	*A. calamus*	66.67	53.33	40.00	23.33	10.00	0.00
		A. indica	70.00	60.00	46.67	30.00	16.67	0.00
		S. nuxvomica	43.33	33.33	16.67	6.67	0.00	0.00
		V. negundo	40.00	30.00	13.00	6.67	0.00	0.00
B. hebetor Adult	Methanol	*A. calamus*	6.67	3.33	0.00	0.00	0.00	0.00
		A. indica	3.33	3.33	0.00	0.00	0.00	0.00
		S. nuxvomica	3.33	0.00	0.00	0.00	0.00	0.00
		V. negundo	3.33	0.00	0.00	0.00	0.00	0.00
B. hebetor Grub	Methanol	*A. calamus*	70.00	56.67	43.33	26.67	10.00	0.00
		A. indica	76.67	63.33	50.00	30.00	13.33	0.00
		S. nuxvomica	46.67	36.67	20.00	6.67	0.00	0.00
		V. negundo	43.33	33.33	16.67	6.67	0.00	0.00
B. hebetor Adult	Aqueous	*A. calamus*	3.33	0.00	0.00	0.00	0.00	0.00
		A. indica	3.33	0.00	0.00	0.00	0.00	0.00
		S. nuxvomica	0.00	0.00	0.00	0.00	0.00	0.00
		V. negundo	0.00	0.00	0.00	0.00	0.00	0.00
B. hebetor Grub	Aqueous	*A. calamus*	60.00	50.00	30.00	20.00	6.67	0.00
		A. indica	63.33	53.33	33.33	20.00	13.33	3.33
		S. nuxvomica	36.67	23.33	13.33	3.33	0.00	0.00
		V. negundo	33.33	20.00	10.00	0.00	0.00	0.00
control			0.00	0.00	0.00	0.00	0.00	0.00

DISCUSSION

The hexane, methanol and aqueous extracts of indigenous plant extracts were proved to be safer to the adults larval parasitoid *B. hebetor* except at higher concentrations. Whereas in case of grubs the mortality was observed maximum at 5.0% concentration of *A. indica* methanol extract (76.67 per cent).

The results of the present study are in accordance with the findings of Schmutterer [13], who found that higher concentrations (40 ppm) of azadirachtin were slightly harmful to *Apanteles glomeratus* and were mainly killed by lack of food and died within their hosts. The fifth instar larvae of *Pieris brassicae* under the influence of metamorphosis-disturbing neem products did not die immediately after uptake of active principles, but reduced food uptake, leading to an increase of interspecific competition among the gregarious larvae of *A. glomeratus*. Earlier, Loke *et al*. [14] reported that adult eclosion of *A. plutellae* was significantly inhibited by 2.5 per cent neem oil and no adult emergence was observed at 10.00% neem oil and the treated cocoons yielded adults with reduced longevities but no morphological deformities were observed. Emmanuel and Dhingra [15] reported slight mortality of the adult *Coccinella septumpunctata* at 10 DAT when treated with methanolic extract of *Caesalpinia crista* seeds at both 5.00 and 10.00%

concentration. Similarly Dhingra *et al.* [16] reported the effect of diverse plant origin insect growth regulators on emergence and survival of endoparasitic wasp, *A. obliqua* (Hymenoptera: Braconidae) and found that 93.34 per cent normal adult emergence by 1.0% methanolic extracts of *C. crista* seeds.

CONCLUSION

All the indigenous plant extracts were proven to be safer to adults of *B. hebetor* where only maximum of 6.67 per cent mortality was observed at the higher concentration tested and at all the lower concentrations the mortality was nil. However, the grub mortality was lowest i.e., 3.33 per cent at 0.05 and 0.50% concentrations of *A. indica* and *S. nuxvomica* aqueous extracts. While, hexane and methanol extracts of *A. calamus* and *A. indica* at 5.00 % concentration reported to cause more than 50.00 per cent *B. hebetor* grub mortality. Hence it is evident from the results that the adults of *B. hebetor* were safe in causing oviposition on the crop pests and is suggested that before incorporating any botanical into pest control schedule, they should be evaluated for their safety to natural enemies present in a particular ecosystem.

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