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Bio-efficacy of Solvent Extracts of Botanicals against Brinjal Shoot and Fruit Borer, *Leucinodes orbonalis* (Guenee) under *in vitro Condition*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the effectiveness of solvent extracts of *A. indica, C. inerme*, and *O. sanctum* against *Leucinodes orbonalis*, a borer of brinjal shoots and fruit. The room temperature solvent extraction method was used to get the acetone, petroleum ether, and methanol extracts of the botanicals, *A. indica* 100% (76.54%) methanol extract had the highest antifeedant efficacy. The acetone and petroleum ether extracts of *A. indica* 100% (65.45%; 60.42%) and *C. inerme* 100% (57.25%; 51.78%) were also effective antifeedants. The per cent larval mortality of *L. orbonalis* was highest in the methanol, acetone and petroleum ether extracts of *A. indica* 100% (89.33%; 80%; 71.33%), *A. indica* 75% (76.67%; 70%; 67%) and *A. indica* 50% (68.67%; 63.67%;58.67%), respectively.

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1. INTRODUCTION

Brinjal (*Solanum melongena* L.), often known as eggplant or aubergine, belongs to the family of Solanaceae and has been cultivated for over 4000 years in India. Among the various biotic and abiotic stresses that constrained the successful cultivation of brinjal crop, the most important factor is the damage caused by insect pests. The crop is susceptible to attack by various insects from seedling to fruiting stage. More than 53 different insect species have been reported as a pest in brinjal [1], among which jasssid, epilachna beetle, aphid, shoot and fruit borers, whitefly and non-insect pest such as mite are considered as important. Among these, a major threat to yield is caused by brinjal shoot and fruitborer (*Leucinodes orbonalis* Guenee) which cause damage levels up to 20.70 – 88.70 per cent in India [2]. It is the most destructive and monophagous pest of brinjal [3]. The pest infestation begins soon after transplanting and, in some circumstances, even before the plants are planted in the field. Even a single plant part can be damaged in extreme situations. Flowers, stems, fruits, inflorescence, and developing structures are all attacked, particularly fruits that are no longer edible following insect infestation. Fruits droop when larvae bore into them, causing severe economic loss. Wilting of the plant is a sign of drilling into the shoots [4]. The larvae penetrate the skin of young fruits, dig tunnels, and feed on internal tissues. Frass blocks the tunnels, making the infested fruits become unfit for consumption and lose their market value [5]. It serves as major limiting factor in quantitative as well as qualitative harvest of brinjal fruits [6].

This pest is managed by spraying conventional insecticides on a regular basis, regardless of the pest's prevalence. Increased reliance on pesticides, calendar-based farmer sprays, and/or short residual action of specific insecticide groups have not only increased production costs but also failed to offer adequate pest control [7]. Pesticides are widely used, which can lead to insect resistance and pollution of the environment.

Non-chemical alternatives have been promoted as a way to prevent chemicals' harmful effects. Biopesticides are gaining popularity because of their environmental safety, target specificity, efficacy, biodegradability, and suitability in integrated pest management (IPM) programmes [8]. Studies on the control of *L. orbonalis* with biopesticides such as botanicals and entomopathogens have been conducted in the past, and more research is needed.

2. MATERIALS AND METHODS

2.1 Mass Culturing of *L. orbonalis*

Brinjal shoot and fruit borer, *L. orbonalis,* were mass reared in the laboratory for the bioassay experiment. Infested brinjal fruits were collected from the farmer's field and kept in plastic trays in the laboratory. The fruits were placed on tissue paper along with dried brinjal leaves and twigs in the plastic trays for pupation. Pupae were collected and transferred to plastic containers where the adults emerged from the pupae. Upon the emergence of moths, they were sexed out (male: female, 1:1), and adults were transferred to small plastic containers. To prevent the moths from escaping and for oviposition, the top of the container was covered with ghada cloth and secured with a rubber band, and 10 % sugar solution soaked in cotton was provided on daily basis as food for the adult moths. The ghada cloth containing the eggs was removed each day and replaced with a new cloth and fresh adult moth feed. The ghada cloth containing the eggs was kept for incubation for hatching.

Alternatively, male and female adults were transferred to an oviposition cage for mating. In the oviposition cage, a 1:1 sex ratio was maintained A conical flask filled with water and a shoot with flowers and buds was placed in an oviposition cage for egg-laying. 10 % sugar solution soaked in cotton was provided on daily basis for the adult moths as food. The adults were allowed to mate for up to two days. After oviposition, leaves with eggs were carefully separated and kept for incubation for hatching.

On hatching, neonate larvae were transferred to fresh-cut brinjal fruits with the help of a fine camel hairbrush. The fruits with larvae were placed in trays on a layer of filter paper at the bottom. The brinjal fruits were changed at periodic intervals to avoid fungal growth. Similarly, the larvae were grown up to the third instar and used for the experiment [9-11].

2.2 Collection of Plant Material and Shade Drying

The leaves of *Azadirachta indica, Ocimum sanctum* and *Clerodendron inerme* were collected from Annamalai Nagar. The collected leaves were washed in clear water and shade dried until they were adequately dry to be grounded (dried for 7 to 10 days). Dried leaves were powdered separately in an electric grinder until a homogenous powder was obtained. The powdered materials were kept in airtight containers and used for extraction later [12].

2.3 Preparation of Solvent Extracts

Plant extracts were obtained using the room temperature solvent extraction method as described by Jaglan et al. [13]. The extraction solvents were petroleum ether (Boiling point: 55^{o}C), acetone (Boiling point: 56.5^{o}C) and methanol (Boiling point: 64.7^{o}C). Separately, 30 g of powdered *A. indica, O. sanctum* and *C. inerme* were macerated with 150 ml each of petroleum ether, acetone and methanol in conical flasks and left at room temperature for three days with frequent shaking. The extracts were filtered using Whatman No.1 filter paper to obtain a clear filtrate. The solvents from the crude extracts were evaporated to air dryness at room temperature until the complete dryness of the solvent. The crude extract was obtained, transferred to amber coloured vials and stored in the refrigerator at4°Cforfurtheruse [14].

2.4 Preparation of Stock Solution

The crude extracts were dissolved in acetone on weight by volume (W/V) basis making it a 100% stock solution. Further concentrations were prepared by diluting the stock solution.

For *in vitro* bioefficacy studies, the extracts of *A. indic*, *O. sanctum*, and *C. inerme* were prepared at 25%, 50%, 75% and 100% concentrations of acetone, methanol and petroleumether [15].

2.5 Antifeedant Activity

The antifeedant activity of solvent extracts of botanicals was done through modified methods of Muthu et al. [16], Pavunraj et al. [17] and Pavunraj et al. [18]. The botanical extracts were tested using the fruit disc, no-choice method. Brinjal fruits were cut into discs of 5 mm thickness and used to assess the antifeedant activity for *L. orbonalis.* The fruit discs were dipped in different concentrations of botanical extracts for ten minutes. After jerking away excess fluid, the fruit discs were allowed for shade drying at room temperature. The weight of the fruit discs was measured and provided to the larvae for consumption. In each plastic petri dish, the wet filter paper was placed to avoid the early death of the test larvae. For each treatment, ten third instar larvae of *L. orbonalis* were introduced into each Petri dish that contains three discs of brinjal fruit. Three replications were maintained for each treatment and laid out in Completely Randomized Design (CRD). The discs of brinjal were dipped in different concentrations of botanical extracts and maintained without larvae to find out the weight loss in the disc due to desiccation at room temperature. Progressive consumption of the fruit discs consumed by *L. orbonalis* larvae was recorded after 24 hours of treatment. After 24 hours, the discs were weighed and the difference between initial and final weights was calculated.

Real consumption was calculated as follows:

Weight loss due to desiccation (D) = Initial weight - final weight
Real consumption = Initial weight - (final weight + D)

The antifeedant activity was calculated according to the formula of [19].

$$\text{Antifeedant activity} = \frac{\text{Consumption in control-Consumption in treated}}{\text{Consumption in control}} \times 100$$

2.6 Larvicidal Activity

The larvicidal activity of botanical extracts of was done through modified methods of Muthu et al. [16]. The fruit disc no-choice method was used to assess larvicidal activity. Brinjal fruit discs were dipped in varying concentrations and were placed in Petri dishes, and the larvae were introduced as in the antifeedant experiment. After 24 hours of treatment, the larvae were continuously reared on the untreated fresh brinjal fruit discs. Every 24 hours, the diet was changed. Larval mortality was recorded at 24, 48 and 72 hours of treatment. Per cent mortality was calculated using Abbott's formula [20].

Abbott's corrected Mortality =
% Mortality in treatment-% mortality in control100 - % mortality in controlx 100

Table 1. Antifeedant activity of solvent extracts of botanicals

S. No.	Treatment	Percent antifeedant activity* (24 HAT)		
		Acetone	Petroleum ether	Methanol
1.	*A. indica* 25%	43.27 (41.13)[h]	40.14 (39.31)[i]	58.52 (49.91)[e]
2.	*A. indica* 50%	48.54 (44.16)[f]	45.36 (42.34)[f]	62.75 (52.39)[c]
3.	*A. indica* 75%	56.73 (48.87)[c]	53.33 (46.91)[c]	68.28 (55.72)[b]
4.	*A. indicia* 100%	65.45 (54.00)[a]	60.42 (51.01)[a]	76.54 (61.03)[a]
5	*C. inerme*25%	41.29 (39.98)[j]	37.47 (37.74)[k]	40.56 (39.56)[j]
6.	*C. inerme*50%	45.60 (42.48)[g]	42.48 (40.68)[h]	43.54 (41.29)[i]
7.	*C. inerme*75%	51.48 (45.85)[d]	49.21 (44.55)[e]	53.67 (47.11)[g]
8.	*C. inerme*100%	57.36 (49.23)[b]	57.00 (49.02)[b]	60.13 (50.85)[d]
9.	*O. sanctum* 25%	38.81 (38.53)[k]	33.73 (35.51)[l]	38.73 (38.49)[k]
10.	*O. sanctum* 50%	42.30 (40.57)[i]	39.26 (38.79)[j]	43.89 (41.49)[i]
11.	*O. sanctum* 75%	49.28 (44.59)[e]	43.54 (41.29)[g]	48.63 (44.22)[h]
12.	*O. sanctum* 100%	57.25 (49.17)[b]	51.78 (46.02)[d]	56.13 (48.52)[f]
13.	Solvent control	3.14 (10.21)[l]	1.79 (7.69)[m]	2.11 (8.35)[l]
14.	Absolute control	2.61 (9.30)[m]	1.00 (5.73)[n]	1.09 (5.99)[m]
	S. Ed	0.106	0.124	0.103
	CD (p =0.05%)	0.217	0.255	0.211

**Mean of three replications; HAT – Hour after treatment*
Values in parenthesis are arcsine transformed values
In a column means followed by a column letter are not significantly different at 5 per cent level (DMRT)

3. RESULTS AND DISCUSSION

3.1 Antifeedant Activity

Among the acetone extracts evaluated, a high range of antifeedant activity was recorded in *A. indicia* 100% (65.45%) followed by *C. inerme*100% (57.36%) which was on par with *O. sanctum* 100% (57.25%), compared to absolute control (2.61%) and solvent control (3.14%) at 24 HAT. Similar types of results were observed by Chauhan and Srivastava [21], who evaluated the antifeedant activity of acetone extract of *O. sanctum* against tobacco caterpillar, *S. litura*. On the basis of preference index, extracts from *O. sanctum,* were extremely antifeedant.

Petroleum ether extracts of botanicals showed that, *A. indicia* 100% (60.42%) recorded the highest antifeedant activity followed by *C. inerme*100% (57%) and *A. indica* 75% (53.33%) at 24 HAT. The present finding is in concordance with Taware et al. [22], who stated that petroleum ether extract of *A. indica* exhibited antifeedant activity against third instar tobacco caterpillar larvae, *S. litura*. Jadhav et al. [23] reported that ethanol crude leaf extract of *C. inerme*showed highest antifeedant potential to *S. litura.*

Among the methanolextracts of botanicals, the maximum antifeedant activity was found in *A. indica* 100% (76.54%) followed by *A. indica* 75% (68.28%). Least per cent antifeedant activity was recorded in acetone, petroleum ether and

Table 2. Larval mortality of *L. orbonalis* by solvent extracts of botanicals at 24, 48 and 72 HAT

S. No.	Treatment	Acetone			Petroleum ether			Methanol		
		24 HAT	48 HAT	72 HAT	24 HAT	48 HAT	72 HAT	24 HAT	48 HAT	72 HAT
1.	*A. indica* 25%	39.67 (39.04)^{f}	44.67 (41.94)^{g}	53.33 (46.91)^{g}	29.67 (33.00)^{f}	40.00 (39.23)^{f}	49.33 (44.62)^{e}	35.00 (36.27)^{e}	46.67 (43.09)^{d}	57.33 (49.22)^{d}
2.	*A. indica* 50%	48.00 (43.85)^{c}	53.33 (46.91)^{d}	63.67 (52.93)^{c}	45.33 (42.32)^{c}	52.00 (46.15)^{c}	58.67 (49.99)^{c}	47.33 (43.47)^{c}	60.00 (50.77)^{c}	68.67 (55.96)^{c}
3.	*A. indica* 75%	53.33 (46.91)^{b}	60.00 (50.77)^{b}	70.00 (56.79)^{b}	50.00 (45.00)^{b}	59.33 (50.38)^{b}	67.00 (54.94)^{b}	52.67 (46.53)^{b}	65.00 (53.73)^{b}	76.67 (61.12)^{b}
4.	*A. indicia* 100%	60.67 (51.16)^{a}	72.67 (58.48)^{a}	80.00 (63.44)^{a}	56.67 (48.83)^{a}	63.00 (52.54)^{a}	71.33 (57.63)^{a}	64.67 (53.53)^{a}	79.33 (62.96)^{a}	89.33 (70.94)^{a}
5	*C. inerme*25%	21.33 (27.51)^{k}	28.67 (32.37)^{k}	36.67 (37.27)^{k}	20.00 (26.57)^{k}	27.33 (31.52)^{k}	32.67 (34.86)^{j}	16.00 (23.58)^{k}	22.67 (28.43)^{k}	35.33 (36.47)^{j}
6.	*C. inerme*50%	28.33 (32.16)^{j}	35.33 (36.47)^{j}	42.67 (40.79)^{j}	23.00 (28.66)^{j}	28.67 (32.37)^{j}	36.00 (36.87)^{i}	19.33 (26.08)^{j}	28.67 (32.37)^{j}	39.33 (38.84)^{i}
7.	*C. inerme*75%	35.00 (36.27)^{g}	43.00 (40.98)^{h}	51.33 (45.76)^{h}	28.67 (32.37)^{g}	34.67 (36.07)^{h}	40.00 (39.23)^{g}	28.00 (31.95)^{g}	32.67 (34.86)^{h}	43.33 (41.17)^{h}
8.	*C. inerme*100%	42.67 (40.79)^{e}	52.00 (46.15)^{e}	60.00 (50.77)^{e}	36.67 (37.27)^{e}	41.00 (39.82)^{e}	47.33 (43.47)^{f}	33.33 (35.26)^{f}	42.67 (40.79)^{e}	50.00 (45.00)^{f}
9.	*O. sanctum* 25%	28.00 (31.95)^{j}	40.67 (39.62)^{i}	46.67 (43.09)^{i}	26.00 (30.66)^{i}	31.33 (34.04)^{i}	37.33 (37.66)^{h}	22.67 (28.43)^{i}	31.67 (34.25)^{i}	42.67 (40.79)^{h}
10.	*O. sanctum* 50%	32.00 (34.45)^{h}	43.33 (41.17)^{h}	52.00 (46.15)^{h}	28.00 (31.95)^{h}	35.33 (36.47)^{g}	40.33 (39.42)^{g}	26.00 (30.66)^{h}	35.00 (36.27)^{g}	47.33 (43.47)^{g}
11.	*O. sanctum* 75%	40.00 (39.23)^{f}	50.00 (45.00)^{f}	56.33 (48.64)^{f}	30.00 (33.21)^{f}	39.67 (39.04)^{f}	49.00 (44.43)^{e}	34.67 (36.07)^{e}	42.00 (40.40)^{f}	52.00 (46.15)^{e}
12.	*O. sanctum* 100%	46.00 (42.71)^{d}	58.67 (49.99)^{c}	62.00 (51.94)^{d}	41.33 (40.01)^{d}	49.33 (44.62)^{d}	53.00 (46.72)^{d}	39.33 (38.84)^{d}	47.00 (43.28)^{d}	58.00 (49.60)^{d}
13.	Solvent control	0.00 (0.442)^{l}	0.00 (0.442)^{l}	0.00 (0.442)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{k}	0.00 (0.44)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{k}
14.	Absolute control	0.00 (0.44)^{l}	0.00 (0.442)^{l}	0.00 (0.442)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{k}	0.00 (0.44)^{l}	0.00 (0.44)^{l}	0.00 (0.44)^{k}
	S. Ed	0.100	0.163	0.252	0.103	0.167	0.259	0.106	0.169	0.267
	CD (p = 0.05%)	0.206	0.334	0.516	0.212	0.342	0.530	0.218	0.348	0.547

**Mean of three replications; HAT – Hour after treatment; Values in parenthesis are arcsine transformed values*
In a column means followed by a column letter are not significantly differentat 5 per cent level (DMRT)

methanol extracts of *O. sanctum* 25% (38.81%; 33.73%; 38.73%).The present findings are in confirmation with Isman et al. [24], who reported that Azadirachtin (tetranortritterpenoid) is one of the major active components in neem which is the most potent natural insect antifeedant. He also stated that Azadirachtin content is highly correlated with both behavioural and physiological effects of neem extracts on lepidopterans.

3.2 Larvicidal Activity

Larvividal activity of acetone extracts of botanicals was highest in *A. indicia* 100% (80%) followed by *A. indica* 75% (70%), while low mortality was seen in *C. inerme* 25% (36.67%) at 72 HAT compared to solvent and absolute controls. A similar trend in mortality rate was recorded at 24 and 48 HAT. Majeed et al. [25] reported that acetone extracts of *A. indica* was most effective against adult female mealybugs. Mamun et al. (2009) showed that Neem extracts showed the highest toxicity against Red flour beetle, *T. castaneum* and among the solvents acetone extract was more toxic. Kamaraj et al. [26] reported that the leaf acetone extract of *O. sanctum* caused greater larval mortality than leaf methanol extract of *O. sanctum* against fourth instarlarva of *S. litura.* Kamaraj et al. [27] showed that the leaf acetone extract of *O. sanctum* caused greater larval mortality than leaf methanol extract of *O. sanctum* against fourth instar larva of *H. armigera.*

Among the various treatments of petroleum ether extracts of botanicals, maximum larval mortality of *L. orbonalis* was recorded in *A. indicia* 100% (71.33%) followed by *A. indicia* 75% (67%) and *A. indicia* 50% (58.67%). Similarly, Ebe et al. [28] reported that petroleum ether extract of *A. indica* was found to be better than aqueous extract of *A. indica* against different instars of *A. gambiae*.

Mximum larval mortality among the methanol extracts of botanicals were recorded in *A. indicia* 100% (89.33%) followed by *A. indicia* 75% (76.67%) and *A. indicia* 50% (68.67%). Similarly, Tulashie et al. [29] reported that methanolic NLE caused 15.5 - 100% larval mortality of *S. frugiperda* and the mortality of larvae increased with increasing concentration of the extracts and increasing period of exposure. Sharma et al. [30] reported that complete larval mortality of *P. xylostella*with neem methanol extract was recorded at 3% concentration. The methanol NLE caused 11 - 70% larval mortality of early-stage larvae and 8 - 27% larval mortality of late-stage larvae of *H. armigera* at 0.5 – 7.5% concentration [13].

4. CONCLUSION

The use of non-chemical substitutes has been endorsed as a means of avoiding the negative effects of chemicals. Because of their environmental safety, target specificity, efficacy, biodegradability, and applicability for integrated pest management (IPM) programmes, biopesticides are growing in popularity. The results of the current investigations showed that the acetone and petroleum ether extracts of *A. indica* 100% (65.45%; 60.42%) and *C. inerme* 100% (57.25%; 51.78%) were likewise efficient antifeedants. The percentage of larval mortality of *L. orbonalis* was highest in the methanol extract of *A. Indica* followed by acetone, and petroleum ether extracts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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