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Efficacy of seed extracts of *Samadera indica* Gaetrn. for the management of epilachna beetle, *Henosepilachna septima* (Dieke)

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ABSTRACT: Snake gourd, a vegetable commonly grown in Kerala is highly prone to the attack of epilachna beetle, *Henosepilachna septima* (Dieke)). There is immense scope for the utilization of botanicals for the management of insects. Niepa bark tree, *Samadera indica* (Gaertn.) is one such potential candidate due to the presence of the bitter principle, quassinoids. In this study, we have analyzed the efficacy of *S. indica* seed extracts in causing feeding deterrence and altering the biology of the pest. Lethal Doses of the extracts effective against the pest was estimated by probit-dose analysis and the LD_{50} values ranged from 8.10 to 22.30 mg per g body weight of the insect and LD_{90} values ranged from 62 to 72.40 mg per g body weight of the insect. The grubs that underwent treatment with LD_{90} of acetone extract consumed relatively lesser quantity of leaves at 72 HAT (0.11 g). Furthermore, there was increase in duration of grubs (14 days) and pupae (7.67 days), reduced pupal weight (1.25 g). Considering the effect on feeding deterrence, mortality and changes in biology of the pest, acetone extract is the most effective extract.

Keywords: acetone, aqueous, ethanol, hexane, *Samadera indica*, snake gourd

INTRODUCTION

Snake gourd is a cucurbitaceous vegetable that is commonly grown in Kerala. The plant is known for its health benefits like detoxification of body, improvement of digestion, management of diabetes etc. as it is a source of various minerals and dietary fibre. Snake gourd is attacked by a number of pests, the major ones in Kerala being pumpkin caterpillar (*Diaphania indica* Saund.), epilachna beetle (*Henosepilachna septima* (Dieke)), snake gourd caterpillar (*Anadevidia peponis* F.) and fruit fly (*Bactrocera cucurbitae* (Coquillett)) etc. Both adults and grubs of epilachna beetle damage leaves by scraping the surface and skeletonising them. There are several chemical means of pest management which were practised by farmers traditionally. However, in this era of 'safe-to-eat' vegetables, people are more inclined towards organic farming. Botanicals could be well exploited as potential organic insecticides. Some of the plants like *Azadirachta indica* A. Juss. (Meliaceae), chrysanthemum etc. are noteworthy.

As this crop generally grows in the homesteads, the pests could be managed only in an environmentally feasible manner. Among various eco-friendly methods of pest management, botanical pesticides own an array of properties. These include toxicity, repellency, antifeedance, insect growth regulatory activities against agricultural pests, non phytotoxicity, systemicity and easy biodegradability. Botanicals like *Azadirachta indica* A. Juss. (Meliaceae), *Chrysanthemum* L. (Asteraceae), *Chromolaena odorata* (L.) R. M. King & H. Robinson (Asteraceae), *Annona squamosa* L. (Annonaceae), *Mikania micrantha* Kunth. (Asteraceae) etc. are some plants with anti-insect properties.

There are still many plants with anti-insect properties in the queue. Many potential plants with promising anti-insect properties await discovery. One such plant is Niepa bark tree (*Samadera indica* (Gaertn.)), in the family Simaroubaceae. Govindachari *et al*. (2001) and Anusree *et al*. (2018) have unveiled that the tree possesses anti-insect properties. In this research paper, we elucidate the efficacy of *S. indica* seed extracts in managing the leaf feeding pest, *H. septima* in snake gourd.

MATERIALS AND METHODS

Maintenance of insect culture

Insect culture of *H. septima* was maintained by rearing the insects at 28±5ºC temperature and 75±5 per cent Relative Humidity. The identity of the insect was confirmed by Dr. J Poorani of NRCB (National Research Centre for Banana), Trichy. Egg masses of the beetle were collected from host plants and kept in a Petri plate for hatching. First instar grubs, on hatching out were transferred to a container using hair brush. The mouth of the container was covered with muslin cloth and the larvae were provided with tender leaves for feeding. Fresh leaves were provided daily and the remnants of feed and insect frass were removed. Fully developed pupae were left undisturbed for adult emergence. Emerging adults

were kept within oviposition chamber and droplets of vitamin E capsules were lightly smeared on the sides of the container/leaves provided as food, if egg laying is delayed. Eggs were laid scattered on the base and walls of the container. The eggs were taken out and kept in a Petri plate for emergence of grubs. The insect culture thus developed was used for further studies.

Seed extracts

The seeds of *S. indica* were procured from various parts of Aleppey district, identification confirmed at University of Kerala, Kariavattom, Kerala and a voucher specimen was kept in the herbarium (Accession Number - KUBH 110627). Seeds shade-dried at 28±5ºC were ground into coarse powder using grinder. Aqueous and solvent extracts of different concentrations, ranging from 0.5 to 15 per cent were prepared from the powder by cold extraction method (Wetwitayaklung *et al*., 2007). For preparing aqueous extracts, 100 g powder was dissolved in 250 mL of distilled water and solvent extracts were prepared by dissolving 20 g of powder in 150 mL of HPLC grade solvents *viz*., n-hexane, acetone and ethanol successively. These were then stirred using a mechanical stirrer for 72 h intermittently and the filtrate was separated out by filtering through muslin cloth and Whatman No. 1 filter paper successively. The filtrate was further subjected to evaporation using Heidolph rotary vacuum flash evaporator or nitrogen gas evapourator to obtain solvent-free extract. This was then dissolved in equal quantity of water to prepare stock solution. A few drops of DMSO (Dimethyl Sulfoxide) were added to the extract to make it completely miscible with water.

Estimation of Lethal Doses

Aqueous and solvent extracts at varying doses from 0.5 to 15 per cent were sprayed onto the grubs (20 no's) using potter precision laboratory spray tower. The insects were provided with fresh leaves of the host plant daily. Mortality of the insects were observed for a period of 72 h and expressed in percentage. The data at 72 HAT, in the range of 20 to 80 per cent was subjected to probit-dose analysis using the software Statistical Package for Social Sciences (SPSS 17.0) to obtain Median Lethal Dose (LD_{50}) and Lethal Dose Ninety (LD_{90}) values. The extracts at LD_{50} and LD_{90} were taken for further experiments involving the assessment of antifeedant effect, insecticidal effect and effect on biology of the insect. Sterile distilled water was adopted as control and all the treatments were replicated thrice.

Assessment of antifeedant effect of *S. indica* seed extracts

The potential of *S. indica* seed extracts in causing feeding deterrence was assessed by no-choice test

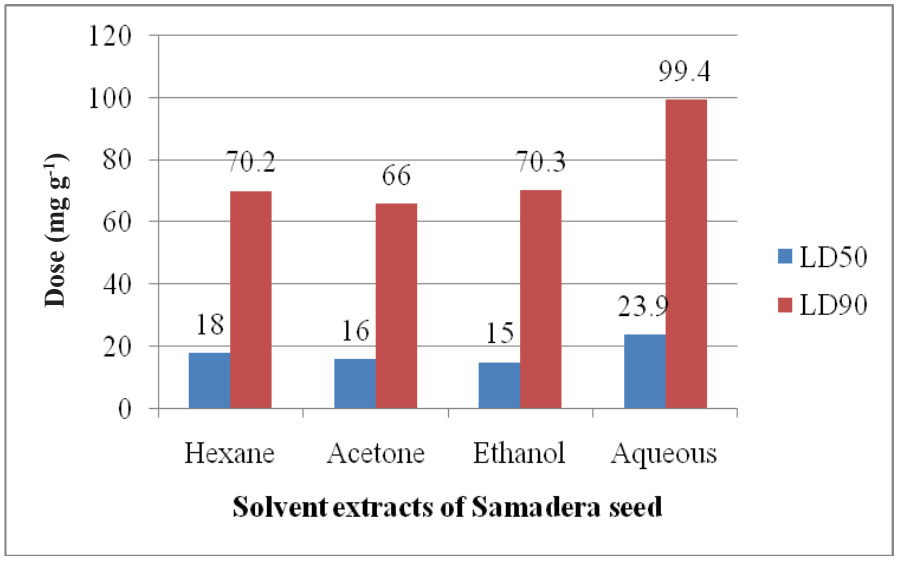

Fig 1. Lethal doses of *S. indica* seed extracts against *H. septima*

Table 1. Antifeedant effect of *S. indica* seed extracts on *H. Septima*

Treatment	Weight of leaf consumed by grubs (g)		
	24 HAT	48 HAT	72 HAT
Hexane extract 17.3 mgg^{-1}	0.27 (0.11)^{c}	0.22 (0.09)^{d}	0.20 (0.08)^{d}
Hexane extract 68 mgg^{-1}	0.23 (0.09)^{d}	0.24 (0.10)^{d}	0.19 (0.08)^{d}
Acetone extract 8.1 mgg^{-1}	0.20 (0.08)^{ef}	0.22 (0.09)^{d}	0.13 (0.05)^{e}
Acetone extract 62 mgg^{-1}	0.19 (0.06)^{f}	0.18 (0.07)^{e}	0.11 (0.05)^{e}
Ethanol extract 16 mgg^{-1}	0.27 (0.10)^{c}	0.22 (0.09)^{d}	0.18 (0.07)^{d}
Ethanol extract 65 mgg^{-1}	0.22 (0.09)^{de}	0.18 (0.07)^{e}	0.12 (0.05)^{e}
Aqueous extract 22.3 mgg^{-1}	0.35 (0.13)^{a}	0.31 (0.12)^{b}	0.29 (0.11)^{b}
Aqueous extract 72.4 mgg^{-1}	0.31 (0.12)^{b}	0.28 (0.11)^{c}	0.25 (0.10)^{c}
NSKE 5%	0.16 (0.07)^{g}	0.13 (0.05)^{f}	0.12 (0.05)^{e}
Control	0.36 (0.13)^{a}	0.35 (0.13)^{a}	0.39 (0.14)^{a}
CD (0.05%)	**(0.008)**	**(0.009)**	**(0.012)**

Figures in parantheses are values after log (x+1) transformation

(Bentley *et al.,* 1984). The extracts at LD_{50} and LD_{90} were sprayed onto leaf discs of snake gourd with approximately five cm diameter and uniform thickness. One grub pre-starved for four hr prior to treatment was allowed to feed on a single leaf. Care was taken to avoid drying of leaves by wrapping moist cotton on the petioles. Each treatment was replicated thrice and each replication contained 10 insects. The portion of the leaf that was fed by the insect (g) was assessed after every 24 h for a period of three days. Neem Seed Kernel Extract (NSKE) 5% was taken as check.

Assessment of insecticidal effect of *S. indica* seed extracts

Insecticidal effect of *S. indica* seed extract was estimated by spraying method, wherein the extract was treated on the grubs topically. Each treatment was replicated thrice and ten insects were treated in one replication. Malathion 50EC @ 2mL per L was taken as check. The data was analysed by Analysis of Variance (ANOVA) after arc sine transformation.

Assessment of effect of *S. indica* seed extracts on biology of the pest

The effect of *S. indica* seed extracts on biology of *H. septima* was studied by topical application of the extracts on grubs. Observations include deformities in grubs, pupae and adults, difference in duration of grubs and pupae, pupal weight (g). NSKE 5% was taken as check treatment. The data was analysed using ANOVA after suitable transformations. Data on pupal weight was subjected to log (x+1) transformation and other data were transformed using square root transformation.

RESULTS AND DISCUSSION

Estimation of Lethal Doses

The LD_{50} values of *S. indica* seed extracts in n-hexane, acetone, ethanol and water against *H. septima* were 18.00, 16.00, 15.00 and 23.90 mg per g body weight of insect, respectively. Meanwhile LD_{90} values were 70.20, 66.60, 70.30 and 99.40 mg per g body weight of insect, respectively (Fig. 1). Our study revealed that seed extracts of *Samadera* are very effective for pest management as indicated by LD_{50} and LD_{90} values. The toxicity of certain plant extracts were studied by various researchers. Ezemuoka *et al*. (2019) reported that LC_{50} values of aqueous extracts from stem bark and leaf of soursop, *Annona muricata* to fourth instar larvae of mosquito, *Aedes aegypti* were 92.5 µg per mL and 26.5 µg per mL, respectively. The findings by Ahmed *et al*. (2020) revealed the toxicity of silvery wormwood, *Artemisia argyi* (L.) to cabbage aphid, *Brevicoryne brassicae* L. with LC_{50} value of 3.91 mg per mL.

Antifeedant effect of *S. indica* seed extracts

The antifeedant effect of seed extracts of *S. indica* against *H. septima*, assessed in terms of weight of leaf consumed by the grubs (g) is furnished in Table 1. Observations at 24 Hours after Treatment (HAT) revealed that among all the extracts, acetone extract 62 mg per g was the best treatment after NSKE 5% which

Table 2. Insecticidal effect of crude extracts of *S. indica* seeds on *H. septima*

Treatment	Mean larval mortality (%)
Hexane extract 17.3 mgg^{-1}	46.67 (43.08)^{c}
Hexane extract 68 mgg^{-1}	85.00 (67.41)^{ab}
Acetone extract 8.1 mgg^{-1}	55.00 (47.88)^{c}
Acetone extract 62 mgg^{-1}	86.67 (68.86)^{ab}
Ethanol extract 1.6 mgg^{-1}	53.33 (46.92)^{c}
Ethanol extract 65 mgg^{-1}	86.67 (68.86)^{ab}
Aqueous extract 22.3 mgg^{-1}	46.67 (43.09)^{c}
Aqueous extract 72.4 mgg^{-1}	83.33 (66.15)^{b}
Check (Malathion 50 EC @ 0.2 mLL^{-1})	91.67 (73.40)^{a}
Control	0^{d}
CD (0.05)	6.05

Figures in parentheses are arc sine transformed values

was revealed by lesser quantity of food consumed by the grubs. The former treatment resulted in only 0.19 g of leaf being fed by the insect and in the latter treatment, it was 0.16 g.

At 48 HAT, acetone extract 62 mg per mL and ethanol extract 65 mg per g, being the second best treatments, after NSKE 5%, with a value of 0.18 g, each. At 72 HAT, the trend was similar with acetone extract at both concentrations being statistically significant and equivalent to NSKE 5%. Similar to this was the treatment with ethanol extract @ 65 mg per g. Quantity of leaf consumed by grubs in these treatments ranged from 0.11 to 0.13 g. This was followed by hexane extracts @ 17.3 and 68 mg per g with leaf weights of 0.20 g and 0.19 g, each. The treatment ethanol extract @ 16 mg per mL was statistically similar to the above treatments and the leaf weight observed was 0.18 g.

In our study, it was observed that acetone extract offered greater feeding deterrence. This finding is in agreement with that of Prajapati *et al*. (2003) who have reported that methanol, acetone and chloroform extracts of Malabar nut, *Justicia adhatoda* L., billy goat weed, *Ageratum conyzoides* L. and Ceylon leadwort, *Plumbago zeylanica* L. strongly deterred the feeding of Bihar hairy caterpillar, *Spilarctia obliqua* Walker at a dose of 10 mg per mL. Erturk (2006) observed that ethanol extract from horse chestnut, *Aesculus hippocastanum* L., common alder, *Alnus glutiosa* (L.) Gaetrn., and sweet amber, *Hypericum androsaemum* L. showed high antifeedant activity against larvae of *Thaumetopoea solitaria*, with the absolute deterrence coefficients being 52.05, 41.18, and 40.06, respectively. This finding is in line with our observation on effectiveness of ethanol extract.

Effect of *S. indica* seed extracts on mortality of grubs

The insecticidal effect of seed extracts of *S. indica* on *H. septima* assessed upto 72 HAT is shown in Table 2. It was observed that the mortality observed with all extracts, with the exception of aqueous extract, was statistically similar to that of malathion 50 EC @ 2 mLL^{-1} and the values ranged from 67.41 to 73.40 per cent. These treatments were followed by aqueous extract 72.4 mg per g, with 66.15 per cent mortality.

In this study, it was noticed that all solvent extracts at LD_{90} value exhibited mortality equivalent to chemical insecticides. Similar to this was the finding in the following research works. The insecticidal effect of acetone extract from leaves of *Millingtonia hortensis* was reported by Kaushik and Saini (2008) who have witnessed a mortality of 98.33 per cent in second instar larvae of *Culex quinquefasciatus* (300 ppm). Similar was the observation by Singh *et al.* (2008) who have observed 100 per cent mortality in third instar larvae of *Anopheles culicifacies* Giles, *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* Linn, when treated with acetone extracts of leaf @ 200 ppm and seed @ 100 ppm. Administration of ethanolic extract of leaves of custard apple, *Annona squamosa* L. (1% and 5%) resulted in 100 per cent mortality after 40 min and 15 min, respectively (Kumar *et al*., 2010).

However, N'Guessan *et al*. (2006) reported high mortality rates of 88.2, 96.6 and 98.5 per cent, against

Table 3. Effect of crude extracts of *S. indica* seed on the biology of *H. septima*

Treatments	Mean larval duration (days)	Mean pupal weight (g)	Mean pupal period (days)
Hexane extract 17.3 mgg^{-1}	12.67 (3.56)^{cd}	1.30 (0.11)^{c}	7.00 (2.65)^{bcd}
Hexane extract 68 mgg^{-1}	13.33 (3.65)^{bcd}	1.29 (0.11)^{d}	7.33 (2.71)^{abc}
Acetone extract 8.1 mgg^{-1}	13.67 (3.70)^{abc}	1.29 (0.11)^{cd}	7.33 (2.71)^{abc}
Acetone extract 62 mgg^{-1}	14.00 (3.74)^{ab}	1.25 (0.10)^{f}	7.67 (2.83)^{a}
Ethanol extract 16 mgg^{-1}	13.33 (3.65)^{bcd}	1.28 (0.11)^{e}	7.00 (2.65)^{bcd}
Ethanol extract 65 mgg^{-1}	13.67 (3.70)^{abc}	1.25 (0.10)^{f}	7.67 (2.71)^{abc}
Aqueous extract 22.3 mgg^{-1}	11.37 (3.36)^{e}	1.34 (0.13)^{b}	6.33 (2.52)^{d}
Aqueous extract 72.4 mgg^{-1}	12.33 (3.51)^{de}	1.28 (0.11)^{e}	6.77 (2.58)^{cd}
Check (Neemazal 1% @ 0.2%)	14.67 (3.83)^{a}	1.25 (0.10)^{f}	7.67 (2.77)^{ab}
Control	11.33 (3.37)^{e}	1.35 (0.13)^{a}	4.33 (2.08)^{e}
CD (0.05)	**(0.154)**	**(0.003)**	**(0.160)**

*Figures in parantheses are square root transformed value
**Figures in parantheses are log(x+1) transformed values

cocoa mirids with neem seed aqueous crude extracts at 10, 20 and 30 per cent respectively at 96 h after treatment.

Effect of *S. indica* seed extracts on biology of the pest

The impact of seed extracts of *S. indica* on the biology of *H. septima* assessed at 72 HAT is shown in Table 3.

Effect on mean duration of grubs

It was observed that there was increase in duration of grubs in treatment with acetone extract 62 mg per g and the value was 14 days. This was noticed to be on par with neemazal 1% with 14.67 days. These treatments were subsequently followed by hexane extract 68 mg per g, acetone extract 8.10 mg per g and ethanol extracts 16 mg per g and 65 mg per g.

There was an increase in larval duration with all treatments and the effect was more pronounced in treatments with acetone extract and ethanol extract. Similarly, Deepthy *et al*. (2010) also observed maximum larval duration of 26.33 days in Asian army worm, *Spodoptera litura* Fab. with the application of methanol extract (6%) of Chinese chaste tree, *Vitex negundo* Linn. The observations by Deepalakshmi and Jeyabalan (2017) were also not indifferent. They have also noticed increase in larval duration in all instars of *Culex quinquefasciatus* (Say) when leaf extracts of *Glochidion neilgherrense* Wight, *Cinnamomum wightii* Meisn. and *Leucas linifolia* (Roth) Spreng. were applied in methanol @ 4%.

Effect on mean pupal weight (g)

Weight of pupae was observed to be similar in the treatments acetone extract 62 mg per g, ethanol extract 65 mg per g and neemazal 1% and the value was 1.25 g. Ethanol extract 16 mg per g and aqueous extract 72.40 mg per g were statistically inferior to above treatments, with mean pupal period being 1.28 days.

Pupal weight was reduced with acetone and ethanol extracts. Similarly, reduced pupal weight with solvent extracts was reported by Malarvannan *et al*. (2008) and Gokce *et al*. (2010). In the former study, it was observed that petroleum ether extract of *Cipadessa baccifera* (Roth) Miq. when treated onto larvae of American bollworm, *Helicoverpa armigera* (Hubner) resulted in reduced pupal weight of 177.5 mg. Latter study showed that pupal weight was reduced in oblique banded leaf roller, *Choristoneura rosaceana* (Harris) when treated with methanol extract of *Humulus lupulus* @ 24.5 µg per insect.

Effect on mean pupal period (days)

Mean pupal duration was found to be statistically similar in grubs treated with all LD_{90} values of solvent extracts (7.33 to 7.67 days) and neemazal 1% (7.67

days). Pupae existed for 6.77 days in treatment with aqueous extract 72.40 mg per g and this was the second best treatment.

Increase in pupal duration observed with acetone and ethanol extracts were in agreement with that of Lucena *et al.* (2017), wherein hexane extract of spiked pepper, *Piper aduncum* L. leaves @ 2.5 mg per mL also increased pupal duration in fall armyworm, *Spodoptera frugiperda* (J.E. Smith) by one day.

Morphogenetic changes

The pupae and adults that were emerged from treated grubs underwent several morphogenetic changes including pupal discolouration and poor sclerotization in the adults.

The anti-insect properties exhibited by various extracts on the larvae were dose-dependent. Changes in biology and morphogenetic effects observed in the larvae may be attributed to the changes in hormonal system.

With regard to antifeedant effect, insecticidal effect and detrimental effect of extracts on pest, acetone extract at LD_{90} value is the most effective extract. Ethanol extract at LD_{90} value was equally good as acetone extract at same concentration in exhibiting antifeedant effect.

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