

Bio-efficacy of botanical soap formulations fortified with essential oils against *Henosepilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae) and evaluation of its shelf life

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ABSTRACT: Experiments were conducted to evaluate the efficacy of botanical soap formulations against epilachna beetle, *Epilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae), and their repellent effect and the shelf life. In the first experiment, neem oil+ citronella oil soap @2 per cent recorded the highest per cent mortality and was on par with treatments, pongamia+ basil oil soap @ 2 per cent, neem oil+ basil oil soap @ 2 per cent, and pongamia+ citronella oil soap @ 2 per cent after 24 hours after treatment. In the second experiment, neem oil+ citronella oil soap @ 2 per cent recorded maximum repellence (80%) and was on par with pongamia oil+ citronella soap @ 2 per cent (73.33%) and neem oil+ citronella soap @ 1 per cent (65%) after 30 minutes after treatment. In the third experiment, neem oil + citronella oil @ 2 per cent exhibited the highest mortality rates under both refrigerated and room conditions and statistically superior to all other treatments. The lowest per cent mortality was recorded for the control. The soap formulations stored under refrigerated conditions (for a period of three months), showed higher insecticidal efficacy than those stored at room conditions, revealing the effectiveness remained for three months.

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KEY WORDS: Epilachna beetle, bioassay, neem oil, citronella oil, basil oil, pongamia oil, repellent effect, shelf life

INTRODUCTION

The hadda beetle, *Epilachna vigintioctopunctata* (Fab.) (Coleoptera, Coccinellidae) is a serious pest of brinjal, *Solanum melongena* L. Both adults and grubs being the damaging stages of the pest, feeds on the epidermal layer of the leaves, reducing the photosynthetic area and cause serious defoliation

(Imura and Ninomiya, 1998). The 3rd and 4th instar grubs were reported to be the most damaging stages. Farmers often rely on harmful chemicals which may prove to be harmful for human health and environment if not used judiciously. Kaur and Singh (2013) reported neem soap @ 1 per cent significantly lowered aphid populations in capsicum followed by pongamia soap @ 2 per cent and

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confidor 17.8 SL @ 0.025 per cent. Pongamia oil soap @2% was found effective in reducing the leaf hopper population, *Amrasca biguttula biguttula* (Ishida) (Thomas and Sreekumar, 2020). Essential oils consist of a mixture of various compounds; Monoterpenes, the primary constituents of essential oils, are lipophilic molecules capable of readily penetrating the insect integument (Tisserand and Young, 2013). Nerio *et al.* (2010) reported that citronella oil possesses repellent effect to insects. Basil oil was reported to contain biologically active constituents that are insecticidal (Chavan and Nikam, 1982), Even though soaps made from plant based insecticides have been proved to be effective in pest management, a combination of botanical soaps fortified with essential oils was lacking. Therefore studies on combined effect of both botanical soaps and essential oils for enhanced pest management were taken up against *E. vigintioctopunctata*.

MATERIALS AND METHODS

The study was carried out at the Department of Entomology, College of Agriculture, Vellayani Kerala, India, during 2021-2023. Botanicals *viz.* neem oil and pongamia oil and essential oils *viz.* basil oil and citronella oil were procured. The saponification value of neem oil, pongamia oil and palm oil estimated were 188, 182, and 192mg KOH/g, respectively. These soft soap formulations were prepared by combining botanicals *viz.*, neem oil and pongamia oil and essential oils *viz.* citronella oil and basil oil. The alkali used for the soap is potassium hydroxide.

Eggs of hadda beetle along with the leaves were collected from infested plants grown in the college campus. The collected egg mass, grubs and adults were placed in separate plastic trays of 28 × 16cm. These trays were covered with white cloth for aeration. Fresh brinjal leaves were provided daily for the grubs and adults. The instars emerged were fed with fresh leaves and placed in new trays. These grubs were reared up to the final instars and the final instar was taken for the laboratory evaluation.

Laboratory bioassay: Leaf dip bioassay was carried out to monitor the toxicity of insecticidal

soaps against the grubs. Laboratory experiments were laid in Completely Randomized Design (CRD) with 19 treatments (Table 1) replicated thrice. Fresh leaves of brinjal were cut into leaf discs of 9 cm diameter and then dipped in different concentrations of botanical soaps. After 10 minutes of exposure, treated leaf discs were kept to dry. After drying, the leaf discs were placed into the Petri plates lined with moist filter paper and 20 grubs representing various treatments were released into each Petri plates. Petri plates were covered with lid to prevent the insect escape. Observations on the mortality were taken after a specific exposure interval of 24 and 48 hours respectively. The corrected mortality was calculated using the following formula (Abbot, 1925). The mortality data were subjected to arcsine transformation and analysis of variance.

$$\begin{aligned} \text{Corrected per cent mortality} = \\ (\% \text{ mortality in treatment} - \% \text{ mortality in control}) \\ \% \text{ mortality in control} \end{aligned}$$

Based on the mortality data, six promising treatments were selected for evaluating repellence effect.

Repellent effect of botanical soap formulations: The repellent effect of different concentrations was determined by modifying the method of Wei *et al.* (2018), with six treatments and three replications in CRD (Table 2). Brinjal leaf discs were cut into two halves (diameter: 3cm) and were placed on the opposite sides of the Petri plates of 9cm diameter. Two halves were separated by gap of 5cm. One half of the brinjal leaves was treated with varying concentration of botanical soaps while the other half was treated with control (water). Following the treatment, the final instars grubs of hadda beetle were released into the centre of the Petri plates and observations were taken at 30 and 60 MAT (Minutes After Treatment). Formula for calculating per cent repellence is given below.

$$PR (\%) = \frac{N_c - N_t}{N_c + N_t} * 100$$

Where PR = per cent repellence, N_c= No. of grubs in control, N_t= No. of grubs in treatment.

Evaluation of shelf life of botanical soap formulations:

The shelf life of best three botanical soap formulations from the bioassay experiments was evaluated under room (27-29°C) and refrigerated conditions (4-7°C) and its effect on epilachna grubs for a period of three months at monthly intervals. The experiment was laid out in the CRD design and data was analysed and values in parentheses were subjected to arc sign transformation. Significant differences between each treatment were compared by using GRAPES (General R-shiny based Analysis Platform Empowered by Statistics) software (Gopinath *et al.*, 2020).

RESULTS AND DISCUSSION

Bioassay: After 24h of exposure, mortality was maximum in the treatment, neem oil+ citronella oil soap @ 2% (54.42%) and was on par with treatments, pongamia+basil oil soap @ 2% (40.89), neem oil+ basil oil soap @ 2% (38.61), and pongamia + citronella oil soap @ 2% (37.56). Palm oil soap @2% (8.60%) and palm oil soap @1% (3.33%) were found to be least effective and were found to be inferior to other treatments (Table 1).

After 48 h of exposure, the treatment neem oil + citronella oil soap @ 2% showed the highest per cent mortality (80.44) followed by neem oil + basil oil soap @2% (70.16). However, both these treatments were found to be on par with each other. Palm oil soap @2% was found to be least effective. The effect of insecticidal effect of neem oil has been proved by many workers. Anam *et al.* (2004) reported that neem oil @ 4% caused highest larval mortality (100%) of *E. dodecastigma*. Similarly, Hanif *et al.* (2021) revealed that neem extract @10 % recorded highest mortality of hadda beetle grubs (73.4%). This might be due to the presence of azadiractin, which alters the feeding habits of insects, and physiological disturbances that lead to the death of insects. Swaminathan *et al.* (2010) reported that pongamia oil @ 5% exhibited a cent percent mortality to epilachna grubs after 7 days of treatment. Lee *et al.* (2004) noticed that the chemical composition of essential oil is lipophilic in nature, which will enter into an insect and cause biochemical dysfunction and mortality.

Table 1. Evaluation of the efficacy of botanical soap formulations on epilachna beetle

Treatments	*Mortality (%)	
	24 HAT	48 HAT
T1: Neem oil + citronella oil soap @1%	20.88 (26.64) ^{bc}	48.07 (43.88) ^{cd}
T2: Neem oil + citronella oil soap @2%	54.42 (47.54) ^a	80.44 (63.83) ^a
T3: Neem oil + basil oil soap @1%	11.93 (19.58) ^{cd}	34.39 (35.82) ^{de}
T4: Neem oil +basil oil soap @2%	38.61 (38.41) ^{ab}	70.18 (57.02) ^{ab}
T5: Pongamia + citronella oil soap @1%	8.51 (16.43) ^{de}	20.53 (26.67) ^{efg}
T6: Pongamia + citronella oil soap@2%	37.56 (37.78) ^{ab}	62.02 (52.00) ^{bc}
T7: Pongamia + basil oil soap @1%	10.35 (18.36) ^d	20.61 (26.59) ^{efg}
T8: Pongamia +basil oil soap @2%	40.89 (39.73) ^{ab}	61.42 (51.66) ^{bc}
T9 : Pongamia oil soap @1%	10.35 (18.36) ^d	17.28 (24.50) ^{fg}
T10- Pongamia oil soap @2%	17.02 (23.69) ^{cd}	24.04 (29.27) ^{efg}
T11: Neem oil soap @1%	10.18 (17.69) ^d	25.79 (30.46) ^{efg}
T12: Neem oil soap @2%	25.53 (30.17) ^{bc}	52.98 (46.81) ^c
T13: Palm oil soap @1%	3.33 (6.14) ^f	5.00 (7.59) ^h
T14: Palm oil soap @2%	8.60 (16.87) ^f	9.33 (10.03) ^h
T15: Palm oil + citronella oil soap@1%	20.88 (26.64) ^{cd}	29.47 (32.63) ^{ef}
T16: Palm oil + citronella oil soap @2%	18.95 (25.45) ^{cd}	34.56 (35.98) ^{de}
T17: Palm oil +basil oil soap @1%	10.35 (18.36) ^{de}	20.61 (26.90) ^{efg}
T18- Palm oil +basil oil soap @2%	12.02 (19.82) ^{cd}	24.04 (29.16) ^{efg}
T19- Control	0.00 (0.00) ^f	0.00 (0.00) ^h

* Mean of three replications

Values in the parenthesis were subjected to arc sine transformation

HAT – Hours after treatment

In a column, the mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Repellent effect of soap formulations: Six most effective treatments from bioassay experiment were further assessed for their repellent effect against the fourth instar grubs (Table 2). Neem oil + citronella oil soap @ 2% recorded maximum repellence (80%) and was on par with pongamia oil + citronella soap @ 2% (73.33%) and neem oil + citronella soap @ 1% (65.00%) after 30 minutes after treatment (MAT). The treatment neem oil + citronella oil soap @ 2% recorded maximum repellence (85%) at 60 MAT and was on par with pongamia oil + citronella oil soap @ 2% (76.67%). Similarly, Ikeura *et al.* (2013) also reported the repellence activity of neem against cabbage armyworms under laboratory conditions and they mentioned that the repellent activity of neem was due to azadiractin content. Manoj (2022) also reported that both basil and citronella oil exhibited 90 to 100 percent repellence against hadda beetle grubs after 30 and 60 minutes of treatment, respectively.

Evaluation of shelf life of botanical soap formulations: The effect of stored botanical soap formulations was evaluated against epilachna grubs on the first, second and third months of storage. After 24 hours of treatment, the freshly prepared soap solutions of neem oil + citronella oil @2% recorded highest mortality (54.42%) and was statistically superior to all other treatments. After

one month of storage, soap formulations stored under refrigerated conditions showed greater mortality compared to those stored at room temperature. Soap formulation of neem oil + citronella oil @ 2% exhibited the highest mortality, under both refrigerated and room conditions (46.71

Table 2. Repellent effect of botanical soap formulations against epilachna beetle grubs

Treatments	*Repellence (%)	
	30 MAT	60 MAT
T1: Neem oil + citronella oil soap @2%	80.00 (63.54) ^a	85.00 (67.40) ^a
T2: Pongamia + citronella oil soap @2%	73.33 (59.32) ^a	76.67 (61.76) ^{ab}
T3: Neem oil + citronella oil soap @1%	65.00 (59.32) ^a	68.33 (55.97) ^{bc}
T4:Neem oil +basil oil soap @2%	55.00 (47.91) ^b	60.00 (50.79) ^{cd}
T5: Pongamia +basil oil soap @2%	55.00 (47.89) ^b	61.67 (51.81) ^{bcd}
T6: Neem oil soap @2%	45.00 (42.09) ^b	50.00 (45.00) ^d

* Mean of three replications; Values in the parenthesis were subjected to arc sine transformation

MAT – Minutes after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Table 3 a. Shelf life efficacy of botanical soap formulations @2% against epilachna beetle after 24 HAT

Treatments	*Mortality (%) – after 1 – 3months of storage						
	Laboratory	First month		Second month		Third month	
		Refrigerated bioassay	Room	Refrigerated	Room	Refrigerated	Room
Neem oil+ citronella oil	54.42 (43.87) ^a	46.71 (43.53) ^a	43.03 (40.91) ^a	41.84 (40.14) ^a	35.33 (34.56) ^a	33.35 (35.07) ^a	31.25 (33.91) ^a
Neem oil + basil oil	38.61 (35.94) ^b	35.39 (36.49) ^b	32.89 (34.79) ^b	35.59 (36.39) ^b	27.76 (30.03) ^b	28.09 (31.77) ^b	26.25 (29.56) ^b
Pongamia oil + citronella oil	37.56 (34.92) ^b	34.14 (35.64) ^b	31.64 (34.06) ^b	29.08 (32.39) ^b	24.14 (28.25) ^b	26.91 (31.17) ^b	23.75 (27.45) ^b
Control	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c	0.00 (0.00) ^c

* Mean of three replications; Values in the parenthesis were subjected to arc sine transformation

HAT – Hours after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

Table 3 b. Shelf life of botanical soap formulations @2% against epilachna beetle after 48 HAT

Treatments	*Mortality (%) – after 1 – 3months of storage						
	Laboratory	First month		Second month		Third month	
		Refrigerated bioassay	Room	Refrigerated	Room	Refrigerated	Room
Neem oil+ citronella oil	80.44 (63.83) ^a	65.09 (53.85) ^a	61.25 (51.79) ^a	61.31 (51.82) ^a	53.75 (47.16) ^a	53.43 (46.99) ^a	47.56 (35.07) ^a
Neem oil + basil oil	70.18 (57.02) ^b	58.36 (49.86) ^a	50.99 (45.71) ^a	53.75 (47.23) ^a	47.64 (43.63) ^a	49.13 (44.50) ^a	44.87 (31.77) ^a
Pongamia oil + citronellaoil	62.02 (52.00) ^b	54.28 (47.53) ^a	48.95 (44.38) ^a	52.70 (346.57) ^a	45.14 (42.18) ^a	45.25 (42.26) ^a	42.43 (31.17) ^a
Control	0.00 (0.00) ^c	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b	0.00 (0.00) ^b

* Mean of three replications; Values in the parentheses were subjected to arc sine transformation; HAT – Hours after treatment; In a column, mean followed by same letter (s) are not significantly different at (P=0.05) by LSD

and 43.03% respectively) and this treatment was found to be superior to all other treatments. The same trend in the mortality of the test insect has been exhibited by soap formulations kept after two and three months of storage respectively (Table 3a).

After 48 hours of treatment, the freshly prepared soap solutions of neem oil + citronella oil @ 2% recorded highest mortality (80.44%) and was statistically superior to all other treatments. After one month of storage, soap formulations kept under refrigerated conditions demonstrated a higher percentage of grub mortality compared to those stored at room temperature. The formulation neem oil + citronella oil @ 2% exhibited the highest mortality, under refrigerated and room conditions (65.01 and 61.25%, respectively) and were found to be superior statistically. The same trend in the mortality of the test insect was showed when the soap formulations were stored after two and three months respectively (Table 3b).

This finding was in line with the findings of Varma (2018), who reported that neem oil garlic soap can be stored for up to three months without reduction in its efficacy. After the storage period, the soap formulations stored under refrigerated conditions showed higher efficacy than those stored at room conditions. This finding was supported by Rowshan *et al.* (2013), who reported that the primary quality

of essential oils was maintained by storing them under refrigerated (4°C) and freezer conditions (-20°C) when compared to room conditions during a period of storage of three months. Farahbakhsh *et al.* (2021) found that the quality of essential oil constituents with low molecular weight decreased over time, especially at room temperature. This quality deterioration may be due to the oxidation, evaporation and other undesired changes in essential oil components during storage. Evaluation of shelf life of these formulations revealed that their effectiveness remained for three months.

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