



RESEARCH ARTICLE

Green arsenal against papaya mealybug (*Paracoccus marginatus* Williams and Granara de Willink): An *in vitro* study of selected botanical oils for sustainable management

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Abstract

Papaya mealybug (*Paracoccus marginatus*) is a highly destructive invasive pest causing severe yield losses in papaya and several other crops. This study evaluated the efficacy of three plant oils, viz., neem seed oil, pongamia seed oil and jatropha seed oil, against the adult stage of papaya mealybug, *Paracoccus marginatus*, by using profenofos 50 EC as a standard check. The study was conducted under laboratory conditions with air temperature ($27 \pm 2^\circ\text{C}$) and relative humidity ($75 \pm 5\%$). The papaya mealybug was reared in potato sprouts under laboratory conditions after collection. A leaf dip bioassay was carried out and also observations were taken and analysed following standard procedures. Experimental findings revealed that among the three plant oils evaluated, neem seed oil was the most effective treatment against the adult stage of papaya mealybug. The highest mortality was recorded at 3.0 % concentration of neem seed oil after 72 hr of treatment. The order of toxicity to *P. marginatus* (adult) with respect to LC_{50} values after 72 hr exposure period was profenofos (0.003 %) > neem seed oil (1.365 %) > pongamia seed oil (2.033 %) > jatropha seed oil (3.262 %). The order of relative toxicity was profenofos 50 EC > neem seed oil > pongamia seed oil > jatropha seed oil for the exposure period of 72 hr. Considering the efficacy, LC_{50} value and relative toxicity it was found that all the evaluated plant oils can be successfully used as efficient alternatives of chemical pesticides in future pest management programmes against papaya mealybug to promote green agriculture. Further field testing can be carried out to develop an eco-friendly management strategy for controlling mealybugs in the field conditions.

Keywords: jatropha (*Jatropha curcas*) oil; neem oil (*Azadirachta indica*) oil; papaya mealybug (*Paracoccus marginatus*); pongamia (*Pongamia pinnata*) oil; Pseudococcidae

Introduction

The papaya plant (*Carica papaya* L.) is a valuable fruit crop from the family Caricaceae (1). The global papaya production was 14.23 mMT in 2023 (2). The papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), is a polyphagous pest and the first specimen was collected in Mexico in 1955. It was first described from the Neotropical region in Belize, Costa Rica, Guatemala and Mexico in 1992 (3). Invasive species have become a major threat to global agriculture, which can adversely affect food security. The papaya mealybug has attained the status of an invasive pest in various countries by spreading across its native range. Now it has become a major threat to agriculture.

In India, the first infestation was reported in papaya plants in the year 2008 from Tamil Nadu Agricultural University and it has gained the status of a major pest in India in 2009 (4, 5). In Assam, it was first reported from Dibrugarh district in the year 2012 now it has spread through various districts of Assam by means of infested

fruit (6, 7). The papaya mealybug starts to attack the crops while approaching three months of age. Secretion of honeydew attracts ants and helps in building up sooty mould. In papaya, infestations are commonly observed along the veins on the underside of leaves, forming dense cottony masses, while waxy secretions on fruits render them unmarketable (8). In dry and warm conditions, the pest becomes more active (9).

One of the primary causes of its spread is the lack of its native natural enemy. It is challenging to regulate this pest because of the presence of wax all over the body (10). Additionally, the species has a strong capability for reproduction, producing 11 generations annually and a generation of 30 to 40 days. Fifteen generations are completed in a year in tropical conditions (11). It has gained the status of a polyphagous pest that infests 49 families of host plants (12). Asteraceae, Malvaceae, Solanaceae and Euphorbiaceae are reported as the most favoured hosts of this mealybug species (13). Infestation by *P. marginatus* can result in yield losses exceeding 70–90 % in severely infested and unmanaged papaya plantations (14, 15).

Hence, the management of this pest has become a major challenge in recent years. To manage high populations of papaya mealybug, systemic organophosphate and contact pesticides are used, but frequent applications are required due to the heavy waxy layer of the pest. Moreover, the use of chemical pesticides causes detrimental effects on non-target beneficial insects and is harmful to our ecosystem (16). In this context, plant-based insecticides have gained importance as safer and sustainable alternatives. Botanical oils possess repellent, antifeedant and toxic properties due to their rich composition of secondary metabolites. Their lipophilic nature enables better penetration through insect cuticles, including wax-coated pests such as mealybugs (17). Botanical oils have been widely evaluated as eco-friendly alternatives to synthetic insecticides for the management of mealybugs, including the papaya mealybug *Paracoccus marginatus*. Essential and fixed oils such as neem (*Azadirachta indica*), garlic (*Allium sativum*), castor (*Ricinus communis*) and citrus oils have shown significant contact toxicity and population suppression of *P. marginatus*, primarily by disrupting the insects' waxy cuticle and respiratory functions (18). Laboratory and field evaluations demonstrated that neem oil-based formulations caused more than 80–90 % mortality of papaya mealybug at higher concentrations and performed comparably to conventional insecticides while being safer to natural enemies (18, 19). Similar efficacy of plant oils has also been reported against other mealybug species, supporting their potential inclusion in integrated pest management programmes for sustainable papaya cultivation (20). Therefore, this study was undertaken to explore the effectiveness of certain plant oils against the devastating pest papaya mealybug under laboratory conditions.

Materials and Methods

The experiment was conducted in the Post Graduate Laboratory, Department of Entomology, Assam Agricultural University, Jorhat, Assam, India, under controlled conditions of 27 ± 2 °C air temperature and 75 ± 5 % relative humidity.

Collection of test insect

The test insect, *P. marginatus*, or papaya mealybug, was collected from infected papaya plants (Fig. 1A) in several locations on the Assam Agricultural University, Jorhat campus, to perform the bioassay. Approximately 200–500 adult and immature mealybugs were collected. Infested papaya leaves containing mature female ovisacs (5–10 ovisacs were collected per day) (Fig. 2A) were examined using a binocular stereoscopic microscope (10X) (Model: AxiocamERc 5s, Make: Zeiss).

Rearing of test insect under laboratory conditions

The lab culture of test insect was maintained on potato sprouts (Fig. 1B) using a slightly modified methodology (21). Seed potatoes were washed, air-dried and treated with 1 % gibberellic acid (GA_3) for 30 min to enhance uniform sprouting, as reported by previous studies (22, 23). The potatoes were sanitised in the sun for three days before being moved and they were then put in trays (43 cm by 30 cm) with damp sand within. With the appearance of sprouts after a week, potatoes with healthy sprouts were selected and cultivated for subsequent processing. With a gentle camel hair brush (No. "0"), the ovisacs that were collected in the field were released into the potato sprouts at the rate of 3–5 ovisacs per potato (24). Within one month, mealybug masses were obtained and the mealybug population was maintained until the completion of the study. The adult female mealybugs were used for the experiment (Fig. 2B).



Fig. 1. Papaya fruit infested: A. by Papaya mealybug, B. Rearing of papaya mealybug in potato sprouts under laboratory conditions, C-D Leaf disc bioassay

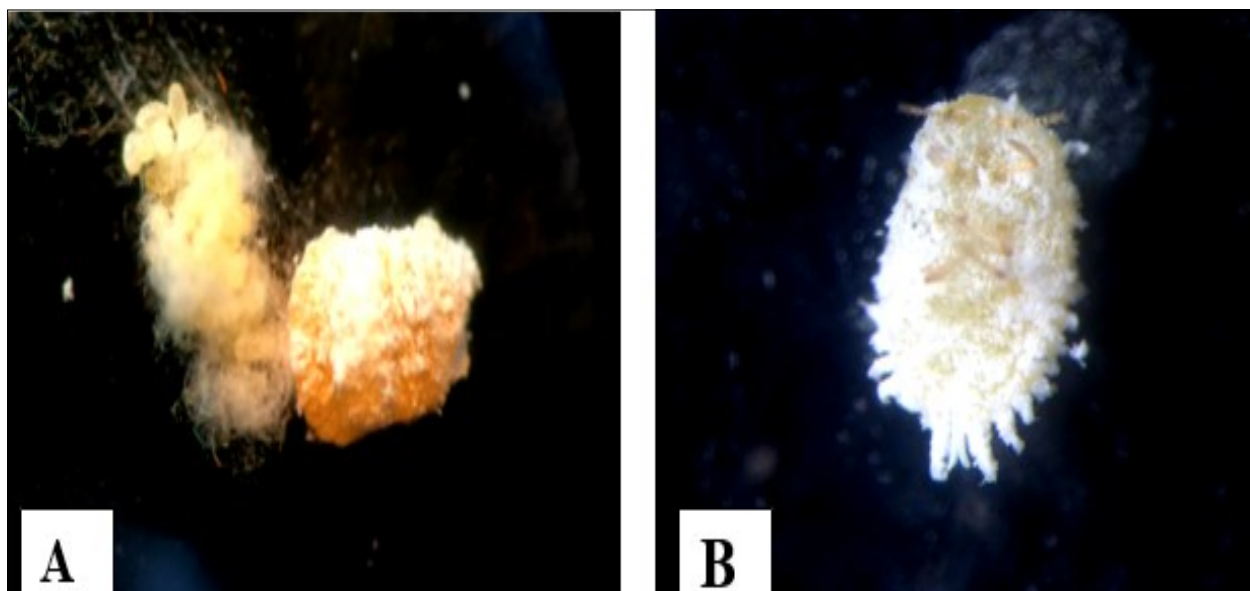


Fig. 2. A. Adult female mealy bug with ovisac, B. Adult female mealy bug

Design and layout of the experiment

A completely randomised design was adopted for the experiment. For each of the plant oils and the standard check, five concentrations were taken, which were replicated 10 times. In each replication, 10 insects were used.

Treatments

In the experiment, five treatments were used: three plant oils, one standard check and the untreated control. The plant oils used are Neem seed oil, *Azadirachta Indica*; Pongamia seed oil, *Pongamia pinnata*; Jatropha seed oil, *Jatropha curcas*. Profenofos 50 EC was used as a standard check. Distilled water was used as the untreated control.

Source of plant oils

The plant oils, neem and pongamia seed oils, along with Profenofos 50 % EC, were purchased from Jaldhara & Company, Jorhat-14 and jatropha seed oil was taken from the Department of Entomology, AAU, Jorhat-13, Assam.

Preparation of botanical oil solution

The collected plant oils were used as the standard (100 %) and stock solutions of known concentration of plant oils were prepared in distilled water from the standard, where washing soap 1 % was added as a surfactant for easy mixing of oil in water. Serial dilution was carried out to obtain subsequent concentrations of the oils.

Preparation of insecticidal solution for the standard check

As a standard check, profenofos 50 EC was used. Distilled water was used to prepare the stock solution with a known concentration and serial dilution was used to get subsequent concentrations.

Leaf dip bioassay against the adult stage of papaya mealybug (*P. marginatus*)

The leaf dip method (Fig. 1C-1D) was used to conduct the bioassay of plant oils against adult stage of *P. marginatus*, with minor modifications made (25). Five different concentrations of each of the plant oil (neem, pongamia and jatropha) and profenofos were prepared after reviewing the literature to determine the LC_{50} value. In the experiment, newly harvested

potato leaves were used and before the treatment, leaves were thoroughly cleaned and allowed to air dry. After being immersed in test solutions, leaves were gently shaken for 30 sec and then placed on tissue paper with the adaxial side facing up to air dry. After being allowed to dry, the treated leaves were placed in 12 cm diameter petri dishes, which were sealed at the mouth with a glass chimney and a muslin cloth tied with rubber band on top to prevent desiccation. In order to record mortality rates, the mass-reared insects were gently released into the petri dishes containing the treated leaves, using a soft camel hair brush to prevent injury. Ten quantities of insects were released per replication, with 10 replications of each concentration of the plant oils and profenofos 50 EC being kept. Furthermore, a control set was also conducted where the leaves were treated only with distilled water and replicated for ten times.

Observation

After 12, 24, 48 and 72 hr of treatment, observations on the mortality rate of the test insect were made using a binocular microscope (4X) (Make: Magnus). A soft camel hair brush was used to gently touch mealybugs; if the insects did not move, they were considered dead. The % mortality was calculated in each treatment. The observed mortality was corrected by using the formula in Eqn 1.

$$\text{Corrected mortality rate} = (X-Y)/X \times 100 \quad (\text{Eqn. 1})$$

Where X = the % living in control, Y = the % living in treatment

Statistical analysis

Subsequent data were subjected to Probit Analysis by using SPSS Statistics version 26.0 (IBM Corp., USA) to determine the LC_{50} value, which was derived using the regression equation. The LC_{50} value of profenofos 50 EC (standard check) was divided by the LC_{50} value of plant oils to estimate the relative toxicity values of neem seed oil, pongamia seed oil and jatropha seed oil.

Relative toxicity =

$$\frac{LC_{50} \text{ value of Standard check (profenofos 50 EC)}}{LC_{50} \text{ value of plant oils}} \quad (\text{Eqn. 2})$$

Results and Discussion

Efficacy of plant oils against *Paracoccus marginatus*

Fig. 1 illustrates the infestation symptoms, laboratory rearing and bioassay procedure, while Fig. 2 shows adult female mealybugs and ovisacs. Table 1-4 provides a detailed account of the efficacy of plant oils against the adult stage of papaya mealybug. From the experiment, it was observed that the % mortality of *P. marginatus* increased progressively with increasing concentrations as well as exposure period, indicating a clear dose-time dependent response. Similar findings were also reported in a study evaluating insecticidal plant parts against *Tribolium castaneum*, where mortality increased with both concentration and duration of exposure (26). A comparable trend was also documented in *Aphis craccivora*, demonstrating a direct proportional relationship between % mortality, concentration and exposure time, thereby supporting the consistency of dose-dependent toxicity across different hemipteran pests (27).

It was evident from the investigation that all the evaluated plant oils exhibited a direct toxic effect against the mealybug. This toxicity may be due to the presence of multiple bioactive compounds such as alkaloids, flavonoids, terpenoids, phenols, amino acids and sugars and other secondary metabolites. Plant essential oils act as repellents and antifeedants against insect pests. Their high lipophilicity enables easy penetration through the insect cuticle. In papaya mealybug,

which is protected by a thick waxy layer, these oils can disrupt and degrade the wax coating, thereby increasing the insects' susceptibility (28).

LC₅₀ and relative toxicity against *P. marginatus* (adults)

Table 5 provides a detailed account of the LC₅₀ and relative toxicity of plant oils against *P. marginatus* (adults). Among the three plant oils evaluated, the neem seed oil registered the highest toxic effect with the lowest LC₅₀ values of 3.427, 2.240, 1.668, 1.365 % after 12, 24, 48 and 72 hr of exposure, respectively. A steady decline in LC₅₀ values with increasing exposure time indicates enhanced toxicity with prolonged contact. Numerically, the LC₅₀ value decreased by approximately 60.2 % from 12 hr (3.427 %) to 72 hr (1.365 %), demonstrating substantial time-dependent toxicity.

The higher efficacy of neem oil may be attributed to its multiple modes of action and the presence of diverse bioactive compounds in it. More than 100 bioactive compounds are present in neem oil, with azadirachtin being the most potent. Neem oil acts primarily as a contact pesticide with both translaminar and systemic effects. Neem oil has a variety of effects, including larvicidal action, feeding deterrent and regulation of insect growth. According to some reports, neem has the highest efficacy against a variety of insects that exhibit insecticidal, repellent, or antifeedant effects, which conforms with the current study (29, 30). Neem oil is effective against a broad range of agricultural pests, including sucking insects,

Table 1. % mortality of *P. marginatus* (adults) by neem seed oil, at different exposure periods

Plant oil	Concentration (%)	Mean mortality (%) at different time intervals			
		12 hr	24 hr	48 hr	72 hr
Neem seed oil	1.0	22.00 (27.74)	31.00 (33.68)	46.00 (42.67)	53.00 (46.73)
		36.00 (36.79)	42.00 (40.36)	50.00 (45.00)	58.00 (49.69)
	1.5	42.00 (40.33)	53.00 (46.70)	58.00 (49.66)	63.00 (52.60)
		51.00 (45.57)	62.00 (51.99)	73.00 (58.78)	80.00 (63.73)
	2.0	60.00 (50.79)	71.00 (57.57)	82.00 (65.20)	85.00 (67.50)
		0 (0.33)	0 (0.33)	0 (0.33)	0 (0.33)
	2.5	1.60 (0.33)	1.84 (0.33)	1.76 (0.33)	1.78 (0.33)
		3.23 (0.33)	3.71 (0.33)	3.55 (0.33)	3.59 (0.33)
	3.0	0 (0.33)	0 (0.33)	0 (0.33)	0 (0.33)
		0.33 (0.33)	0.33 (0.33)	0.33 (0.33)	0.33 (0.33)
	S.Ed (±)	1.60	1.84	1.76	1.78
	CD (P = 0.05)	3.23	3.71	3.55	3.59

**Data are means of 10 replications, each with 10 individuals, % = percent. Figures within parentheses are transformed angular values. Means within a column differ significantly at $P \leq 0.05$ according to one-way ANOVA.

Table 2. % mortality of *P. marginatus* (adults) by pongamia seed oil at different exposure periods

Plant oil	Concentration (%)	Mean mortality (%) at different time intervals			
		12 hr	24 hr	48 hr	72 hr
Pongamia seed oil	1.5	29.00 (30.94)	39.00 (38.57)	42.00 (40.36)	49.00 (44.42)
		38.00 (37.97)	44.00 (41.53)	49.00 (44.42)	55.00 (47.88)
	2.0	47.00 (43.26)	51.00 (45.57)	54.00 (47.30)	65.00 (53.77)
		50.00 (45.00)	59.00 (50.24)	64.00 (53.17)	73.00 (58.84)
	2.5	55.00 (47.88)	67.00 (54.98)	72.00 (58.15)	81.00 (64.39)
		0 (0.33)	0 (0.33)	0 (0.33)	0 (0.33)
	3.0	0.33 (0.33)	0.33 (0.33)	0.33 (0.33)	0.33 (0.33)
		0.33 (0.33)	0.33 (0.33)	0.33 (0.33)	0.33 (0.33)
	3.5	1.56 (0.33)	1.56 (0.33)	1.31 (0.33)	1.50 (0.33)
		5.33 (0.33)	3.14 (0.33)	2.65 (0.33)	3.01 (0.33)
	S.Ed (±)	2.65	1.56	1.31	1.50
	CD (P = 0.05)	5.33	3.14	2.65	3.01

**Data are means of 10 replications, each with 10 individuals, % = percent. Figures within parentheses are transformed angular values. Means within a column differ significantly at $P \leq 0.05$ according to one-way ANOVA.

Table 3. % mortality of *P. marginatus* (adults) by jatropha seed oil at different exposure periods

Plant oil	Concentration (%)	Mean mortality (%) at different time intervals			
		12 hr	24 hr	48 hr	72 hr
Jatropha seed oil	2.5	19.00	25.00	32.00	42.00
		(25.45)	(29.82)	(34.29)	(40.36)
	3.0	28.00	34.00	43.00	52.00
		(31.81)	(35.54)	(40.93)	(46.15)
	3.5	33.00	43.00	51.00	59.00
		(35.01)	(40.96)	(45.57)	(50.21)
	4.0	42.00	48.00	63.00	72.00
		(40.33)	(43.82)	(52.60)	(58.24)
	4.5	51.00	60.00	71.00	82.00
		(45.57)	(50.81)	(57.51)	(65.20)
	control	0	0	0	0
		(0.33)	(0.33)	(0.33)	(0.33)
	S.Ed (±)	1.71	1.82	1.63	1.62
	CD ($P = 0.05$)	3.43	3.60	3.28	3.27

**Data are means of 10 replications, each with 10 individuals, % = percent. Figures within parentheses are transformed angular values. Means within a column differ significantly at $P \leq 0.05$ according to one-way ANOVA.

Table 4. % mortality of *P. marginatus* (adults) by profenofos 50 EC at different exposure periods

Plant oil	Concentration (%)	Mean mortality (%) at different time intervals			
		12 hr	24 hr	48 hr	72 hr
Profenofos 50 EC	0.005	29.00	48.00	57.00	64.00
		(32.42)	(43.84)	(53.17)	(53.17)
	0.010	42.00	60.00	67.00	76.00
		(40.33)	(50.84)	(55.04)	(60.92)
	0.050	52.00	65.00	73.00	81.00
		(46.20)	(53.77)	(58.84)	(64.39)
	0.070	65.00	69.00	79.00	87.00
		(53.86)	(56.37)	(63.21)	(70.30)
	0.100	71.00	74.00	82.00	91.00
		(57.57)	(59.65)	(65.35)	(75.47)
	control	0	0	0	0
		(0.33)	(0.33)	(0.33)	(0.33)
	S.Ed (±)	2.13	1.92	2.07	2.82
	CD ($P = 0.05$)	4.29	3.86	4.16	5.67

Data are means of 10 replications, each with 10 individuals, % = percent. Figures within parentheses are transformed angular values. Means within a column differ significantly at $P \leq 0.05$ according to one-way ANOVA.

Table 5. Median lethal concentration (LC_{50}) values of different treatments to *P. marginatus* (adults) after 72 hr

Treatment	Regression equation	Standard error of the regression coefficient	Chi square	LC_{50} (%)	Fiducial limit		Relative toxicity	Order of toxicity
					Lower limit	Upper limit		
Neem seed oil	$Y = -0.155 + 1.145X$	0.034	54.944	1.365	1.232	1.481	0.0021	I
Jatropha seed oil	$Y = -1.251 + 2.436X$	0.108	35.340	3.262	3.147	3.372	0.0009	III
Pongamia seed oil	$Y = -0.426 + 1.364X$	0.055	29.193	2.033	1.888	2.162	0.0014	II
Profenofos 50 EC	$Y = 0.985 + 0.385X$	0.060	118.20	0.003	0.001	0.005	1.0000	-

Y = Probit kill, X = log concentrations. Mortality based on 10 replications, each with 10 individuals

caterpillars and stored-product pests and is commonly integrated into eco-friendly and IPM strategies because of its biodegradability and low toxicity to non-target organisms. Beyond pest management, neem seed oil is also used in medicine and pharmaceuticals, cosmetics and personal care products, veterinary applications and as a biofertilizer and soil conditioner, highlighting its multifunctional value in agriculture and allied sectors.

The order of toxicity based on LC_{50} and relative toxicity values was profenofos 50 EC > neem seed oil > pongamia seed oil > jatropha seed oil across all exposure periods. Neem seed oil showed the lowest LC_{50} value among botanical treatments, highlighting its strong potential as a plant-based alternative. This ranking remained consistent across 12, 24, 48 and 72 hr, indicating stability in toxic performance over time. Previous works supported the efficacy of neem oil in mealybug suppression. For instance, 3.0 % neem significantly reduced the population of cotton mealybug, *Phenacoccus solenopsis* (24).

Similarly, 2.0 % neem seed oil resulted in consistent suppression of pink mealybug, *Maconellicoccus hirsutus* (31). The LC_{50} values obtained in the present study (1.365–3.427 %) fall within a comparable concentration range, supporting the efficacy of neem seed oil across different mealybug species.

The results of the present bioassay are also supported by some earlier works where *Thymus vulgaris*, *Mentha longifolia* and *Cyperus articulatus* essential oils were reported to be the most significant toxic essential oils after 24 and 72 hr of treatment (31). The LC_{50} values were 29.03, 34.32 and 54.69 ppm, after 24 hr of treatment, respectively, whereas after 72 hr, they were 15.04, 24.93 and 29.21 ppm. From an ecological perspective, plant oils offer advantages because of their biodegradability, lower persistence in the environment and reduced risk to non-target organisms. Hence, the lowest LC_{50} value of neem oil across all exposure periods indicates its suitability for integration into sustainable pest management.

Conclusion

The present study demonstrated that neem, pongamia and jatropha seed oils possess significant insecticidal activity against adult *P. marginatus*. Neem seed oil was the most effective botanical, exhibiting the lowest LC₅₀ value and highest relative toxicity. These findings highlight the potential of neem oil as a sustainable, eco-friendly alternative to chemical insecticides. However, since the study was conducted under laboratory conditions, further field-level validation and formulation development are required before large-scale application. The integration of neem seed oil into IPM programmes may reduce reliance on synthetic organophosphates and contribute towards residue-free papaya production.

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Authors' contributions

BB¹ conducted the experiments, performed insect rearing and data collection; contributed to the conception, design and coordination of the study; participated in statistical analysis; and assisted in manuscript writing. IG contributed to the conception, design and coordination of the study. BB² contributed to the conception, design and coordination of the study. AB assisted in insect rearing and leaf collection. NK assisted in insect rearing and leaf collection. PB participated in the design of the study and performed statistical analysis. DS, MS and JS contributed to manuscript writing. All authors read and approved the final manuscript. [BB¹ - Bondita Bora and BB² - Badal Bhattacharyya].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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