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RESEARCH ARTICLE

Entomology

Insecticidal, repellent and histopathological effects of selected botanical extracts on the rice leaf folder (*Cnaphalocrocis medinalis*)

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Abstract: The rice leaf folder (*Cnaphalocrocis medinalis*) is a key pest in Asian rice fields that causes significant yield loss. This study evaluated the insecticidal potential of 10 methanolic botanical extracts from *Azadirachta indica, Calotropis* sp., *Ricinus communis*, *Justicia adhatoda, Aristolochia bracteolate, Cassia auriculata*, *Acorus calamus, Nicotiana tabacum, Pongamia pinnata,* and *Lawsonia inermis*. The extracts were tested at concentrations of 1%, 3%, 5%, 10%, 15% under laboratory conditions. Larval mortality was recorded at 24, 48, and 72 h and repellency was assessed using the filter paper disc method. Histopathological changes were examined in thoracic muscles, gastrointestinal tracts, and Malpighian tubules. The overall findings demonstrated that *A. indica* and *J. adhatoda* consistently caused the most larval mortality across all concentrations and time intervals, with one-way ANOVA indicating highly significant effects at 24, 48, and 72 h ($p < 0.001$). Probit analysis established lethal concentration values with an LC_{50} value of 28.5 ppm for *A. indica*, which is consistent with the previously reported efficacy of neem-based botanicals against rice leaf folder larvae. *A. indica and P. pinnata* showed 100% repellent activity while *C. auriculata and L. inermis* were the least effective (<30%). *A. indica* extracts caused intense histopathological effects resulting in significant tissue alterations with an H-score ranging from 255 to 285 while a lower H-score was recorded for the control. Overall, the selected botanical extracts caused significant larval mortality and pronounced histopathological alterations in *C. medinalis* larvae.

Keywords: Botanical extracts, *Cnaphalocrocis medinalis*, eco-friendly pest control, larval mortality

INTRODUCTION

Rice is a way of life and a cultural heritage that is essential in most Asian countries (Samarasekara et al., 2018). In Sri Lanka, paddy cultivation occupies ~29% of the total cultivated area and is the main source of food security, feeding most people and providing vital income for many farming families. According to statistics from 2022-2023, the annual average yield was 3.68 million tons (Department of Agriculture, Sri Lanka, 2025). Although Sri Lanka is able to cultivate rice intensively due to its favourable weather and climate, recurring insect pests are a source of biotic stress affecting both yield and quality of rice (Deutsch et al., 2018; Jena et al., 2018).

One such key pest is the rice leaf folder (*Cnaphalocrocis medinalis*), a common lepidopteran pest that has become widespread in Asia. The infective stage is the larva, which is able to roll the leaf and scrape off the green mesophyll tissues affecting photosynthesis, retarding plant growth and causing significant damage to yield (Zhuang et al., 2021; Bairwa et al., 2023).

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When leaf folders appear, farmers resort to synthetic insecticides as their main form of protection. However, the instantaneous results of chemical insecticides come with long-term consequences to the environment such as the presence of persistent chemicals in the soil and water, elimination of other helpful insects such as pollinators, and potential health concerns to people (Kampermann et al., 2023; Kashyap et al., 2024; Shareefdeen & Elkamel, 2024). Additionally, using synthetic chemicals in farming leads to the development of resistance in pests so that common pest treatments cease to function. This highlights the urgent need for environmentally safe, flexible and practical pest control methods (Nile et al., 2019; Fenibo et al., 2022).

The use of botanical insecticides shows promise in pest control. These extracts, derived from plants, have a broad range of effects, including insecticidal, ovicidal, repellent, growth regulatory and antifeedant actions. Studies have shown that plant extracts that include bioactive phytochemicals like alkaloids, flavonoids, terpenoids, tannins and saponins have an important role in controlling pests (Damalas & Koutroubas, 2020; Ahmed et al., 2022). More importantly they are biodegradable, safe for non-target organisms and usually slow down the development of resistance. In addition, using native or traditional plants makes their application more practical and economical for smallholder farmers (Monsreal-Ceballos et al., 2018; Ngegba et al., 2022).

However, the current literature lacks consistent and in-depth comparative analysis. Most studies consider a limited set of plant species or pests with little replication across geographical areas or environmental settings. Incompatible results on efficacy are not uncommon, often attributed to variations in extraction procedures, solvents, and bioassay conditions, which prevent the generalisation of the results (Nikolaou et al., 2021; Assadpour et al., 2023). Moreover, although larva mortality rates are widely used parameters, there are few studies addressing the physiological or histopathological alterations that could explain the described phenomenon. To address this gap, the present study tested the effectiveness of different botanical extracts to control *C. medinalis* larvae under laboratory conditions. The study also determines the bioactive phytochemicals present in the most effective extracts and examine how they affect larval tissue. By linking the activity of botanical insecticides with insect physiology through tissue studies, this research aims to contribute to eco-friendly pest control and a better understanding of their mode of action. The research results will help to promote the use of botanicals to control pests as part of eco-friendly rice farming in Sri Lanka.

Our hypothesis is that methanolic extracts of selected botanical species have significant insecticidal and repellent activities against *C. medinalis* larvae and induce distinct histopathological alterations in their larval tissues. These findings will provide scientific evidence supporting the potential application of these botanical extracts for environmentally sustainable rice pest management.

MATERIALS AND METHODS

Rearing of rice leaf folder larvae

Field collection

Rice leaf folder larvae and pupae were randomly collected from rice fields by hand-picking infested rice leaves. After collection, the insects were transferred onto clean potted rice plants placed inside net cages and labelled with the collection date.

Growing of host plant

Larvae and pupae were raised on rice seedlings in cages at a temperature of 27 ± 2 °C and a 12 h light cycle. Rice seeds of a susceptible variety (Bg352) were soaked in water for 3 – 4 days, placed on moss at 5 °C for 2 – 3 weeks and then planted in peat moss trays. The trays were kept at 25 °C under a 16 h light and 8 h dark period. The plants were watered well to keep them moist.

Introduction of adult rice leaf folder to the host plant

Male and female adult moths were confined in polyethylene cages containing cotton soaked with a 5% honey solution as a food source. The cages were placed over potted rice plants to allow controlled oviposition under laboratory conditions. Adults were not released freely as confinement was necessary to prevent flight and ensure uniform egg laying on rice leaves.

After 5-7 days, eggs were laid on the rice leaves, and larvae emerged naturally, resulting in uniform larval infestation of the rice plants. The emerged larvae were reared and maintained on rice plants within the cages and subsequently used for bioassay and histopathological studies.

Pupae were collected approximately on day 20 and transferred to separate containers lined with moistened cotton and supplied with a 5% honey solution until adult emergence. The rearing cycle was continuously maintained through laboratory handling to obtain the required number of larvae throughout the study period. All rearing procedures and developmental timelines were followed according to standard protocols (Tsuda et al., 2005; Padmavathi et al., 2013).

Preparation of plant extracts

In this study, 10 plant species were chosen and identified based on their English common names and scientific names as follows: Neem (*Azadirachta indica*), Giant milkweed (*Calotropis* sp.), Castor (*Ricinus communis*), Malabar nut (*Justicia adhatoda)*, Worm killer (*Aristolochia bracteolata*), Tanner cassia (*Cassia auriculata*), Sweet flag (*Acorus calamus*), Tobacco (*Nicotiana tabacum*), and Pongam (*Pongamia pinnata*),

Specific plant parts (leaves, flowers, or roots) were collected based on the species of the plants (Table 1). The plant materials were thoroughly washed with tap water followed by distilled water and allowed to dry in the shade for 3-4 weeks at room temperature. The dried plant materials were ground to a fine powder using an electric grinder, sieved and stored in dark airtight containers before extraction.

Table 1: Selected plant species, and their parts used in the study

Scientific name	Family	English common name	Plant part used
Azadirachta indica	Meliaceae	Neem	Leaves
Calotropis sp.	Apocynaceae	Giant milkweed	Leaves
Ricinus communis	Euphorbiaceae	Castor	Leaves
Justicia adhatoda	Acanthaceae	Malabar nut	Leaves
Aristolochia bracteolata	Aristolochiaceae	Worm killer	Leaves
Cassia auriculata	Fabaceae	Tanner's cassia	Flowers
Acorus calamus	Acoraceae	Sweet flag	Rhizome
Nicotiana tabacum	Solanaceae	Tobacco	Leaves
Pongamia pinnata	Fabaceae	Pongam	Leaves
Lawsonia inermis	Lythraceae	Henna	Leaves

Stock solution preparation

A sample of 25 g of the powdered plant part was mixed with 250 ml of methanol and allowed to shake at 350 rpm on a mechanical shaker for two days. The concentrate was obtained after filtration with Whatman No.1 filter paper and a crude extract was prepared after evaporating the filtrate in a rotary evaporator at 40 °C. Then, 10 g of the crude extract was dissolved in 10 ml of methanol to prepare the 1 g/ml stock solution. The five bioassay concentrations of the extract (10 mg/ml or 1%, 30 mg/ml or 3%, 50 mg/ml or 5%, 100 mg/ml or 10%, 150 mg/ml or 15%) were prepared using the stock solution. These specific concentrations were determined based on previous range-finding bioassays and previously reported dose-response data for botanical extracts for lepidopteran pest control (Ghabbari et al., 2018; Ahmed et al., 2022; Vasantha-Srinivasan et al., 2023). The 150 mg/ml concentration is considered the most effective (non-phytotoxic) concentration that produces biological activity under laboratory conditions, as suggested by standard procedures for botanical bioassays (Sarangi et al., 2025; Seni et al., 2025). In order to determine how different doses produce various physiological responses, the above concentrations were used for the evaluation of dose-dependent responses and provide evidence of both sub-lethal and lethal effects.

Bioassays

Larvicidal bioassay

Plant extracts were added to each beaker containing 100 ml of paddy water. Fourth instar larvae of leaf folders were then exposed to the plant extracts in the beaker. A control was maintained without adding any plant extract. For each trial, 10 ± 2 larvae were used per concentration. The larvae were fed with dry yeast powder (25 mg/100 ml) during the exposure period. The motility of larvae in the test and control beakers was observed at

24, 48 and 72 h. Five replicates were conducted for each concentration of all 10 plant extracts.

The percent mortality was calculated using the following formula [1] (Abbott,1925).

$$Corrected\ mortality = \frac{(x-y)}{(100-y)} \times 100 \qquad ...(01)$$

Where,
X = Percent mortality in the treatment
Y = Percent mortality in the control

Repellent activity

The filter paper disc method was used to evaluate repellent activity. Petri dishes containing filter paper discs (9 cm in diameter) were prepared for each of the five different concentrations. Each filter paper disc was divided into two equal halves, one half with the plant extract and the other half treated with methanol as the control. Ten fourth-instar larvae were placed on the central line of each filter paper disc per replicate, and the experiment was conducted with five replicates for each concentration. After 2 h of exposure, the position of larvae on the treated and untreated halves was recorded, and repellency percentage was calculated using the standard formula (2) (Loko et al., 2017),

$$PR = \frac{(Nc-Nt)}{(Nc+Nt)} \times 100 \qquad ...(02)$$

Where,
PR= Percentage of repellence
Nc =Number of insects on untreated seedlings.
Nt =Number of insects on treated seedlings.

Histopathological analysis

Tissues from treated larvae were immersed in 10% neutral-buffered formalin for 7 days and then dehydrated, embedded, sectioned using a microtome and stained with haematoxylin and eosin. Parts of the gut thoracic muscles and Malpighian tubules were examined under a light microscope. The tissues were graded using an H-score (0–300), reflecting the level of severity of the plant extracts on the larval tissues (Christina and Walker, 2014; Pintong et al., 2020). The H-score was calculated by multiplying the percentage of affected cells by the severity score of tissue damage, where 0 = no damage, 1 = mild damage, 2 = moderate damage, and 3 = severe damage.

RESULTS AND DISCUSSION

Concentration depended larval mortality analysis

The risk of larval mortality increased as the dose was increased (Figure 1). The control group (0 mg/L) showed the lowest mortality (M = 1.31, SD = 0.788), proving minimal natural mortality and showing the accuracy of the experiments, an increased number of larval deaths were observed as the concentration of extracts increased and significant mortality was observed at 150 mg/L

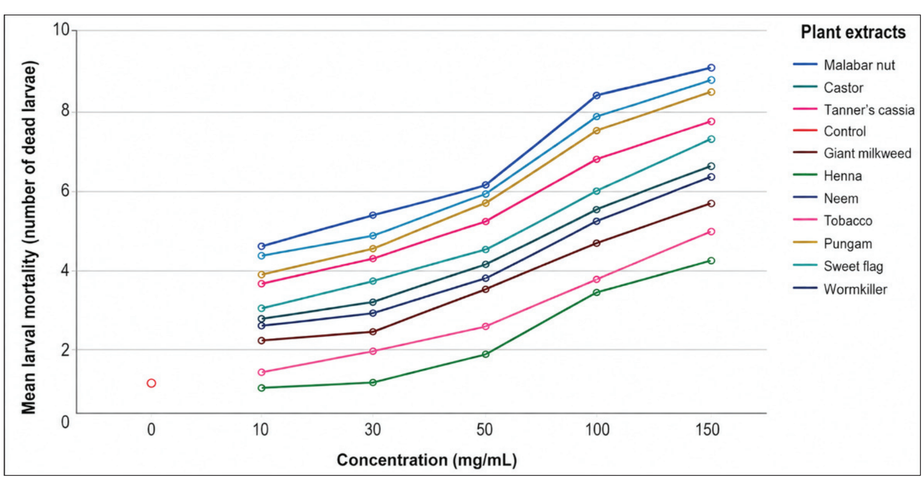

Figure 1: Larvicidal effect of various plant extracts at different concentrations (10 mg/ml (1%), 30 mg/ml (3%), 50 mg/ml (5%), 100 mg/ml (10%) and 150 mg/ml (15%)

(M = 6.97, SD = 1.778). This shows a close positive relationship between the concentration of the extract and its larvicidal strength.

Out of all the plant extracts tested, Neem (*Azadirachta indic*a) performed the best with a mean mortality of 6.75 (SD = 2.014) followed in efficiency by Malabar nut (*Justicia adhatoda*) with a mortality mean of 6.43 (SD = 1.918) and Pungam (*Pongamia pinnata*) with a mean of 6.12 (SD = 1.945). The potent bio-compounds such as azadirachtin, vasicine and karanjin in these plants contributed to larval mortality. In contrast, Henna (*Lawsonia inermis)* and Tanner's cassia (*Cassia auriculata*) showed the lowest average mortality with 2.35 and 2.97 respectively, revealing weaker action against rice leaf folder larvae.

The one-way ANOVA showed that larval mortality significantly varied among the different concentration groups, indicating that concentration is a major factor affecting mortality. Evidence of a strong impact of concentration on mortality was also found using the Welch test, which corrected for unequal variances (Welch's $F(5, 369.38) = 296.75$, $p < 0.001$). All groups in the concentration experiments differed significantly from the control group ($p < 0.001$). The risk of mortality was higher in larvae exposed to higher concentrations than to lower concentrations. At 150 mg/L, there was a marked increase in mortality compared to 100 mg/L (by an average of 0.95, $p < 0.001$) and all lower concentrations, demonstrating a strong dose-response relationship. These results indicated that smaller concentrations of extracts have reduced lethality.

Table 2: Summary of two-way ANOVA

Tests of Between-Subjects Effects

Dependent variable: Mortality (number of dead larvae)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3993.173[a]	50	79.863	121.027	.000
Intercept	16411.117	1	16411.117	24869.873	.000
Extracts	1471.115	9	163.457	247.708	.000
Concentration	1656.941	4	414.235	627.744	.000
Extracts * Concentration	34.339	36	0.954	1.445	.046
Error	510.747	774	0.660		
Total	21062.000	825			
Corrected Total	4503.920	824			

a. R Squared = 0.887 (Adjusted R Squared = 0.879)

Two-way ANOVA analysis was used to examine how plant extract type and concentration independently and together affect larval mortality. As shown in Table 2, both main factors, i.e., plant extract type and concentration had significant effects on larval mortality (Extracts: $F(9, 774) = 247.708$, $p < 0.001$; Concentration: $F(4, 774) = 627.744$, $p < 0.001$). Additionally, there was a significant difference ($p = 0.046$) in the efficacy of extracts due to changes in the concentrations used in testing. For instance, Neem and Malabar nut caused high mortality at all tested levels, compared to Henna and Tanner's cassia, which required higher doses to achieve similar mortality rates.

An excellent fit and reliability of results were demonstrated by the fact that the overall model accounted for 88.7% of the larval mortality variation (R^2=0.887, Adjusted R^2=0.879). The data indicate that some plant extracts are consistently effective, while higher concentrations of some extracts are needed to achieve similar outcomes. This shows that the botanical pesticides could be tailored with specific concentrations to control pests more effectively (Tiyagi et al., 2004)

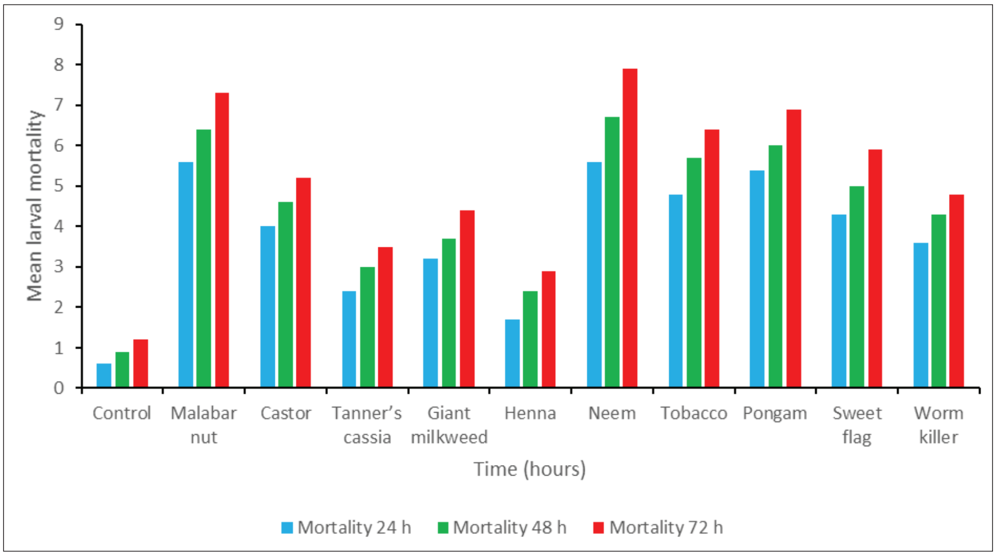

Figure 2: Mean larval mortality of *Cnaphalocrocis medinalis* after treatment with various plant extracts at 24, 48, and 72 hours.

Time depended larvicidal analysis

A repeated measures analysis of variance (ANOVA) was used to study the impact of different plant extracts on insect mortality at 24, 48 and 72 hrs. Significant differences were shown for the time and type of extract.

Most extracts showed a progressive increase in mortality. At 24 h, the highest average death count occurred among treated groups (M=5.68 for Malabar nut and Neem; and 5.40 for Pungam) while the control group had the lowest mortality (M = 0.60). After 72 h, the Neem extract treatment had the highest mortality. Meanwhile, both Malabar nut and Pungam had slightly lower mortality rates compared to Neem (about 70% and 69% of mortality) with insignificant mortality in the control group, showing the effectiveness of these plants over the treatment period.

Results from the multivariate tests showed that time played a highly significant role (Wilks' Lambda = 0.197, F (2,288) = 588.41, $p < .001$, $\eta^2 = .803$) in explaining differences in mortality among different time periods. An important relationship between time and extracts (Wilks' Lambda = 0.666, F (20,576) = 6.49, $p < .001$, $\eta^2 = .184$) indicated that insecticidal activity, as measured by time, was affected by the plant extracts. Subsequent analysis revealed that Neem, Malabar nut and Pungam extracts significantly differed from the control and from several others, showing higher insecticidal activity. Therefore, it is evident from Figure 2 that prolonged exposure to botanical insecticides is more important for their effectiveness than short exposure times, and pest control efforts may be more effective if repeated applications are utilized (Scott et al., 2005)

Probit analysis

The results of the probit analysis for the larvicidal action of ten botanical extracts on *C. medinalis* larvae are summarized in Table 3, showing LC_{50}, LC_{90}, and LC_{99} values together with lower and upper confidence limits (LCL – UCL).

There is a significant variability in the larvicidal toxicity among the tested botanical extracts, indicating differences in the dose-response relationship. Neem had the highest larvicidal efficacy as shown by the lowest values for LC_{50} (25.8 ppm), LC_{90} (75.6 ppm), and LC_{99} (120.0 ppm). Lower concentrations of neem extract were required to produce larval mortality at 50%, 90%, and 99%, highlighting its toxic potential. On the other hand, Henna demonstrated the highest lethal concentrations (LC_{50} = 70.1 ppm; LC_{90} = 150.3 ppm; LC_{99} = 250.0 ppm) of the tested botanical extracts, which indicates lower larvicidal effectiveness under experimental conditions.

Table 3: Probit analysis of larvicidal activity of selected botanical extracts against *Cnaphalocrocis medinalis* larvae

Plant extract (English common name)	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	LC_{99} (ppm) (LCL-UCL)
Malabar nut	35.2 (30.1-40.5)	95.8 (90.0-100.8)	155 (150.0-160.5)
Worm killer	45.1 (40.0-50.5)	110.2 (105.0-115.8)	180.5 (175.0-185.8)
Castor	50.4 (45.0-55.8)	120.3 (115.0-125.8)	200 (195.0-205.8)
Tanner's cassia	60.3 (55.0-65.5)	135.4 (130.0-140.5)	220.5 (215.0-225.8)
Giant milkweed	55.6 (50.0-60.8)	128.5 (123.0-133.8)	210 (205.0-215.8)
Henna	70.1 (65.0-75.5)	150.3 (145.0-155.8)	250 (245.0-255.8)
Neem	25.8 (20.0-30.8)	75.6 (70.0-80.5)	120 (115.0-125.8)
Tobacco	40.7 (35.0-45.8)	105.7 (100.0-110.5)	175.5 (170.0-180.8)
Pongam	30.9 (25.0-35.8)	85.4 (80.0-90.5)	140 (135.0-145.8)
Sweet flag	38.5 (33.0-43.8)	98.7 (93.0-103.5)	160.5 (155.0-165.8)

The remaining botanical extracts demonstrated a moderate level of larvicidal activity, with lethal concentration values falling between those of Neem and Henna. For example, Pongam and Malabar nut had lower LC_{50} values than Castor, Giant milkweed, and Tanner's cassia, which indicated higher toxicity at lower concentrations. The progressive increase in LC values among the different botanical extracts demonstrates a consistent dose-dependent response, which is an essential characteristic of a valid probit model.

In addition, the relatively narrow LCL-UCL confidence intervals for the LC_{50}, LC_{90}, and LC_{99} values among all plant extracts demonstrated the precision and reliability of the probit estimates. The lack of excessively wide confidence limits also suggested consistent larval mortality responses across replicates and concentrations. In general, the probit analysis confirms the significant differences in larvicidal potency among the tested botanical extracts, providing a quantitative basis for comparing the toxic effect on *C. medinalis* larvae.

Repellent activity of selected botanical extracts against rice leaf folder larvae

Repellency plays a vital role in suppressing larval settlement, feeding damage and subsequent crop injury by rice leaf folder larvae, even in the absence of direct

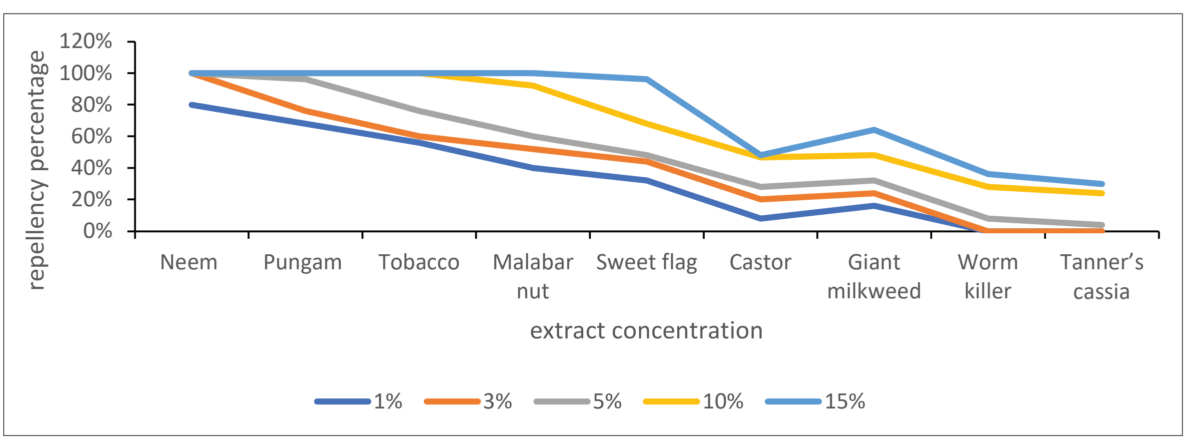

Figure 3: Repellency percentage of ten plant extracts against rice leaf folder (Cnaphalocrocis medinalis) larvae at five different concentrations (10 mg/ml (1%), 30 mg/ml (3%), 50 mg/ml (5%), 100 mg/ml (10%) and 150 mg/ml (15%)

lethality. Hence, repellency trials were incorporated as complementary assessments to mortality analysis offering insights into the behavioural disruption potential of botanical extracts in an integrated pest management (IPM) program.

Figure 3 shows the percentage of repellence of ten botanical extracts on rice leaf folder larvae at five concentrations (1, 3, 5, 10 and 15%). Repellency response depended on concentrations in all tested extracts with highly different levels of activity across plant species.

Out of the botanicals tested, Neem and Pongam showed good repellent activity with maximum repellency recorded at 3%. The repellency levels remained high at higher concentrations implying a strong repulsive action on larval movement and feeding behaviour. There was also significant repellent behaviour observed with tobacco, especially at 10% and 15%, implying that there was a concentration level beyond which strong behavioural avoidance was elicited.

Malabar nut and Sweet flag exhibited moderate repellency with high levels of activity at the highest concentration (15%). Castor, showed a gradual and steady increase in repellency with increasing concentration ranging with increasing values of about 32% at a 1% concentration to 96% at a showing a dose-dependent behavioural response.

Reduced repellence was observed in Giant milkweed, *Aristolochia bracteolata* (Worm killer) and *Cassia auriculata* (Tanner cassia). Even though repellence was concentration-dependent, the highest repellency values were comparatively low (48, 64 and 36%, respectively), indicating that there was no significant deterrent capacity under the experimental circumstances. *Lawsonia inermis* (Henna) was the most inactive, with no more than about 30% repellency at the highest concentration. This implies that its use as a repellent against rice leaf folder larvae is limited.

The variation in repulsive behaviour between plant extracts could be due to the differences in the nature and concentration of bioactive phytochemicals, including alkaloids, terpenoids, and phenolic compounds which are known to influence insect sensory perception and behaviour. In general, the findings of the repellency experiments indicate that some botanical extracts can alter larval behaviour, which reduces feeding pressure and complements their larvicidal properties. These results show the potential of repellency as an additional mode of action to botanical-based management of rice leaf folder.

Histopathological effects of Neem extract on larval tissues

Histopathological analysis indicated significant tissue-based effects in the neem extract-treated larvae compared to the untreated control larvae (Figure 4). The degree of tissue injury was evaluated semi-quantitatively based on an H-score from 0 (no injury) to 300 (maximum tissue damage).

Histologically, in the control larvae, the structure of each of the three tissues was viz, gut, thoratic muscle an Malpigian tubule well preserved. In addition, the epithelial cells lining the midgut had normal organization,

Table 4: Histopathological damage scores (H-score) of gut, thoracic muscle, and Malpighian tubules of *Cnaphalocrocis medinalis* larvae treated with Neem extract.

Sample ID	Parts of larvae	% at 0 (No damage)	% at 1 (Mild)	% at 2 (Moderate)	% at 3 (Severe)	H-Score (% of damage cells*score)
	Gut	5	-	-	-	0
Control	Thoracic muscle	-	25	-	-	25
	Malpighian tubules	8	-	-	-	0
	Gut	-	-	-	95	285
Neem Extracts	Thoracic muscle	-	-	-	85	255
	Malpighian tubules	-	-	-	86.67	260

Note. H-score values ranged from 0–300, with higher scores indicating greater tissue damage.

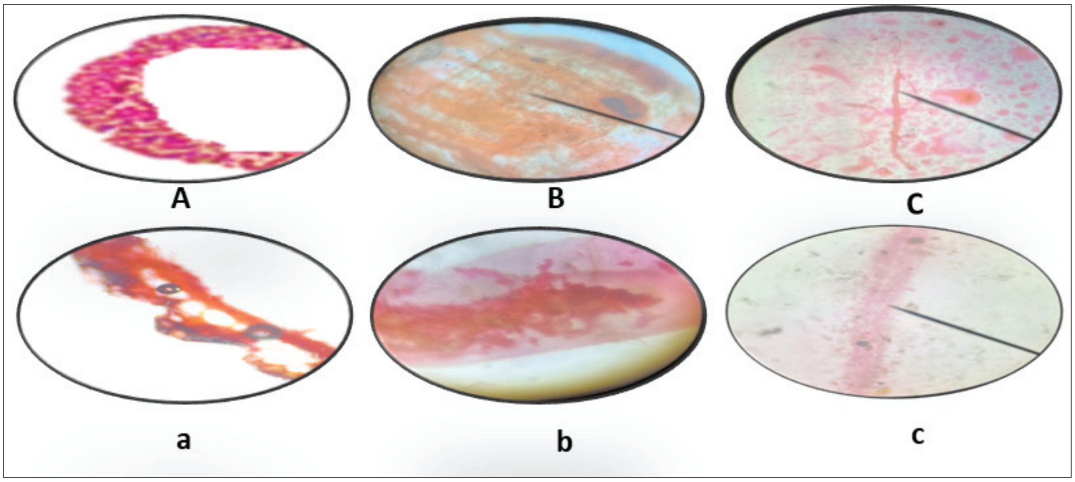

Figure 4: Histological changes in the larvae: Control groups. A: Gut, B: Thoracic muscle, C: Malpighian tubules. Neem extract treated larvae. a: Gut, b: Thoracic muscle, c: Malpighian tubule

with only slight alteration of less than 5% of the cells and the Malpighian tubules had only slight structural changes affecting < 10% of cells. As such, the H-scores for these two tissues were 0, indicating that the tissues were healthy. The thoracic muscles in the control larvae also had slight histological alterations with some breakage of the individual muscle fibres but this represented a low H-score of 25. These observations suggested that the tissues studied were not injured during the preparation for microscopy, and thus represent a valid reference point for comparing the effect of the neem treatment.

On the other hand, the larvae that were treated with neem extract had extensive histopathological injury to all the tissues that were studied. In particular, the midgut had a high level of cellular injury with >95% of the epithelial cells showing extensive damage including epithelial disintegration, cytoplasmic vacuolization, and loss of normal tissue structure and architecture, and consequently received an H-score of 285. It is reasonable to assume that the extensive damage to the midgut epithelial cells would cause an impairment of nutrient absorption and, subsequently, a reduction in digestive capacity and increased likelihood of larval death.

Similar to the midgut, the thoracic muscles also had extensive histological alterations due to the neem extract, including massive fragmentation and disintegration of the individual muscle fibres, granulation of the cytoplasm, and necrotic areas, resulting in an H-score of 255. Injury to the thoracic muscles could reduce or eliminate the ability of larvae to move and feed, and therefore enhance the toxic effects of the extract. Finally, the Malpighian tubules had extensive structural injury to approximately 87% of the cells, including shrinkage and vacuolation of the cytoplasm, and degradation of the tubular architecture, and therefore received an H-score of 260. This suggested that the excretory and osmoregulatory functions of the Malpighian tubules were impaired.

In general, the microscopic studies demonstrate that epithelial rupture occurred in the midgut, degeneration and necrosis occurred in the thoracic muscle fibres, and extensive vacuolization and tissue degeneration occurred in the Malpighian tubules of the larvae treated with the neem extract. The histopathologic alterations to the tissues supported the previously described larvicidal activity of the neem extract. The histopathologic effects are in agreement with the mechanism of action of azadirachtin, a major active compound of neem, which damages cellular membranes, causes cell death through apoptosis and reduces membrane fluidity in insect tissues (Shao et al., 2016). Therefore, the results of the histopathological studies provide additional evidence supporting the toxic mechanism of action of neem extract at the cellular and tissue level against *C. medinalis* larvae.

Possible mechanisms of bioactive compounds which act as larvicidal compound

The larvicidal effect of botanical extracts is due to the synergistic effect of a variety of phytochemicals that are known to interfere with the biochemical and physiological functions of insects. These chemicals can disrupt neural control, hormonal balance, energy production and the integrity of the cell membranes, which can result in larval death.

Alkaloids are known to inhibit acetylcholinesterase (AChE), an important enzyme involved in the termination of nerve impulses at cholinergic synapses. AChE inhibitors prevent the breakdown of acetylcholine (ACh), leading to continual nerve stimulation, paralysis of the insect's nervous system, and death. Additionally, certain alkaloids can act on ion channels and neurotransmitter receptors, which also interfere with the normal neural function of the brain (Colović et al., 2013; Abou-Donia et al., 2016).

Flavonoids can exhibit toxicity to insects through the generation of oxidative stress in insect cells. High concentrations of reactive oxygen species (ROS) may cause damage to lipids, proteins, and DNA, leading to mitochondrial dysfunction, and triggering apoptosis and necrosis. Larval tissues are more vulnerable to oxidative damage, particularly in the Malpighian tubules and midgut, which could be the reason for histopathological changes observed in the present study (Mao et al., 2024).

Terpenoids and saponins may cause leakage of ions and enzymes from cellular and mitochondrial membranes, leading to the loss of electrochemical gradients and ultimatly causing cell lysis. Tannins can bind digestive enzymes or even structural proteins in the insect gut, reducing the absorption of nutrients and damaging the gut epithelium (Konuk & Ergüden ,2020; Ergüden, 2021).

Together, these mechanisms can affect several organ systems such as digestive, nervous, muscular and excretory, which can lead to poor feeding, diminished mobility, physiological dysfunction and mortality of the larvae.

Comparison with existing studies

The final results of this study conclude a high insecticidal potential of neem, malabar nut and pongam at all concentrations. These findings align with the existing literature, which has demonstrated comparable larvicidal activity of neem on lepidopteran pests (Charleston et al., 2005; Kamaraj et al., 2008; Dua et al., 2009; Nicoletti et al., 2012; Adhikari et al., 2020). The study included neem and also evaluated the larvicidal activity of various native botanical extracts, including malabar nut, pongam, tobaccco and castor under laboratory conditions. These extracts showed different degrees of larvicidal activity on rice leaf folder larvae, and thus have potential applications in botanical pest control.

Histopathological observations revealed severe tissue changes in neem-treated larvae characterized by gut epithelium degeneration, as well as Malpighian tubules and muscle fibre disruption. These results indicate systemic cellular toxicity. The comparable pathological alterations were identified in experimental studies with neem-based extracts (Coventry & Allan, 2001, Agbaje et al., 2023, Nagini et al., 2023), which suggests to the hypothesis that the abnormal digestive and excretory processes are caused by active phytochemicals, including azadirachtin and flavonoids. Similar to the histological changes reported in previous studies, the present study also observed comparable alterations in the gut, which may be attributed to oxidative damage as well as inhibition of digestive enzymes; the degeneration of Malpighian tubules indicated a disruption of osmoregulation and excretion. Neuromuscular dysfunction may occure through interference with neurotransmission leading to muscle tissue damage. These processes can have a strong connection with larval behavioural pattern such as decreased feeding and locomotion throughout the bioassays. .

CONCLUSIONS

The present study evaluated the larvicidal and repellent activity as well as the histopathological effects of ten botanical extracts against *Cnaphalocrocis medinalis* under laboratory conditions. neem and malabar nut showed the highest larvicidal activity resulting in significant mortality at all tested concentrations and exposure times ($p < 0.001$). These extracts additionally exhibited lower LC_{50} values compared to other botanical treatments, implying greater potency at lower concentrations, as confirmed through probit analysis.

In repellency assays, neem and pongam demonstrated strong repellent effects, suggesting their potential to reduce larval settlement and feeding damage. Histopathological examination of larvae treated with neem extract revealed severe damage to the midgut, thoracic muscles, and Malpighian tubules, providing mechanistic evidence for its toxic effects at the tissue level.

In general, the results support the efficacy of selected botanical extracts against *C. medinalis* and suggest their use in environmentally friendly rice pest management stratergies. However, additional research is needed to test these extracts under field conditions, develop appropriate formulaions, isolate the active ingredients, and evaluate their non-target effects before practical field applications.

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