

Original Research

Biopesticidal Effects of Plant Extracts Against Cigarette Beetle *Lasioderma serricorne* (Coleoptera: Anobidae), a Major Stored Insect Pest

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Abstract

Frequent use of phosphine over decades for the control of cigarette beetles (*Lasioderma serricorne*), has led to serious negative effects, including strong insecticide resistance, disruption of biological control by natural enemies, and environmental and human health concerns. As an environmentally friendly alternative to synthetic pesticides, plant-derived pesticides have been the focus of modern research. In this research, the toxicity and repellency effect of plant species extracts of *Adhatoda vasica* Nees, *Azadirachta indica*, *Nigella sativa*, *Parthenium hysterophorus*, and *Thuja orientalis* against the adult of *L. serricorne* were investigated. There were six concentrations 250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L, 1250 mg/L and 1500 mg/L of each plants species extracts. Both contact and residual toxicity were checked for 24, 48, 72, and 96 h. The experiment was replicated four times using Completely Randomized Design and a Probit analysis was done to determine the LC₅₀ and LC₉₀. The phytochemical profile showed the presence of phytosterols, saponins, di-terpenes, flavonoids, and alkaloids. In both contact and residual toxicity test, the highest level of toxicity was exhibited by *A. indica* with the lowest LC₅₀ (0.46 mg/L, 1.15 mg/L) and LC₉₀ value of (7.62 mg/L, 8.05 mg/L) followed by *A. vasica* having LC₅₀ (0.92 mg/L, 0.95 mg/L) and LC₉₀ value of (6.58 mg/L, 8.70 mg/L) respectively. *A. indica* and *A. vasica* also showed 100% repellency effect against

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L. serricornis after 96 h exposure period. The findings of the present study suggested the inclusion of *A. indica* and *A. vasica* extracts in the manufacture of novel biopesticides for mitigating *L. serricornis*.

Keywords: cigarette beetles, integrated pest management, mortality, repellency, phytochemicals

Introduction

The cigarette beetle, *Lasioderma serricornis* (Fabricius) (Coleoptera: Anobiidae), is a major threat to food and other plant-derived products [1]. In the tobacco industry, the economic impact of this pest is most severe. This beetle causes annual 0.7%-1.0% losses globally to tobacco production [1]. The traits of the cigarette beetle further complicate the control of this pest. It is very difficult to distinguish between males and females because the species showed minimal sexual dimorphism. During the life span, a female can lay over 100 eggs and create holes in the packaging and stored products for oviposition [2]. Larval stages are primarily responsible for damaging stored products, but the presence of adult insect fragments can also reduce product quality.

Presently, like pest control in stored food and processing factories, management of *L. serricornis* in cigarette manufacturing factories has always depended heavily on sanitation [3]. For the management of *L. serricornis*, contact and residual chemical insecticides are used as a control measure [4]. The applications of organophosphate and carbamate pesticides in cigarette manufacturing factories are inadequate because of the unpleasant smell and higher mammalian toxicity [4]. The positive aspect of Pyrethroid insecticides with broad-spectrum neurotoxins is that they have less toxicity to nontarget organisms, minimum environmental persistence, and no bioaccumulation. Moreover, they have been widely used to control *L. serricornis* in cigarette manufacturing factories and food processing factories with positive effects [2]. Continued use of pesticides against insect pests can lead to the development of resistance, reducing their overall effectiveness. Consequently, researchers have shifted away from relying on potent insecticides and are now focusing on environmentally sustainable pest control strategies. The first report of pyrethroid resistance was confirmed in 1985 in the population of *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) in West Texas [5]. Due to the injudicious use of pyrethroids and other insecticides, it is also observed that Coleoptera stored product pests, including *Sitophilus zeamais*, *Tribolium confusum*, and *Tribolium castaneum*, developed resistance against various levels of insecticides [6].

The tobacco industries of developing countries have employed various strategies to protect stored tobacco products from insect infestations. Among these strategies, the use of synthetic insecticides in the form of fumigants is the most common. However, extensive use of synthetic insecticides has led to several issues, including insect resistance, adverse effects on non-target organisms, and overall environmental contamination

[7], [8] and [9]. Consequently, the search for alternative management strategies is crucial that is environmentally safe to manage *L. serricornis* while maintaining product quality. One promising approach is the use of botanical pesticides. The biocompounds derived from various plant species offer a more environmentally friendly and sustainable approach to pest management [10]. Moreover, these biocompounds are readily biodegradable with low mammalian toxicity and less harm to the environment [11] and are an effective alternative for controlling insect pests with a novel mode of action, and insect pests encounter challenges in developing resistance [11].

Gaining knowledge on the efficacy of plant extracts, including those derived from chrysanthemum, garlic, or neem, can help to develop eco-friendly pest control techniques by enabling these extracts to potentially act as insecticidal agents against *L. serricornis* [12].

In order to develop an integrated pest management approach for the sustainable management of *L. serricornis* in the tobacco sector, this study aims to examine and evaluate the toxicity and repellency responses of *L. serricornis* against the investigated botanicals.

Materials and Methods

Laboratory Culture of *L. serricornis*

Lasioderma serricornis were collected from Pak Hill (Private company) godown and were reared in jars containing diets (wheat feed/yeast, 10:1, w/w) in incubators in the dark (29±1°C, 70-80% r.h.). All the insects used in all the experiments were about the same age and similar size regardless of gender [13].

Plant and Sample Preparation

The leaves of five different plant species at the vegetative stage used in the experiment were collected in and around the University of Haripur, located in Northern Pakistan (Table 1). For the authentication of plant species, they were identified by a botanist in the Department of Biology, the University of Haripur, and the plant specimens were deposited in Herbarium (Voucher no. F.No. UH/Hort/2514). For fifteen days, the plant leaves were air-dried at room temperature. The dried samples were ground to make a fine powder. One kg of each sample was separated by maceration (1 * 2L) in 12 h intervals using concentrations of 50% at room temperature. The macerated samples were filtered, and under low temperatures and pressure, the solvents were

removed using a rotary evaporator (Buchi, R-210) in the Food Science Laboratory at the University of Haripur. The yield obtained was *Parthenium hysterophorus* 46 g, *Thuja orientalis* 63 g, *Azadirachta indica* 51 g, *Adhatoda vasica* 48, and *Nigella sativa* 55 g, which were then stored at less than 4°C temperature.

Preliminary screening: Preliminary screening of extracts from five different plant species was carried out at various concentrations based on yield (500-4,000 mg/L) for their efficacy against adults of *L. serricornis*. Six different concentrations, i.e. (250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L, 1250 mg/L, and 1500 mg/L) were selected on the basis of preliminary data and evaluated against *L. serricornis* in the final bioassays.

Qualitative Analysis of Plant Species Extracts

Preparation of Plant Extracts

Whole or coarsely ground plant drugs were macerated (for fluid extraction) by keeping them in contact with the solvent using a lidded vessel for a predetermined amount of time while stirring frequently until a homogenized mixture was achieved [14].

Wegners tests for alkaloids: The herbal extract samples were separately mixed with dilute HCl (1.5%) and filtered through filter paper (Whatman No. 1). A small amount of potassium iodide was added to the filtrate. The occurrence of alkaloids in the sample was indicated by the presence of reddish-brown precipitates [15].

Ferric Chloride Test for phenols: To assess the plant extracts for phenols, a few drops of ferric chloride solution were added to the extract in a 5 ml test tube. The presence of phenols was indicated by the emergence of a bluish-black color in the solution [16].

Salkowski's test for phytosterols: Chloroform was added to the plant extract solution. After this, the mixture was subjected to filtration using Whatman no. 1 filter paper. Three to four drops of H₂SO₄ (concentrated) were then added to the filtrate, which was vortexed and allowed to rest for some time. The solution turned golden yellow due to the presence of phytosterol [16].

Tests for di-terpenes: To identify terpenes, copper acetate solution (2-3 drops) was added to the plant extracts. The development of a bright green hue suggested the presence of diterpenes [16].

Tests for Saponins: In a test tube, 2 ml of plant extract was diluted with distilled water, followed by vortexing for five minutes. The presence of white foam at the surface for more than 10 minutes confirmed the presence of saponins [15].

Alkaline reagent tests for flavonoids: To determine whether flavonoids were present in the plant aqueous extracts, a few drops of lead acetate solution and diluted acid were added. The appearance of a brilliant yellow hue indicated the presence of flavonoids in the extracts [17].

Residual Toxicity

A 6x6 factorial design was used for the current experiment. Residual toxicity of five plant species was tested against adult of *L. serricornis* by the Potter spray method as per the standard method [18]. Briefly, six concentrations of plant extracts (250-1500 mg/L) were used. We placed a paper filter at the bottom of the Petri dishes, and 10 adults of *L. serricornis* were released at the center of each Petri dish. The treatment was replicated thrice. Six different concentrations (25 mg/L to 1500 mg/L) were sprayed and incubated under controlled conditions. The mortalities of beetles were observed at 24 h intervals and up to 96 h. Distilled water was used as a control. Mortality data were corrected using Abbott's formula. The LC₅₀ and LC₉₀ were determined via probit analysis [19].

Mortality (%)

$$= \frac{\text{number of dead insects} \times 100}{\text{number of insects introduced}} \quad (1)$$

Topical (Direct) Toxicity

A 7x7 factorial design was used for the topical toxicity. 210 adults of *L. serricornis* were used. In each treatment, 30 adults of *L. serricornis* were used (three replications per treatment, and each replicate contained 10 beetles). The Beetle mortalities were checked after post-treatment of 24 h and up to 96 h. *L. serricornis* adults were considered dead when they failed to respond to gentle touch with a camel hairbrush or finger grasping. The mortality was assessed at 24 h intervals to 96 h. Abbott's formula was employed to correct

Table 1. List of plant species tested against *L. serricornis* at six different concentrations.

S. No	Local name	Technical name	Concentrations used
1	Parthenium	<i>Parthenium hysterophorus</i>	250, 500, 750, 1000, 1250 and 1500 mg
2	Morpankh	<i>Thuja orientalis</i>	250, 500, 750, 1000, 1250 and 1500 mg
3	Adua	<i>Adhatoda vasica</i>	250, 500, 750, 1000, 1250 and 1500 mg
4	Kalongi	<i>Nigella sativa</i>	250, 500, 750, 1000, 1250 and 1500 mg
5	Neem	<i>Azadirachta indica</i>	250, 500, 750, 1000, 1250 and 1500 mg

the mortality rate, and LC_{50} and LC_{90} were determined via probit analysis [19].

Repellent Effect of Plant Extracts Against *L. serricorne*

The repellent effect of five plant species with six different concentrations was checked using the method of [20] with a slight modification against *L. serricorne*. We used Petri plates of 16 cm diameter and divided the bottom of Petri plates by cutting into two halves of Whatman filter paper no. 1 and then past it. We treated half portion of the filter paper with plant species extract (250 mg/L, 500 mg/L, 750 mg/L, 1000 mg/L, 1250 mg/L, and 1500 mg/L), and the other half was treated with distilled water. The filter paper was air dried for 20 minutes and, afterward, carefully placed into uniform and equally edged by edge.

Twenty newly emerged adult beetles, mostly of the same size and age, were arranged in ten pairs and released in the center of the treated area within the Petri dishes. To prevent insect escape, the Petri dishes were secured with muslin cloth and kept in an incubator at $27\pm C$ and a relative humidity of $65\pm 5\%$. For each treatment, 30 beetles were used, each treatment contained three replications, and 10 beetles were present in each replication. The distribution of the insects was observed every hour for 1 h, 12 h, 24h, 48 h, 72 h, and post 96 h. After this exposure period, the percentage repellency (PR%) was calculated according to [21] using the formula shown below:

$$PR(\%) = \frac{Nc - Nt}{Nc + Nt} \times 100 \quad (2)$$

Nc = Number of beetles counted in the untreated arena

Nt = Number of beetles counted in the treated arena

where Nc represents the number of insects in the control segment, and Nt represents the number of insects in the treated section [22]. Different classes were assigned as per the average repellence percentage, which included Class 0 = PR of 0-0.1%; Class I (0.2-10%); Class II (20.1-40%); Class III (40.1-60%); Class IV (60.1-80%); and Class V (80.1-100% [23].

Data Analysis

The mortality data of *L. serricorne* based on the residual and direct toxicity of plant species extracts were compiled. The median lethal concentration values (LC_{50} and LC_{90}) and other regression parameters were determined by Probit [24, 25] using SPSS 10 software, version 16. Similarly, the percentage mortality data against *L. serricorne* were also analyzed by one-way analysis of variance, and the means were compared using Tukey's post hoc test.

Results

Qualitative Analysis of Phytochemicals from Selected Plant Species: Phytochemical Screening of Plant Extracts for Their Chemical Constituents

The results in Table 2 showed that all the tested plant species' aqueous extracts contain phytochemicals. Among the plant species, alkaloids, flavonoids, phenols, phytosterol, di terpenes, and saponins are higher in *A. indica* as compared to other plant species. *A. vasica* also exhibited higher alkaloids and saponins. Saponins and flavonoids are also higher in *P. hysterophorus* as compared to *N. sativa* and *T. orientalis*. In *T. orientalis*. The percentage occurrence of all the phytochemicals was low.

Potency of Plant Extracts on *L. Serricorne* According to a Topical Toxicity Bioassay

The lethal effects of the plant extracts increased as their concentrations increased. Among the plant species after 24 h exposure, *A. indica* exhibited the lowest $LC_{50} = 6.80$ mg/L and $LC_{90} = 95.78$ mg/L, respectively (Table 3). Among the tested plant species extracted at 3.0% concentration, *A. indica* exhibited 52.50% mortality, whereas *T. orientalis* exhibited 35.00% lowest mortality against *L. serricorne* (Fig. 1a). After 48 hours, *A. vasica* exhibited the lowest $LC_{50} = 3.40$ mg/L and $LC_{90} = 25.60$ mg/L, respectively, against *L. serricorne*. Similarly, *A. indica* with $LC_{50} = 3.71$ mg/L and $LC_{90} = 71.78$ mg/L were the second most lethal plant extracts. The mean percentage mortality among

Table 2. Occurrence of phytochemicals in five plant species extracts.

Plants Species	Alkaloids	Flavonoids	Saponins	Di-terpenes	Phyto-sterol	Phenols
<i>T. orientalis</i>	+	+	+	+	+	+
<i>P. hysterophorus</i>	+	++	++	+	+	+
<i>N. sativa</i>	+	+	+	+	+	+
<i>A. vasica</i>	++	+	++	+	+	+
<i>A. indica</i>	+++	+++	++	++	+++	+++

+++Highly present, + moderately present, - Absent

Table 3. Direct toxicity of plant extracts to *L. serricornis* after 24 h, 48 h, 72 h, and 96 h.

Plant species	Slope \pm SE	LC ₅₀ mg/L (95% CLs)	LC ₉₀ mg/L (95% CLs)	P	χ^2
24 hr					
<i>A. indica</i>	1.06 \pm 0.22	6.80 (4.43-10.26)	95.78 (33.70-835.18)	0.99	0.39
<i>A. vasica</i>	0.93 \pm 0.20	10.58 (61.48- 437.69)	236.80 (61.48- 8437.69)	0.94	0.85
<i>N. sativa</i>	0.83 \pm 0.23	13.62 (8.45-57.59)	461.16 (85.10- 8293.90)	0.90	1.11
<i>P. hysterophorus</i>	1.15 \pm 0.25	13.10 (7.98-30.82)	161.60 (50.13-2108.41)	0.87	1.29
<i>T. orientalis</i>	0.92 \pm 0.23	19.30 (9.91-102.46)	455.70 (89.16- 5296.40)	0.97	0.62
48 hr					
<i>A. indica</i>	0.91 \pm 0.19	3.71 (1.96-3.52)	71.78 (27.68-798.90)	0.88	1.12
<i>A. vasica</i>	1.25 \pm 0.19	3.40 (1.81-2.80)	25.60 (15.41-70.91)	0.89	1.18
<i>N. sativa</i>	1.25 \pm 0.24	3.58 (2.10-3.16)	28.78 (16.87-77.03)	0.90	1.11
<i>P. hysterophorus</i>	1.08 \pm 0.23	4.92 (3.19-5.06)	48.78 (25.31-199.31)	0.61	2.74
<i>T. orientalis</i>	1.59 \pm 0.21	4.81 (3.29-4.58)	25.61 (15.69-53.61)	0.96	0.68
72 hr					
<i>A. indica</i>	0.95 \pm 0.20	1.22 (0.57-1.67)	25.40 (14.62-138.40)	0.80	1.51
<i>A. vasica</i>	1.33 \pm 0.20	1.15 (0.74-1.52)	8.30 (7.00-17.53)	0.32	2.76
<i>N. sativa</i>	1.26 \pm 0.21	1.60 (2.15-2.01)	17.10 (11.35-37.56)	0.80	1.50
<i>P. hysterophorus</i>	1.20 \pm 0.20	1.73 (1.26-2.18)	18.27 (12.00-49.00)	0.50	1.22
<i>T. orientalis</i>	1.02 \pm 0.22	1.85 (1.24-3.40)	31.29 (17.26-142.40)	0.87	1.09
96 hr					
<i>A. indica</i>	1.10 \pm 0.21	0.46 (0.16-1.81)	6.62 (3.79-10.85)	0.57	2.81
<i>A. vasica</i>	1.40 \pm 0.23	0.92 (0.55-2.24)	6.58 (5.69-12.57)	0.31	3.80
<i>N. sativa</i>	1.69 \pm 0.19	1.53 (0.82-3.14)	8.70 (6.62-23.51)	0.10	4.30
<i>P. hysterophorus</i>	1.36 \pm 0.22	0.98 (0.60-2.32)	9.71 (5.33-16.26)	0.48	3.11
<i>T. orientalis</i>	1.41 \pm 0.22	1.82 (1.46-3.20)	12.86 (10.61-26.73)	0.22	3.80

** 95% confidence intervals (CLs) are used to define lethal concentrations (LC).

the plant extracts was higher for *A. vasica* (69.00%) (Fig. 1b) (Table 3). Similarly, the LC₅₀ = 1.15 mg/L and LC₉₀ = .30 mg/L of the *A. vasica* extract were significantly lower than those of the other plant species extracts after 72 h against *L. serricornis*. Among the plant species, the least toxic plant species was *T. orientalis*, which exhibited LC₅₀ = 1.85 mg/L and LC₉₀ = 31.29 mg/L. At a maximum 3.0% concentration, *A. vasica* caused 87.00 % mean percent mortality to *L. serricornis* (Fig. 1c) (Table 3). After 96 h exposure period among the plant species extracts, maximum toxicity was observed with *A. indica* having LC₅₀ = 0.46 mg/L and LC₉₀ = 6.62 mg/L and *A. vasica* with LC₅₀ = 0.92 mg/L and LC₉₀ = 6.58 mg/L against *L. serricornis*. The least effective among the plant species was *T. orientalis*, which exhibited the least toxicity, having LC₅₀ = 1.82 mg/L and LC₉₀ = 12.86 mg/L (Table 3). Furthermore, the mean percentage mortality

of *L. serricornis* was higher at 90.00% for *A. indica* and 89.00% for *A. vasica* at 3.00% concentration (Fig. 1d)).

Residual Toxicity of Plant Species Extracts Against *L. Serricornis*

With increasing concentrations of botanical extracts, the LC₅₀ and LC₉₀ values also decreased. *A. indica* was showed the most toxic plant extract due to its lowest LC₅₀ = 10.02 mg/L and LC₉₀ = 108.61 mg/L, respectively, after 24 h, while the least effective plant species was *T. orientalis*, having LC₅₀ = 14.12 mg/L and LC₉₀ = 188.12 mg/L (Table 4). The highest mean mortality of 77.00% was observed for the *A. indica* and the lowest mortality of 40.00% were recorded for *T. orientalis* against *L. serricornis* at 3.00% concentrations (Fig. 2a). After 48 h, *A. indica* was shown to be the most effective, with the lowest

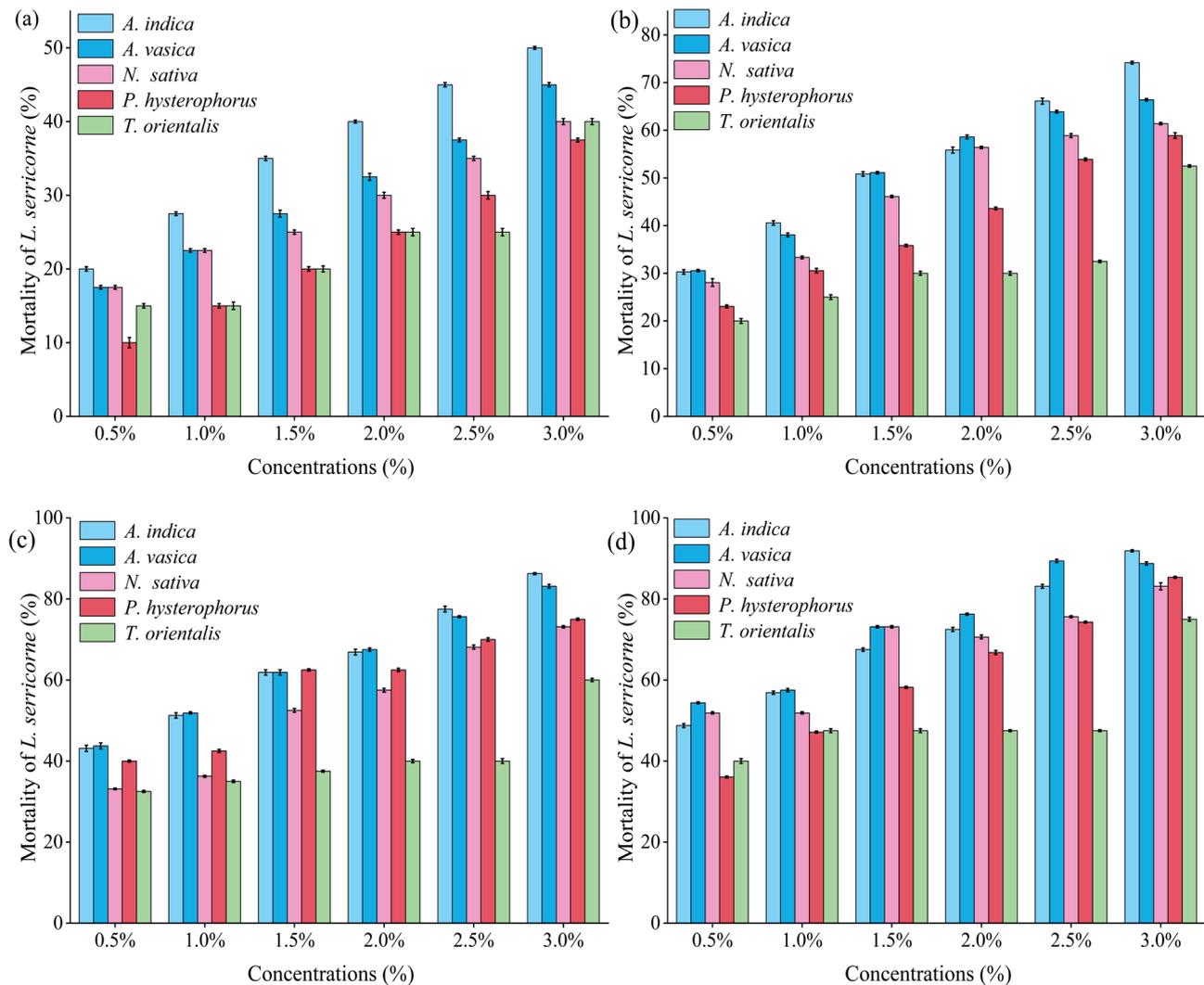


Fig. 1. Mean percentage mortality of *L. serricorne* on five plant extracts after (a) 24h, (b) 48h, (c) 72h, and (d) 96 h exposure in contact toxicity test. Statistically significant differences among the various treatments are indicated by the error bars at a 0.5% significance level.

$LC_{50} = 2.60$ mg/L and $LC_{90} = 28.77$ mg/L against *L. serricorne* (Table 4). Among the plant species extracts at 3.00% concentrations, maximum mean% mortalities of 74.11% were seen with *A. indica*, and the minimum mortalities were shown with *T. orientalis* (52.00%) against *L. serricorne* (Fig. 2b). After 72 h exposure period, *A. indica* exhibited maximum toxicity to *L. serricorne* in terms of lowest $LC_{50} = 1.33$ mg/L and $LC_{90} = 12.08$ mg/L. Among the plant species extracts, the least toxic plant species was *T. orientalis*, which exhibited $LC_{50} = 4.16$ mg/L and $LC_{90} = 79.41$ mg/L, as shown in Table 4. At 3.00% concentration, the highest mean percentage mortality of *L. serricorne* was observed with *A. indica* (86.25%), whereas *T. orientalis* showed minimum mean% mortality (60.00%) against *L. serricorne* (Fig. 2c). *A. vasica* among the plant species extracts showed maximum toxicity to *L. serricorne* in terms of $LC_{50} = 0.95$ mg/L and $LC_{90} = 8.70$ mg/L followed by *A. indica* having $LC_{50} = 1.15$ mg/L and

$LC_{90} = 8.05$ mg/L against *L. serricorne*. Similarly, *T. orientalis* showed the least toxicity, having $LC_{50} = 3.40$ mg/L and $LC_{90} = 40.50$ mg/L, as shown in (Table 4). Furthermore, the mean percentage mortality after 96 h was higher for *A. vasica* (90.00%), while the lowest mean percent mortalities was observed for *T. orientalis* (50.83%) against *L. serricorne* after 96 h at 3.00% concentrations (Fig. 2d).

Repellency of *L. Serricorne* Towards Plant Extracts

L. serricorne repellency was directly correlated with the increase in the concentration of plant extracts such that at 0.5% concentration, the repellency was minimal, whereas it was at the peak when the concentration of plant extracts increased to 3% and vice versa.

After one hour of exposure to various plant extracts, the repellency of *L. serricorne* to *A. indica* reached a maximum (Class III) at 250 mg/L, 500 mg/L, 750 mg/L,

Table 4. Residual toxicity of plant species extracts to *L. serricornis* on five after 24 h, 48 h, 72 h, and 96 h.

Plant species	Slope \pm SE	LC ₅₀ mg/L (95% CLs)	LC ₉₀ mg/L (95% CLs)	P	χ^2
24 hr					
<i>A. indica</i>	1.02 \pm 0.20	10.02 (7.05-21.02)	108.61 (39.91-870.98)	0.95	0.61
<i>A. vasica</i>	0.98 \pm 0.21	10.07 (7.74- 25.11)	182.97 (56.15-3448.06)	0.82	1.45
<i>N. sativa</i>	1.21 \pm 0.23	13.49 (8.81-38.80)	262.61 (66.51-9295.78)	0.94	0.62
<i>P. hysterophorus</i>	1.15 \pm 0.23	13.05 (9.51-32.94)	141.39 (50.07-1461.75)	0.86	1.15
<i>T. orientalis</i>	1.20 \pm 0.21	14.12 (10.40-45.40)	188.12 (58.90-3096.20)	0.77	1.74
48 hr					
<i>A. indica</i>	1.23 \pm 0.21	2.60 (3.01-3.14)	28.77 (16.85-79.01)	0.86	1.11
<i>A. vasica</i>	1.37 \pm 0.21	3.44 (2.01-3.92)	19.55 (14.03-47.91)	0.60	2.61
<i>N. sativa</i>	1.11 \pm 0.21	4.42 (3.29-5.10)	47.60 (23.65-220.44)	0.83	1.35
<i>P. hysterophorus</i>	1.14 \pm 0.22	5.41 (3.58-6.91)	53.82 (26.91-230.70)	0.61	1.55
<i>T. orientalis</i>	0.76 \pm 0.20	14.20 (8.65-60.03)	491.22 (90.01- 105.42)	0.56	2.72
72 hr					
<i>A. indica</i>	1.39 \pm 0.22	1.33 (0.96-1.70)	12.08 (8.02-20.10)	0.23	4.90
<i>A. vasica</i>	1.11 \pm 0.21	2.05 (0.55-2.44)	17.52 (8.50-39.70)	0.40	2.95
<i>N. sativa</i>	1.15 \pm 0.19	3.04 (2.52-3.53)	30.42 (15.34-80.08)	0.55	1.75
<i>P. hysterophorus</i>	1.30 \pm 0.22	2.80 (1.75-3.70)	19.13 (14.07-49.46)	0.31	3.82
<i>T. orientalis</i>	0.92 \pm 0.20	4.16 (3.41-3.26)	79.41 (30.75- 1040.11)	0.91	1.04
96 hr					
<i>A. indica</i>	1.50 \pm 0.22	1.15 (0.40-1.90)	8.05 (5.92-40.00)	0.23	4.80
<i>A. vasica</i>	2.40 \pm 0.22	0.95 (0.18-1.07)	8.70 (3.70-50.80)	0.07	4.18
<i>N. sativa</i>	1.05 \pm 0.21	2.80 (0.35-2.24)	13.10 (9.56-39.50)	0.60	3.70
<i>P. hysterophorus</i>	1.55 \pm 0.21	2.00 (1.22-2.90)	11.20 (8.09-20.05)	0.28	3.23
<i>T. orientalis</i>	0.80 \pm 0.20	3.40 (1.40-3.60)	67.50 (24.80-867.30)	0.28	3.91

** Lethal concentrations (LC) are indicated with 95% confidence limits (CLs).

and 1000 mg/L; however, the repellency of insects to *A. vasica* extracts was slightly lower than the repellency of the test insect recorded at 1250 mg/L and 1500 mg/L (Table 5). Nevertheless, there was no statistically significant difference between the repellency of *L. serricornis* on various concentrations of *A. indica* and *A. vasica*; however, it was significantly greater than that of *T. orientalis*, *N. sativa*, and *P. hysterophorus* (Class II). The mean percentage repellency of *L. serricornis* was highest with the *A. indica* extracts (34.9%), and the lowest percentage repellency was observed against *T. orientalis* (24.1%) (Fig. 3a). After 12 h and 24 h, the insects in the experiment exhibited the greatest repellency to all concentrations of *A. indica* (31.44%, 35.30%, 43.35%, 49.10%, 52.37% and 63.54%) (Class IV), whereas all concentrations of *T. orientalis* resulted in the least repellency (20.50%, 24.22%, 28.74%, 33.63%, 38.50% and 43.92%) (Class III)

(Table 5). Similarly, the mean percentage repellency of *L. serricornis* was significantly greater (47.8% and 53.30%) on *A. indica* than on the other plant extracts. On the other hand, *T. orientalis* exhibited significantly lower repellency (31.6% and 41.5%) to *L. serricornis* after 12 h and 24 h, respectively (Fig. 3b and c). After exposure of *L. serricornis* to various concentrations of the five plant extracts, its repellency was significantly greater at all six concentrations of *A. indica* (45.10%, 54.22%, 59.25%, 67.50%, 77.62%, and 85.60%) (Class-V). In contrast, *T. orientalis* was the least avoided extract by the test insect, as indicated by the lowest percentage repellency of the insect against its six concentrations (30.05%, 36.55%, 43.05%, 52.00%, 60.22%, and 65.25%) (Class IV) (Table 5). The mean percentage repellency of the *A. indica* extracts was significantly greater than that of the *T. orientalis* and *P. hysterophorus* extracts, whereas no significant difference was found in the repellency

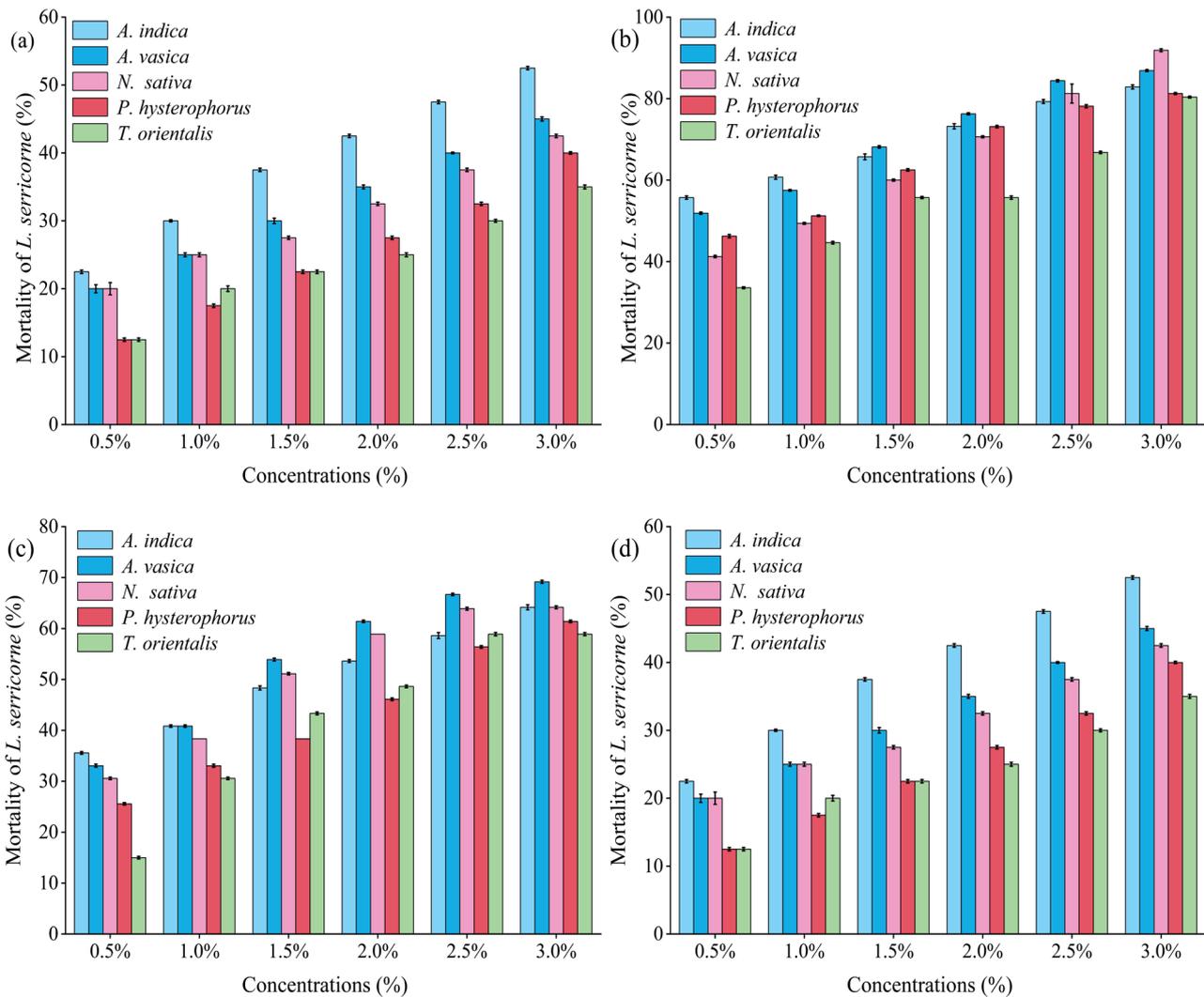


Fig. 2. Mean percentage mortality of *L. serricornis* on 5 plant extracts after (a) 24 h, (b) 48 h, (c) 72 h, and (d) 96 h in residual toxicity test. Statistically significant differences among the various treatments are indicated by the error bars at a 0.5% significance level.

of the *A. indica*, *N. sativa*, and *A. vasica* extracts. Repellency caused by *T. orientalis* and *P. hysterothorus* was significantly lower than that caused by the other three plant species extracts (Fig. 3d). After 72 h and 96 h, *L. serricornis* had significantly greater repellent effects on six concentrations of *A. indica*: 54.50%, 63.23%, 71.12%, 79.10%, 85.50% and 93.20% and 62.10%, 78.72%, 84.52%, 92.10%, 100.00% and 100.00% (Class V). Although the repellency of *T. orientalis* increased with exposure time, the repellency of *T. orientalis* remained significantly lower at all six concentrations (35.225%, 40.62%, 46.32%, 52.50%, 58.22% and 65.22% and 42.22%, 51.23%, 58.64%, 62.70%, 71.33% and 78.33%, respectively) (Class-IV) (Table 5). Similarly, the mean percentage repellency of the test insects was significantly greater (74.4% and 86.2%, respectively) for *A. indica* and significantly lower (49.7% and 60.7%, respectively) for *T. orientalis* (Fig. 3e and f).

Discussion

The active phytochemicals present in the plant species extracts may be involved in the insecticidal activities of the plant extracts, and phytochemical screening gave an idea about the qualitative nature of active compounds. The phytochemical analysis of aqueous extracts of *P. hysterothorus*, *T. orientalis*, *A. indica*, *A. vasica*, and *N. sativa* indicated the presence of alkaloids, flavonoids, saponins, di tarphenes, phytosterol, and phenols. Other researchers have also reported the presence of phytochemicals in aqueous acetone and methanol extracts, along with other plant species extracts, that are responsible for killing stored insect pests [26, 27, 28]. Based on our recent investigations, a considerable knockdown was observed for most plant species extract, but immediate mortality was not observed. Moreover, this knockdown eventually resulted in mortality, even though the insects were removed from the treated Petri plates [29, 30]. In the current research, *A. indica* exhibited the greatest contact toxicity, followed closely

Table 5. Repellency of *L. serricornis* on various concentrations of five plant species extracts after 1 h, 12 h, 24 h, 48 h, 72 h, and 96 h exposure.

Plant species	250 mg/L	500 mg/L	750 mg/L	1000 mg/L	1250 mg/L	1500 mg/L	Repellency class
1 h							
<i>T. orientalis</i>	15.20 c	19.35 abc	23.45 abc	27.00 abc	29.10 abc	30.72 abc	Class - II
<i>N. sativa</i>	17.42 c	19.45 abc	24.92 abc	29.22 abc	31.2 abc	35.6 abc	Class - II
<i>A. vasica</i>	22.42 abc	27.85 abc	31.80 abc	35.00 abc	39.00 a	45.50 a	Class - III
<i>P. hysterophorus</i>	17.62 c	19.50 abc	23.33 abc	26.77 abc	30.24 abc	33.60 abc	Class - II
<i>A. indica</i>	29.30 abc	28.40 abc	32.10 abc	37.32 abc	38.45 a	43.70 a	Class - III
12 h							
<i>T. orientalis</i>	20.50 j	24.22 ij	28.74 hij	33.63 q-t	38.50 b-e	43.92 a-e	Class - III
<i>N. sativa</i>	29.64 hij	34.85 d-j	39.10 b-e	45.55 a-e	50.60 abc	57.32 ab	Class - III
<i>A. vasica</i>	30.60 stu	34.34 d-j	41.05 m-p	47.44 a-e	52.45 abc	60.40 a	Class - IV
<i>P. hysterophorus</i>	25.25 hij	31.70 f-j	36.62 o-r	42.44 l-o	47.32 i-l	53.25 ab	Class - III
<i>A. indica</i>	31.44 f-j	35.30 d-j	43.35 a-e	49.10 a-d	52.37 ab	63.54 a	Class - IV
24 h							
<i>T. orientalis</i>	27.05 z	33.22 xy	40.60 tuv	45.12 rs	49.22 op	53.72 lmn	Class - III
<i>N. sativa</i>	32.42 xy	42.32 tuv	49.00 op	55.32 lm	61.30 ij	69.60 ab	Class - IV
<i>A. vasica</i>	35.52 wx	44.02 rst	50.40 nop	57.15 kl	64.25 hi	73.12 a	Class - IV
<i>P. hysterophorus</i>	30.50 yz	37.05 vw	45.42 qrs	52.32 mno	59.20 jk	65.62 h	Class - IV
<i>A. indica</i>	39.33 uv	47.35pqr	53.12 mn	59.20 jk	67.15gh	77.62 a	Class - IV
48 h							
<i>T. orientalis</i>	30.05 w	36.55 uv	43.05 rs	52.00 mno	60.22 ijk	65.25 fgh	Class - IV
<i>N. sativa</i>	39.55 st	45.12 qr	52.62 mno	60.12 ijk	66.40 fg	72.32 c	Class - IV
<i>A. vasica</i>	40.30 tu	48.15 pq	55.62 lm	62.30 ijk	70.10 d	77.25 b	Class - IV
<i>P. hysterophorus</i>	33.25 v	41.25 st	49.20 op	57.60 kl	63.40 ghi	70.20 de	Class - IV
<i>A. indica</i>	45.10 qr	54.22 mn	59.25 jk	67.50 ef	77.62 b	85.60 a	Class - V
72 h							
<i>T. orientalis</i>	35.22 p	40.62 o	46.32 mn	52.50 kl	58.22 ij	65.22 g	Class - IV
<i>N. sativa</i>	46.25 mn	55.32 jk	60.52 hi	69.22 f	77.10 de	85.40 c	Class - V
<i>A. vasica</i>	48.10 m	56.32 jk	62.72 gh	73.10 ef	85.12 c	90.70 a	Class - V
<i>P. hysterophorus</i>	42.42 no	50.02 lm	55.32 jk	64.10 gh	72.56 f	80.55 d	Class - V
<i>A. indica</i>	54.50 jk	63.23 gh	71.12 f	79.10 d	85.50 c	93.20 a	Class - V
96 h							
<i>T. orientalis</i>	42.22 s	51.23 r	58.64 pq	62.70 no	71.33 m	78.33 kl	Class - IV
<i>N. sativa</i>	55.32 q	60.92 op	70.43 m	80.23 jk	91.30 ef	100.00 a	Class - V
<i>A. vasica</i>	58.60 pq	66.05 n	77.24 kl	86.90 gh	95.15 cd	100.00 a	Class - V
<i>P. hysterophorus</i>	49.62 r	56.52 q	63.42 no	75.20 kl	82.12 ij	91.25 ef	Class - V
<i>A. indica</i>	62.10 o	78.72 k	84.52 hi	92.10 de	100.00 a	100.00 a	Class - V

by *A. vasica* when used against *L. serricornis*. This finding suggests that there is a possibility for the mass production of these specific plant species as grain protectors, given that they were able to achieve a 50% mortality rate of *L. serricornis* with the smallest amount of extract after a 96-hour exposure period. Recently, numerous investigations have been conducted to assess the efficacy of plant extracts in combating *L. serricornis*. According to the reports of [31], the extracts from

Elsholtzia densa have a higher contact effect, having $LD_{50} = 24.29$ mg/L when used against *L. serricornis*. Similar results were also revealed by [32], who stated that extracts of *Artemisia lavandulaefolia* showed a promising contact toxicity $LD_{50} = 13.51$ mg/L against *L. serricornis*. *A. indica* showed the greatest toxicity against *C. maculatus*, a phenomenon that can be attributed to the presence of azadirachtin, which has been identified as highly toxic to insect pests. The toxic

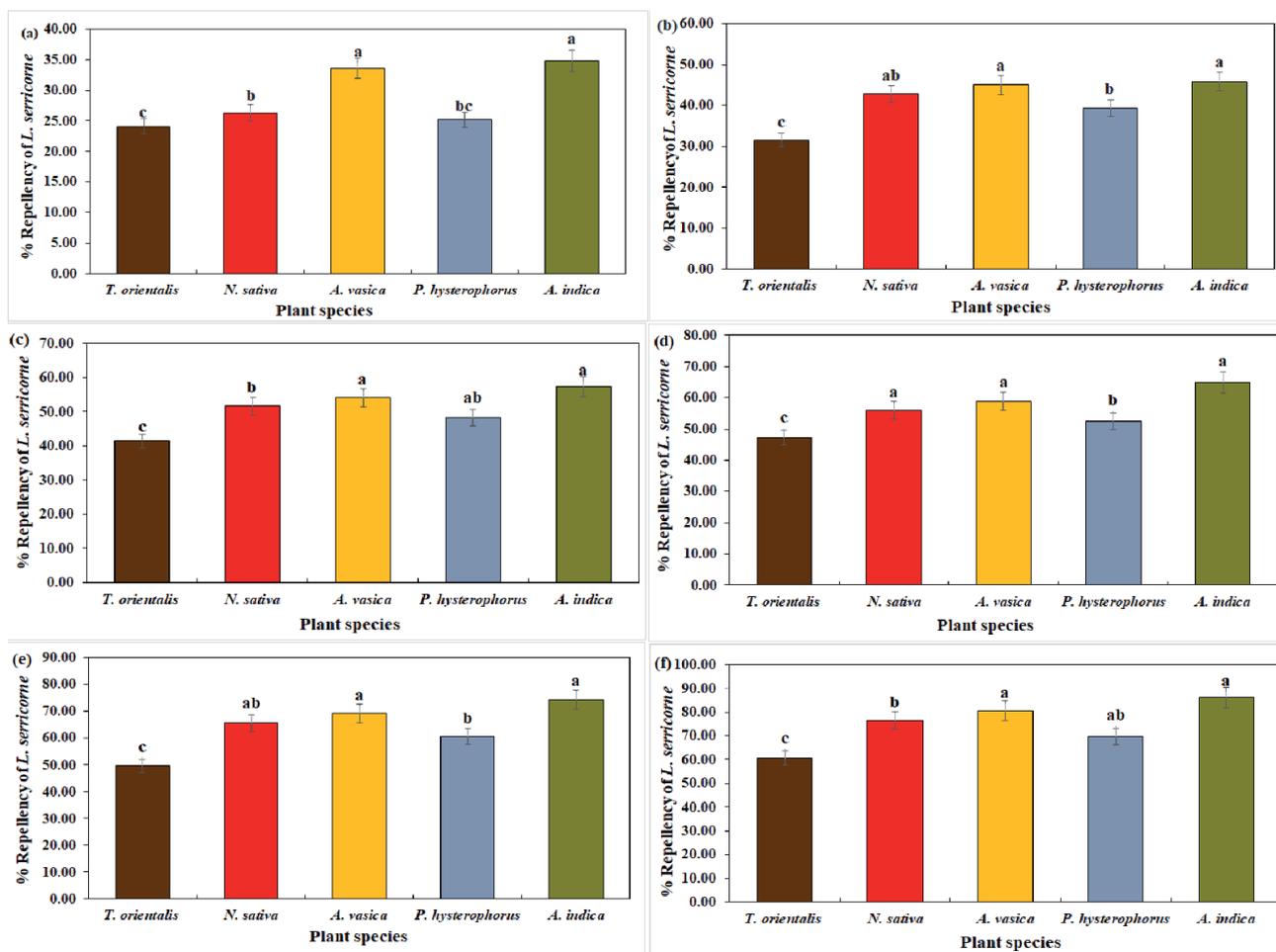


Fig. 3. Mean percentage repellency of *L. serricornis* on five plant species extract after exposure of (a) 1 h, (b) 12 h, (c) 24 h, (d) 48 h, (e) 72 h, and (f) 96 h. The different lowercase letters above the error bars indicate significant differences among the various treatments at a 0.5% significance level.

nature of botanical pesticides can be attributed to their ability to disrupt nervous and hormonal systems (e.g., azadirachtin) or interfere with mitochondrial functions (e.g., rotenone) [33]. Furthermore, it has been reported that *A. vasica* possesses insecticidal properties, with the major constituents of this plant being vasicine, vascinone, and saponine [34]. The observed activity of *A. vasica* can be attributed to the collective or independent effects of these compounds. The anthelmintic activity of *A. vasica* water extract has also been documented [35]. *A. indica* exhibited the greatest toxicity against *C. maculatus*, which could be attributed to the presence of azadirachtin, a compound that is highly toxic to insect pests. The toxicity of botanical pesticides can stem from their ability to disrupt the nervous and hormonal systems of insects, such as azadirachtin, or interfere with mitochondrial function, such as rotenone [18].

Likewise, *P. hysterophorus* exhibited both toxic and repellent effects on *L. serricornis*, which can be attributed to the presence of parthenin, the active ingredient in *P. hysterophorus*. Notably, the use of the entire plant extract of *P. hysterophorus* against fifth

instar larvae of *S. litura* resulted in an adverse impact on the growth of the insects [36]. The compound parthenin, which is present in *P. hysterophorus*, exhibits a diverse array of biological characteristics. These include cytotoxic, antitumor, allergic, antimicrobial, antifeedant, phytotoxic, and insecticidal properties [37], [38]. The increased mortality observed with these botanical substances can be ascribed to their capacity to disrupt fundamental metabolic, biochemical, behavioral, and physiological aspects in insects. Moreover, elevated concentrations of these substances can induce insect mortality by influencing nerve cells in insects [39]. The potential toxicity or repellent characteristics of these essential extracts vary based on the chemical composition of the oil and the sensitivity displayed by the insect [40]. A study conducted by [32] revealed a total of 32 components, with the majority being monoterpenes such as p-cymene (38%), carvacrol (5-11%), and -pinene (5-14%). These specific components have been scientifically proven to possess insecticidal activities against various phytophagous insects, including *Tribolium confusum*, *Ceratitis capitata*, and *Rhopalosiphum padi* [41, 42].

Although the current study revealed a significant reduction in insect infestation in packaged storage goods treated with plant extracts, any infestation in packaged food is still unacceptable to its consumer market. Therefore, it is essential to address whether infestation can be entirely prevented by employing plant extracts. The efficacy of plant extracts on *L. serricornis* and their potential for use in insect-resistant materials are influenced by various factors, predominantly the application dose of these extracts, their application techniques, the developmental stages of the insects, and other variables. Further research is essential to determine the optimal formulation, appropriate dosage, suitable application methods, effects of environmental conditions, and comprehensive composition analysis of the plant extract for effectively protecting stored products from *L. serricornis* infestations.

Conclusions

The present study demonstrated that plant species, particularly *A. indica* and *A. vasica*, exhibit strong potential for controlling *L. serricornis* through both toxicity and repellency. *A. indica* showed the highest topical and repellent effectiveness, while *A. vasica* excelled in residual toxicity. These findings suggest that plant-based extracts offer an environmentally friendly, cost-effective, and socially acceptable alternative to synthetic pesticides, especially in tropical and subtropical regions. Incorporating these botanicals into pest management strategies could reduce environmental pollution and health risks associated with chemical insecticides.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

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