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Original Research

Comparative Study of Aqueous Plant Extracts and Deltamethrin against Red Flour Beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract

The red flour beetle (*Tribolium castaneum* Herbst), a major pest of stored grains in tropical and subtropical regions, causes significant economic losses in several agricultural storage items. This research investigates the use of aqueous plant extracts and synthetic insecticide (deltamethrin) for the sustainable and safe management of the red flour beetle. Aqueous extracts from five plant species – *Alpinia galanga, Azadirachta indica, Curcuma longa, Nicotiana tabacum,* and *Nicotiana rustica* – were evaluated at six different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%) along with deltamethrin against *T. castaneum*. Toxicity (both contact and residual) was assessed after 24, 48, 72, and 96 hours using a completely randomized design with four replications. Probit analysis was carried out to determine LC_{50} and LC_{90} values. Results revealed that, generally, the mortality of *T. castaneum* increased as the concentration and exposure time increased. Deltamethrin exhibited the highest contact toxicity overall, with an LC_{50} of 0.88 ppm and LC_{90} of 4.74 ppm, and the highest residual toxicity showed

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the lowest LC_{50} (0.93 ppm) and LC_{90} (3.83 mol/L) after a 96 h exposure period. Among the plant species extracts in contact toxicity, *N. rustica* showed the highest toxicity with an LC_{50} of 1.00 g/L and LC_{90} of 5.38 g/L, followed by *N. tabacum* (LC_{50} of 1.09 g/L and LC_{90} of 6.50 g/L). In residual toxicity tests, among the plant species, *N. rustica* again had the highest toxicity in residual tests with an LC_{50} of 0.79 g/L and LC_{90} of 10.37 g/L, followed by *N. tabacum* (LC_{50} of 1.18 g/L and LC_{90} of 9.02 g/L) and *A. indica* (LC_{50} of 1.82 g/L and LC_{90} of 22.36 g/L). The findings suggest that deltamethrin is recommended for managing *T. castaneum*; however, *N. rustica, N. tabacum,* and *A. indica* can effectively control *T. castaneum* and may be useful in developing novel biopesticides.

Keywords: aqueous extracts, insecticidal activity, contact toxicity, residual toxicity, integrated pests management

Introduction

Grain commodities need to be preserved when supply surpasses demand, as is characteristic of the global grain markets. So, when the grains are stored for a long time period, different insect pests attack the grains, which causes a devaluation in the quality of grains. Stored grains, cereals, and their products are significant sources of food on a worldwide scale; it is crucial for human survival to conserve this precious resource effectively [1, 2]. Wheat, rice, and maize are among the most commonly consumed grains, while chickpeas serve as a significant source of animal feed and are, therefore, important to help meet global food demands [3, 4]. These crops make up a significant proportion of Pakistan's agricultural yield. Post-harvest, these commodities are kept in storage for up to a year in order to maximize market prices for the yield [5, 6]. During this extended storage, these valuable resources are susceptible to several losses, particularly those brought on by insect pest infestation [7, 8].

An estimated 10 to 40% qualitative and quantitative loss to stored agricultural goods occurs globally due to these destructive insect pests of grains. *Tribolium castaneum* (Herbst) is one of the most destructive insect pests that reduce the grain's weight by up to 40% [9]. It attacks the endosperm of the seeds, leaving them with a decaying smell and coagulating texture [10-12]. To prevent these grains from rotting, an efficient control method is necessary. At the moment, synthetic insecticides are the backbone of the stored grain industry. These substances not only cause a major health risk to consumers but also have a negative effect on the environment [13-15]. Due to the indiscriminate use of synthetic insecticides against insect pests of stored grain, these insect pests developed resistance against the synthetic insecticides. Because of the harmful effects of chemical pest control, plant species contain natural compounds that can be considered for use to combat these pests without any threats to human health or affecting grain quality [16].

Chemicals derived from organic and natural plants may be a source of such discoveries. Regarding this, new research has identified several promising plant extracts that naturally ward off insects in grain storage systems [17]. In order to aid in pest control, these botanical extracts may do a variety of wonders by not only deterring pests or keeping them from feeding and egg-laying but concurrently acting as insecticides [18]. Plant-based insecticides have gained significant attention as eco-friendly alternatives to synthetic chemicals due to their biodegradable nature, low toxicity to non-target organisms, and minimal environmental persistence. Several studies have explored the efficacy of plant extracts, essential oils, and phytochemicals against *T. castaneum*, demonstrating their potential in stored grain pest management. For example, neem (*Azadirachta indica*) extracts, particularly rich in azadirachtin, have shown strong repellent, antifeedant, and toxic effects against *T. castaneum* [12, 19]. It functions as an insect growth regulator, repellent, sterilant, and feeding inhibitor. In addition, it may also prevent oviposition [19]. Likewise, nicotine, another plant derivative (from *N. tabacum*), acts as an acetylcholine inhibitor and has effectively controlled insect pests' infestation, with LD_{50} reported in the range of 50 to 60 mg/kg. Similarly, turmeric is also well known for its insecticidal properties, making it beneficial against several agricultural and household insect pests. It also shows some insect-repellent properties. Research shows turmeric products are a safe and affordable alternative to synthetic insecticides, according to Sulhath et al. [20]. *C. longa* demonstrates promise as a biopesticide against *T. castaneum*. Carvacrol (monoterpenoid phenol) is an acetylcholinesterase inhibitor and has been shown to be toxic to *T. confusum* [21]. The use of *C. longa* and *A. galanga* in pest control is supported by their bioactive compounds, such as curcuminoids and terpenes, which disrupt insect growth and reproduction [22]. These plants have shown efficacy against a range of insect species; their specific activity against *T. castaneum* highlights the potential for integrating them into pest management strategies. Furthermore, the phytochemical composition of these plants, including alkaloids, phenols, flavonoids, and saponins, contributes to their synergistic insecticidal effects [23, 24].

Investigating the biopesticidal effect of plant species extracts against *T. castaneum* is of immense significance for sustainable pest management in agriculture. This research aligns with the growing

global interest in developing eco-friendly alternatives to conventional chemical pesticides, contributing to the broader framework of integrated pest management (IPM) strategies [25-27]. By exploring the efficacy of plant extracts against *T. castaneum*, a notorious pest affecting stored grains, the study adds to the body of knowledge on botanical insecticides, aligning with the principles of green chemistry and environmentally friendly pest control [28]. The identification of potent plant-derived biopesticides not only addresses concerns about the ecological impact of synthetic pesticides but also offers practical solutions for sustainable agriculture, aligning with the goals of the United Nations Sustainable Development Agenda. As the research unfolds the biopesticidal potential of various plant species, it contributes to a paradigm shift towards more sustainable and ecologically responsible pest control practices in agriculture.

While research on the comparative effect of plant species extracts against *T. castaneum* has made notable progress, there remains a significant gap in understanding the mechanisms underlying the observed effects and the variability in efficacy among different plant extracts. Further investigation is needed to unravel the specific bioactive compounds responsible for insecticidal properties and their mode of action against *T. castaneum*. Additionally, comprehensive studies that systematically compare the biopesticidal potential of diverse plant species are scarce, limiting our knowledge of the most effective botanical agents for controlling this pest. Addressing this research gap is crucial for developing targeted and optimized plant-based biopesticides, enhancing their practical applicability in integrated pest management strategies. Furthermore, there is a need for long-term studies assessing the sustainability and ecological impact of these plant extracts on non-target organisms and the overall agroecosystem. This will ensure that the adoption of plant-based biopesticides aligns with the principles of environmentally sustainable pest management practices. Consequently, it is crucial to compare their effectiveness to that of other readily available plant species [29-31]. Plant-based products can serve as effective substitutes for synthetic chemicals. As a result, the negative effects of synthetic pesticides will be greatly reduced. The current study sought to examine the efficacy of potential new botanical pesticides in managing the storage pest *T. castaneum* in its adult stages. The findings of this study might help improve the efficiency of pesticide use, enhance the ecological advantages of phytochemicals, and broaden their uses in other agricultural domains [15]. The study aims to study the contact and residual toxicity of aqueous extracts from five plant species and deltamethrin against *T. castaneum.* To determine the most effective treatments, compare the mortality rates of *T. castaneum* when exposed to six concentrations and exposure times of the plant extracts and deltamethrin.

Materials and Methods

The current investigation was carried out in the Entomological Laboratory at the University of Haripur, Pakistan. *T. castaneum* was collected from various godowns in the district of Swabi, Khyber Pakhtunkhwa, and raised on wheat flour. The culture was maintained under laboratory conditions at a relative humidity of 65%±5% and an average temperature of 27±2ºC [32].

Collection of Plant Species and Preparation of Powders

Plant materials, such as the leaves of *Nicotiana rustica* and *Nicotiana tabacum*, roots of *Curcuma longa, s*eeds of *Azadirachta indica,* and rhizome of *Alpinia galanga* were collected from different areas of the district Swabi, in Khyber Pakhtunkhwa, Pakistan (Table 1). For the authentication of plant species, they were identified by a botanist in the Department of Biology at the University of Haripur, and the plant specimens were deposited in the Herbarium in the Department of Horticulture. After collection, the plant materials were cleaned to remove debris and then air-dried in a well-ventilated, shaded area to preserve their bioactive compounds. Over 15 days, the plant-selected parts 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 a 12 h interval using different 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.

Table 1. List of plant species.

Common Name	Botanical Name	Family	Parts used
Neem	*Azadirachta indica*	Meliaceae	Seed
Turmeric	*Curcuma longa*	Zingiberacae	Roots
Virginia tobacco	*Nicotiana tabacum*	Solanacaae	Leaves
Ginger	*Alpinia galanga*	Zingiberaceae	Rhizome
White Patta	*Nicotiana rustica*	Solanacaae	Leaves

The yields obtained were *N. rustica and N. tabacum,* 46 g each; *C. longa,* 63 g; *A. indica,* 51 g; and *A. galanga,* 55 g then kept at less than 4ºC temperature [32].

Preparation of Crude Extract for Insecticidal Activity

We accurately weighed 40 g of fine powders of each plant species and dissolved them in 400 mL of distilled water in an Erlenmeyer flask, and subjected them to a Stuart SSL1 Orbital Shaker (180 rpm) at 30±5ºC for 8 h. The resultant aliquots were filtered through Whatman filter paper No.1, and the residues were washed with 20 mL of de-ionized water. Finally, water was evaporated under a vacuum at 40ºC, and dried extracts were weighed to calculate the percentage yield of crude aqueous extracts. The plant species crude extracts, i.e., 10%, were stored in airtight bags at 4ºC in the dark until further required [33].

Detection of Phenols, Alkaloids, Terpenes, Phytosterols, and Flavonoids in Plant Extracts

A few drops of potassium iodide and iodine were dissolved separately in diluted hydrochloric acid (1.5%). The plant extracts were mixed with 1 mL of this reagent for 5 min. The formation of dark red precipitates in the samples indicates the occurrence of alkaloids in plant extracts [34]. 3 to 4 drops of ferric chloride solution were mixed with the plant aqueous extracts to detect phenols. The presence of phenols in the extract is indicated by the extract's appearance as bluish-black [34]. Similarly, chloroform-cured extracts of the chosen plants were filtered to look for phytosterols. A few drops of concentrated sulfuric acid were added to the filtrate and allowed to stand for a short while. The development of a golden yellow hue suggested the presence of phytosterols [35]. Diterpenes were detected by adding 3 to 4 drops of copper acetate to the plant aqueous solution. The appearance of an emerald green color indicates diterpenes [36]. Likewise, flavonoids were investigated in plant extracts by adding 2-3 drops of lead acetate solution to the plant aqueous extracts. The emergence of a vivid yellow color confirms that flavonoids are present. When a few drops of weak acid were applied, the color vanished [36].

Contact Effect of Plant Aqueous Extracts against *T. castaneum*

The designed experiment was conducted in a 6x6 factorial treatment arrangement, with 5 plant species extracts, 1 synthetic insecticide, and 6 concentrations as factors, making a total of 36 treatments. All the plant species were separately applied on Whatman No. 1 filter paper cut into small circles of 9 cm diameter and allowed to stand at 35ºC for 30 min to evaporate the solvent [37]. The filter paper was then placed in the Petri dishes and covered with muslin cloth for aeration. Then, ten adult beetles of the same age and size were released at the center of the arena. For the control, we used distilled water. Dead beetles were counted after 24, 48, 72, and 96-hour exposure periods. LC_{50} and LC_{90} values were found after correcting the mortality using Abbott's equation [38]. Each treatment was replicated four times.

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

The correction for natural mortality was done by following the Abbott's formula:

$$\text{Mortality (\%)} = \frac{\%\text{Mo} - \%\text{Mc}}{100 - \%\text{Mc}} \times 100 \quad (2)$$

Where, %Mc = percentage of mortality in control, %Mo = percentage of observed mortality.

Residual Effect of Plant Aqueous Extracts against *T. castaneum*

To study the residual effect of five plant species extracts and deltamethrin, sterilized flour was treated with six concentrations, i.e., 0.5, 1, 1.5, 2, 2.5, and 3% of the mentioned materials, whereas treatment with distilled water served as control. Flours were tested at each concentration. In order to achieve full blending with botanical elements and leaves, the mixture was shaken vigorously for half an hour. Treated flour was placed in plastic Petri dishes (12 cm) with a total floor area of 23 cm^2. Adult beetles were released into each Petri dish with treated flour. Mortality of the beetle was recorded after 24, 48, 72, and 96-hour of exposure. The dead beetles from these petri dishes were discarded every 24 h [32].

Data Analysis

While normality was checked using the Shapiro-Wilks test, the assumption of homogeneity of variance was evaluated using Levine's test. The Tukey HSD analysis was applied at a 5% probability of Type I error to separate the means of the obtained data from recent research using Analysis of Variance (ANOVA). Statistical analyses were carried out using STATISTIX 8.1 [39]. The mortality percentages were corrected using Abbott's formula. Log-Probit model analysis was then applied to the percentage mortality of adult *T. castaneum* to determine the 50% and 90% lethal concentrations (LC_{50}/LC_{90}) [40]. The Analysis of Variance and the Probit analyses were done using the Statistical Package for the Social Sciences (SPSS) version 20.

Results

Phytochemical Screening

A variety of phytochemicals such as phenols, alkaloids, diterpenes, phytosterols, and flavonoids were detected in *N. rustica* and *N. tabacum* plant extracts; however, *A. indica* contained only a few of them. Of these, phytosterol was found to have the highest concentration. Moreover, the plant aqueous extracts also contained saponins (Table 2).

Contact Mortalities of Plant Extracts and Deltamethrin against *C. castaneum*

From Table 3, the results showed that deltamethrin exhibited the highest toxicity against *T. castaneum* in terms of the lowest LC_{50} of 13.93 ppm and LC_{90} of 185.26 ppm after 24 h of exposure. Among the plant species extracts, *N. rustica* showed the highest toxicity, having an LC_{50} of 27.25 g/L and an LC_{90} of 356.20 g/L. The lowest toxicity was recorded with *A. galanga,* having a maximum LC_{50} of 43.19 g/L and LC_{90} of 851.23 g/L. The mortality of *T. castaneum* at six different concentrations was significantly higher with deltamethrin (10.00, 15.00, 22.50, 30.00, 30.00, and 32.50%). Among the plant species extracts, *N. rustica* exhibited maximum mortality at all six concentrations (10.00, 15.00, 17.50, 22.00, 25.00, and 27.00%) and minimum mean percent mortality (5.00, 10.00, 12.50, 15.00, 17.00, and 20.00%) was recorded at all concentrations of *A. galanga (df* = 25*, F* = 0.64*, P* = 0.8978*)* as shown in Fig. 1a).

After a 48-hour exposure period, deltamethrin showed maximum toxicity, having an LC_{50} of 4.60 ppm and an LC_{90} of 54.28 ppm against *T. castaneum.* Among the five plant species extracts, a minimum LC_{50} of 5.75 g/L and LC_{90} of 94.80 g/L were recorded for *N. rustica,* followed by *N. tabacum* exhibiting an LC_{50} of 9.62 g/L and LC_{90} of 235.80 g/L against *T. castaneum.* The least effective plant species against *T. castaneum* was *A. galanga,* with an LC_{50} of 14.08 g/L and an LC_{90} of 443.32 g/L, as shown in Table 3. Among the plant species, the significantly highest mean percent mortality of *T. castaneum* was recorded at all concentrations of *N. rustica* (22.00, 30.00, 37.50, 42.00, 47.00, and 52.00%), and the lowest mean percent mortality (12.50, 17.00, 22.50, 27.00, 32.00, and 40.00%) was recorded with all concentrations of *A. galanga.* Deltamethrin showed maximum (25.00, 30.00, 37.00, 45.00, 52.00, and 60.00%) mean percent mortality against *T. castaneum* (*df* = 25, *F* = 0.84, *P* = 0.6792) as shown in Fig. 1b).

With an increase in the exposure time of *T. castaneum* to plant species extracts, the lethal effects also increased after 72 h. In plant species extracts, the highest toxicity was observed with *N. rustica* having an LC_{50} of 1.92 g/L and an LC_{90} of 12.36 g/L, and the least effective plant species was *A. galanga,* having an LC_{50} of 3.89 g/L and an LC_{90} of 47.78 g/L. Meanwhile, deltamethrin showed the highest toxicity effect, with the lowest LC_{50} of 1.77 ppm and LC_{90} of 11.75 ppm against *T. castaneum,* as shown in Table 3. Deltamethrin showed significantly higher mean percent mortality (40.00, 50.00, 57.50, 67.00, 75.00, and 87.00%) with all concentrations against *T. castaneum.* Among the plant species extracts, the overall mean percent mortality to *T. castaneum* was significantly higher (37.00, 47.00, 56.00, 63, 74, and 85.00%) with all concentrations of *N. rustica* and significantly minimal (27.00, 35.00, 40.00, 47.00, 57.00, and 62.00%) with all concentrations of *A. galanga.* Deltamethrin showed the highest mean percent mortalities (40.00, 50.00, 75.50, 66.00, 75.00, and 87.50%) to *T. castaneum* as shown in Fig. 1c) (*df* = 25, *F* = 1.37, *P* = 0.1358).

Likewise, after 96 h of exposure, the mortality rate of *T. castaneum* increased as exposure to deltamethrin and plant species increased, and vice versa. Deltamethrin showed a maximum toxicity effect with the least LC_{50} of 0.88 ppm and LC_{90} of 4.74 ppm. Among the plant species extracts, the highest toxicity was observed with *N. rustica* having a minimum LC_{50} of 1.00 g/L and LC_{90} of 5.38 g/L, and the maximum toxicity was observed with *A. galanga* having an LC_{50} of 1.72 g/L and LC_{90} of 9.31 g/L Table 3. In the overall mean percent mortality, deltamethrin at 6 different concentrations exhibited maximum (60.00, 67.50, 77.00, 85.00, 92.00, and 97.00%) mortality to *T. castaneum.* On the other hand, among the plant species extracts, significantly higher mean percent mortality (55.00, 63.00, 75.00, 84.00, 91.00, and 95.00%) was recorded with all concentrations of *N. rustica,* and significantly lower (40.00, 50.00, 59.00, 68.00, 77.00, and 88.00%) mean percent mortality was recorded with

Table 2. Composition of phytochemicals in aqueous extracts of plant species.

Plant species	Phytochemical constituents of five plant species					
	Phenols	Alkaloids	Diterpenes	Phyto-sterol	Flavonoids	Saponins
A. indica	Low	highly	Low	Low	Low	Low
C. longa	Moderately	Moderately	Low	Moderately	Moderately	Low
N. tabacum	Low	highly	highly	Low	Low	Low
A. galanga	Low	Low	Moderately	Low	Low	Low
N. rustica	highly	Moderately	highly	Moderately	Moderately	Low

Table 3. Contact toxicity of deltamethrin and plant species extracts against *T. castaneum* after 24, 48, 72, and 96 hours' exposure time.

Time interval	Treatments	LC_{50} (95% CLs)	LC_{90} (95% CLs)	χ^2	*P*	Slope±SE
24 h	*A. indica*	29.02 (13.11-360.48)	792.32 (116.03-417.39)	0.17	0.99	0.89±0.24
	C. longa	40.86 (16.18-956.61)	997.60 (128.62-934.98)	0.25	0.99	0.86±0.26
	N. tabacum	31.88 (13.74-515.70)	944.79 (125.67-897.29)	0.36	0.98	0.87±0.24
	A. galanga	43.19 (16.98-1014.72)	851.23 (117.92-776.29)	0.08	0.99	0.99±0.27
	N. rustica	27.25 (13.60-181.13)	356.20 (79.09-237.97)	0.13	0.99	1.14±0.27
	Deltamethrin	13.93 (8.80-39.39)	185.26 (56.63-309.40)	0.86	0.93	1.14±0.23
48 h	*A. indica*	12.08 (7.97-29.82)	160.15 (52.23-210.30)	1.28	0.86	1.02±0.23
	C. longa	12.62 (7.44-56.63)	460.16 (84.12-829.88)	1.10	0.89	0.92±0.21
	N. tabacum	9.62 (6.37-25.75)	235.80 (60.50-843.74)	0.84	0.93	0.92±0.21
	A. galanga	14.08 (8.13-65.35)	443.32 (84.33-612.91)	0.62	0.96	0.85±0.21
	N. rustica	5.75 (4.42-9.26)	94.80 (35.89-834.23)	0.38	0.98	1.05±0.20
	Deltamethrin	4.60 (3.73-6.26)	54.28 (25.79-240.52)	2.84	0.58	1.19±0.20
72 h	*A. indica*	2.85 (2.37-3.39)	23.78 (14.64-56.08)	2.37	0.66	1.39±0.20
	C. longa	2.65 (2.11-3.23)	31.14 (17.02-100.70)	1.25	0.87	1.19±0.20
	N. tabacum	2.31 (1.80-2.79)	24.55 (14.40-66.90)	1.17	0.88	1.24±0.20
	A. galanga	3.89 (3.18-5.05)	47.78 (23.30-201.21)	2.73	0.60	1.77±0.20
	N. rustica	1.92 (1.53-2.26)	12.36 (8.92-20.97)	5.96	0.20	1.58±0.20
	Deltamethrin	1.77 (0.92-2.44)	11.75 (6.83-55.67)	7.84	0.09	1.56±0.20
96 h	*A. indica*	1.46 (1.14-1.74)	7.43 (5.92-10.44)	6.05	0.19	1.81±0.21
	C. longa	1.33 (0.54-1.91)	6.89 (4.52-21.43)	9.88	0.04	1.79±0.21
	N. tabacum	1.09 (0.16-1.78)	6.50 (3.98-44.25)	10.36	0.01	1.65±0.21
	A. galanga	1.72 (0.92-2.34)	9.31 (5.85-31.63)	8.67	0.07	1.74±0.21
	N. rustica	1.00 (0.70-1.27)	5.38 (4.39-7.25)	5.49	0.24	1.76±0.22
	Deltamethrin	0.88 (0.22-1.41)	4.74 (3.27-11.93)	9.27	0.05	1.75±0.22

** Lethal concentrations (LC) are indicated with 95% confidence limits (CLs). Lethal concentrations of plant extracts g/L, while Lethal concentrations of deltamethrin ppm.

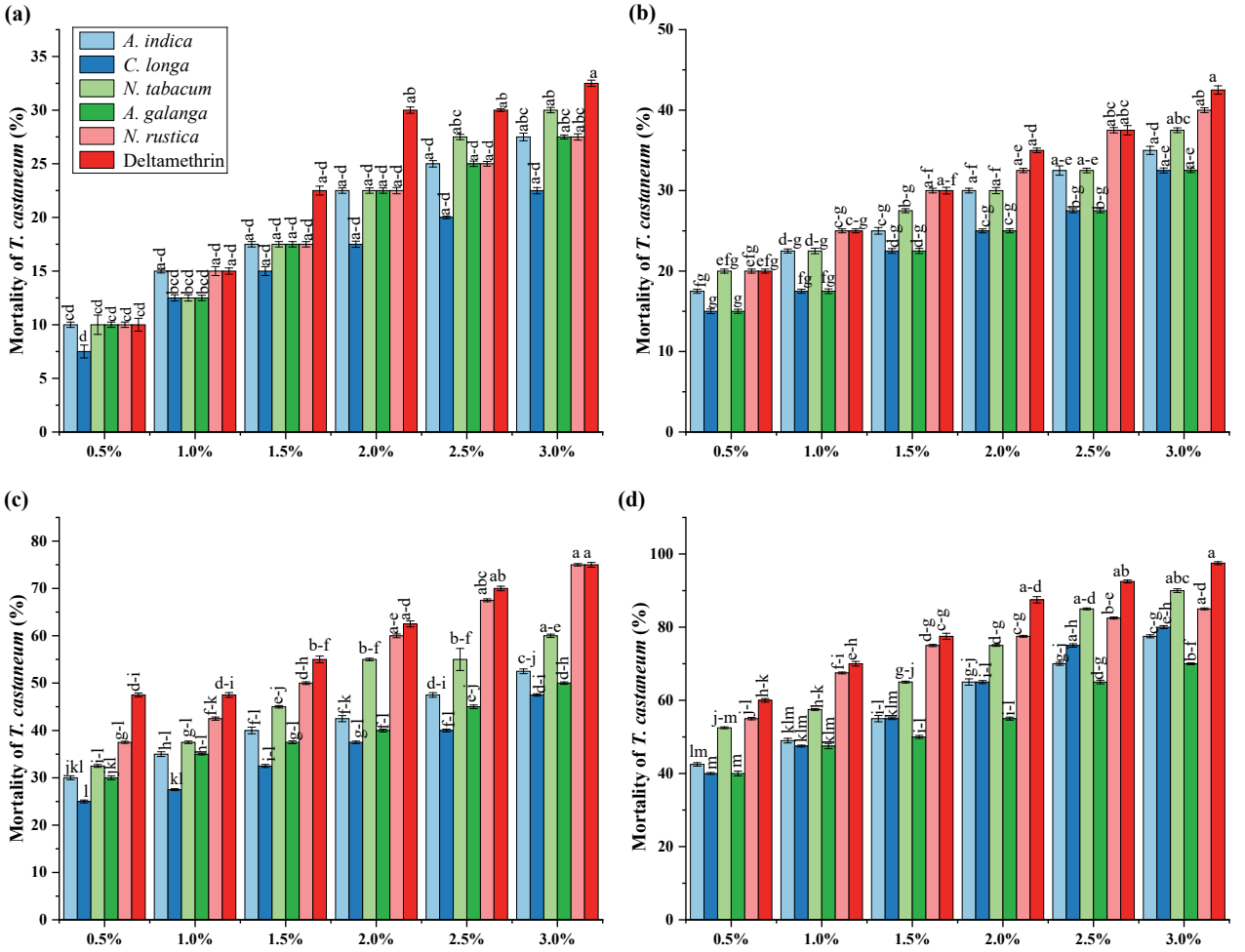

Fig. 1. Mean percentage mortality of *T. castaneum* treated with plant extracts and deltamethrin in contact toxicity test. a) after 24 h, b) after 48 h, c) after 72 h d) after 96 h. The bars with different lowercase letters indicate that the means are significantly different from each other at $p = 0.05$.

all concentrations of *A. galanga* against *T. castaneum* (*df* = 25, *F* = 1.11, *P* = 0.3470) Fig. 1d).

Residual Effect of Plant Species Extracts and Deltamethrin against *T. castaneum*

Table 4 shows the residual toxicity of five plant species extracts and deltamethrin against *T. castaneum*. After a 24-hour exposure period, deltamethrin showed the highest toxicity in all treatments, having an LC_{50} of 13.93 ppm and an LC_{90} of 185.26 ppm against *T. castaneum*. Among the plant species extracts, *N. tabacum* exhibited the highest level of toxicity, with an LC_{50} of 19.96 g/L and an LC_{90} of 322.17 g/L. *C. longa* was the least effective extract, having an LC_{50} of 47.95 g/L and an LC_{90} of 1531.88 g/L against *T. castaneum*. In overall mean percent mortality, deltamethrin at all concentrations showed significantly higher mean percent mortality (10.00, 15.00, 22.50, 30.00, 30.00, and 32.00%), as shown in Fig. 2a). Among the plant species extract, the overall mean percent mortality was significantly higher (10.00, 12.50, 17.00, 22.00, 25.50, 27.00%) with all concentrations of *N. tabacum,* and lower mean percent mortality (7.00, 12.00, 15.00, 17.00, 20.00, and 22.00%) was recorded with all concentrations of *C. longa* against *T. castaneum* (*df* = 25, *F* = 0.25, *P* = 0.999).

After a 48-hour exposure period of 5 plant species extracts and deltamethrin, the highest toxicity effect was observed with deltamethrin having LC_{50} 11.52 ppm and LC_{90} 394.06 ppm against *T. castaneum,* as shown in Table 4. In the case of plant species extracts, *N. rustica* exhibited the highest toxicity, with the lowest LC_{50} of 13.93 g/L and LC_{90} of 658.48 g/L. The least toxic plant species were *A. galanga and C. longa,* having the same LC_{50} of 29.78 g/L and LC_{90} of 1574.98 g/L against *T. castaneum*. From Fig. 2b), it was also clear that the overall mean percent mortality was significantly maximum (25.00, 31.00, 39.00, 46.00, 53.00, and 60.50%) with all concentrations of deltamethrin. In the case of plant species extracts, the highest mean percent mortality (22.00, 30, 37.00, 42.50, 47.00, and 52.00%) was recorded with all concentrations of *N. rustica,* and the lowest mean percent mortality (12.00,

18.00, 23.00, 28.00, 33.00, and 40.00%) was seen with all concentrations of *A. galanga* against *T. castaneum* (*df* = 25, *F* = 0.21, *P* = 1.000).

Among the plant species extracts, the highest toxicity effect was observed with *N. rustica,* having a minimum LC_{50} of 2.30 g/L and an LC_{90} of 24.46 g/L, and the least effective plant species extract was *A. galanga,* having an LC_{50} of 9.84 g/L and an LC_{90} of 475.09 g/L against *T. castaneum*. Deltamethrin exhibited a maximum toxicity effect, having the least LC_{50} of 1.64 ppm and LC_{90} of 36.11 ppm, as shown in Table 4. As the time exposure period of plant species and deltamethrin increases, the mortalities of *T. castaneum* also increase and vice versa. Deltamethrin showed significantly maximum mean

Table 4. Residual toxicity of five plant species extracts and deltamethrin on *T. castaneum* after 24, 48, 72, and 96 hours' exposure time.

Time interval	Treatments	LC_{50} (95% CLs)	LC_{90} (95% CLs)	χ^2	*P*	Slope±SE
24 h	*A. indica*	29.02 (13.11-360.48)	792.32 (116.03-4170.39)	0.17	0.99	0.89±0.24
	C. longa	47.95 (17.17- 240.61)	1531.88 (154.06-1164.55)	0.05	1.00	0.85±0.25
	N. tabacum	19.96 (11.00-91.48)	322.17 (76.27-1453.46)	0.97	0.91	1.06±0.24
	A. galanga	29.02 (13.11-360.48)	792.32 (116.03-4173.39)	0.17	0.99	0.89±0.24
	N. rustica	26.34 (12.65- 227.18)	584.61 (101.44-1170.44)	0.55	0.96	0.95±0.24
	Deltamethrin	13.93 (8.80-39.39)	185.26 (56.63-3093.40)	0.86	0.93	1.14±0.23
48 h	*A. indica*	22.12 (10.15-418.38)	1367.58 (138.90-1142.21)	0.22	0.99	0.71±0.21
	C. longa	29.78 (12.38-845.56)	1574.98 (152.81-1546.66)	0.64	0.95	0.74±0.22
	N. tabacum	22.74 (9.96-771.31)	1980.62 (158.61-1615.04)	0.64	0.95	0.66±0.21
	A. galanga	29.78 (12.38-845.75)	1574.98 (152.81-1546.66)	0.64	0.95	0.74±0.22
	N. rustica	13.93 (7.58-88.02)	658.48 (98.27-4025.07)	0.27	0.99	0.76±0.21
	Deltamethrin	11.52 (7.03-44.64)	394.06 (77.62-4913.54)	0.41	0.98	0.83±0.21
72 h	*A. indica*	6.13 (4.24-15.89)	359.22 (65.67-1082.52)	0.73	0.94	0.72±0.20
	C. longa	8.02 (4.95-46.43)	895.92 (97.71-1479.40)	0.91	0.92	0.62±0.20
	N. tabacum	3.51 (2.73-4.78)	78.16 (29.59-783.37)	1.35	0.85	0.95±0.20
	A. galanga	9.84 (6.10-39.51)	475.09 (81.25-1482.63)	1.59	0.81	0.76±0.20
	N. rustica	2.30 (1.80-2.79)	24.46 (14.35-66.35)	4.69	0.32	1.25±0.20
	Deltamethrin	1.64 (0.98-2.16)	36.11 (16.91-211.28)	5.00	0.98	0.95±0.19

96 h	*A. indica*	1.82 (1.30-2.27)	22.36 (13.04-63.98)	3.81	0.43	1.17±0.20
	C. longa	2.24 (1.55-2.88)	51.89 (21.87-402.85)	3.12	0.53	0.94±0.19
	N. tabacum	1.18 (0.29-1.83)	9.02 (5.27-59.36)	8.96	0.06	1.45±0.21
	A. galanga	1.88 (1.45-2.27)	15.78 (10.50-32.14)	4.86	0.30	1.38±0.20
	N. rustica	0.79 (0.37-1.16)	10.37 (6.97-22.71)	0.19	0.99	1.04±0.21
	Deltamethrin	0.93 (0.28-1.33)	3.83 (2.53-13.31)	7.58	0.00	2.08±0.24

** Lethal concentrations (LC) are indicated with 95% confidence limits (CLs). Lethal concentrations of plant extracts g/L, while Lethal concentrations of deltamethrin ppm.

percent mortality (47.00, 47.00, 55.00, 62.00, 70.00, and 75.00%) with all concentrations against *T. castaneum*. Among the plant species extracts, overall mean percent mortality was significantly higher (37.00, 47.00, 55.00, 65.00, 73, and 86.00%) with all concentrations of *N. rustica,* and the lowest mean percent mortality (27.00, 34.00, 38.00, 46.00, 56.00, and 63.00%) was recorded with all concentrations of *A. galanga* against *T. castaneum* as shown in Fig. 2c) (*df* = 25, *F* = 1.31, *P* = 0.1735) after a 72-hour exposure period.

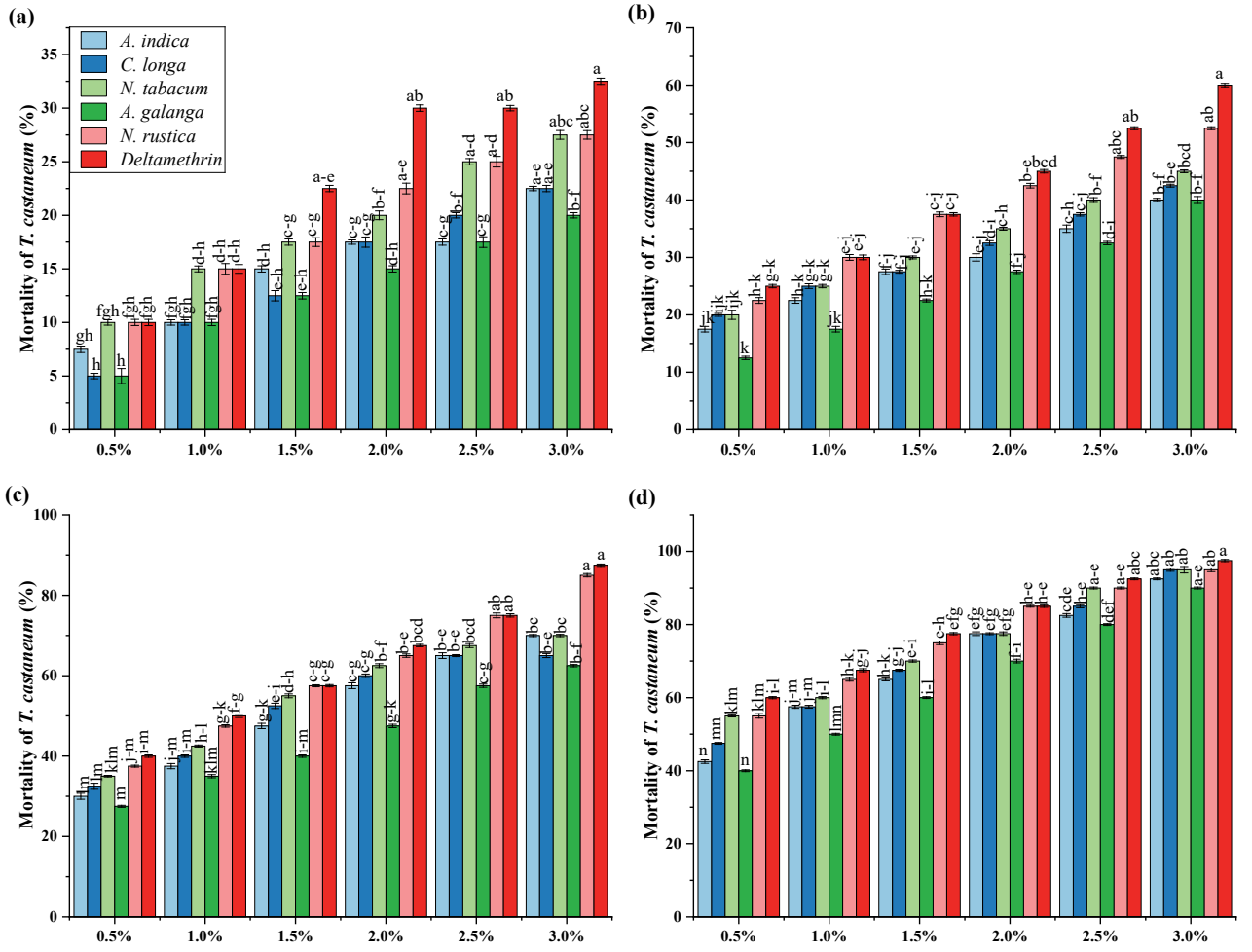

Fig. 2. Mean percent mortality of *T. castaneum* treated with plant extracts and deltamethrin after a) 24h, b) 48h, c), 72h, and d), 96 h exposure (residual effect). The bars with different lowercase letters indicate that the means are significantly different from each other at *p*=0.05.

From Table 4**,** it was observed that deltamethrin showed the highest toxicity effect with the least LC_{50} of 0.93 ppm and LC_{90} of 3.83 ppm. Among the plant species extracts, the highest toxicity was observed with *N. rustica,* having a minimum LC_{50} of 0.79 g/L and an LC_{90} of 10.37 g/L. The second most effective plant species extract was *N. tabacum,* having an LC_{50} of 1.18 g/L and an LC_{90} of 9.02 g/L against *T. castaneum.* After a 96-hour exposure period, deltamethrin showed significantly maximum mean percent mortality (60.00, 70.00, 77.00, 86.00, 92.00, and 98.00%) with all concentrations against *T. castaneum*. On the other hand, among the plant species extracts, *N. rustica* exhibited significantly higher mean percent mortality (55.00, 65.00, 76.00, 83.00, 89.00, and 94.00%) with all concentrations and lower mean percent mortality (40.00, 51.00, 60.00, 72.00, 80.00, and 88.00%) was seen with all concentrations of *A. galanga* against *T. castaneum,* as shown in Fig. 2d) ($df = 25$, $F = 1.36$, $P = 0.1440$).

Discussion

The results of our study demonstrate that the detected phytochemicals phenols, alkaloids, terpenes, phytosterols, flavonoids, and saponins play a crucial role in the insecticidal activity observed against *T. castaneum*, a major pest in stored grains. These phytochemicals have been widely recognized for their bioactive properties, contributing to their toxicity against various insect species. Phenols, recognized for their antioxidant properties, can induce oxidative stress in insects by damaging cellular components, leading to metabolic disturbances and eventual mortality [41]. Alkaloids, which are potent neurotoxic compounds, act by interfering with the insect's central nervous system. By blocking neurotransmission or inhibiting acetylcholinesterase, alkaloids can cause paralysis and death in pests like *T. castaneum* [42]. Terpenes, particularly monoterpenes and sesquiterpenes, have been shown to affect the respiratory system or disrupt feeding behavior, contributing to their insecticidal activity through both fumigant and repellent effects [43]. Phytosterols disrupt hormonal regulation in insects and inhibit ecdysteroid synthesis, preventing molting and leading to developmental abnormalities in *T. castaneum* [44]. Flavonoids act as toxicants by disrupting cellular membranes and inhibiting metabolic enzymes, leading to reduced feeding and eventual pest death [44]. Saponins, with their surfactant properties, disrupt insect cell membranes, causing leakage of cellular contents and dehydration, which contributes to the insecticidal activity against pests like *T. castaneum* [45, 46].

Tables 3 and 4 showed the percentage mortality of *T. castaneum* adults in contact and residual toxicity tests after exposure to different concentrations of plant extracts for 24, 48, 72, and 96 h. Of the five plant species extracts, *N. tabacum*, *N. rustica, C. longa,* and *A. indica* demonstrated the most encouraging results in terms of high mortality. This may be due to the neurotoxicity and gastrotoxicity of *N. tabacum* [47, 48]. These variations are thought to result from both the chemical composition of plant extracts from distinct plant species and the physiological makeup of insects. Additionally, the effectiveness of the plant extracts varied depending on the exposure time.

This can be explained by the duration of exposure or is thought to be linked to the penetration ability of the active chemical compound. The efficacy of plant extracts has been reported in recent literature [49, 51], which is in line with the current study's findings. The percentage mortality of red flour beetle was directly related to the increase in the concentration of *C. longa* extracts. These results are in line with [52], who also reported an increase in the mortality rate of *T. castaneum* with increased concentration and exposure time. Similarly, [53], in their study, showed the repellency effect of turmeric (*Curcuma longa*) extracts on *Oryzaephilus surinamensis, T. castaneum, Sitophilus oryzae, Corcyra cephalonica,* and *Cryptolestes ferrugineus.* [54] Investigated the toxicity of essential oil in turmeric leaf extracts in contact bioassay and as a fumigant on *Sitophilus oryzae*, *T. castaneum*, and *Rhyzopertha dominica's*, eggs, juveniles, and the rate of adult oviposition. Adults of *Rhyzopertha dominica* were found to be significantly susceptible to contact activity. Moreover, in *T. castaneum,* exposure to the essential oils caused a significant decline of 72 and 80% in oviposition and egg hatching, respectively. The various chemicals isolated from turmeric powder also demonstrated a repellent effect on *T. castaneum* [55].

Neem seed extracts demonstrated a diverse effect on *T. castaneum.* The mortality rate of the red flour beetle increased with the increase in concentration of neem seed extract as well as exposure time, and experimental concentrations exhibited a high insecticidal effect compared to the control. Maximum mortality was reported when the pest was exposed to the treatments for 96 h. Many recent studies confirm the effectiveness of plant derivatives against stored grain pests. For instance, [56] reported that azadirachtin found in Neem extracts was more noxious for *T. castaneum* than control. As per their findings, the number of F1 adults declined significantly with all the test doses. Likewise, [57] tested neem extracts at 50, 100, and 200 ppm against *T. confusum*, *Sitophilus oryzae* (L.), and *Rhyzopertha dominica* (F.). Their findings showed an increase in mortality of the pests with an increase in dose and duration of exposure. *A. galangal* plant extract exhibited a significantly high insecticidal effect on *T. castaneum*. This may be attributed to the occurrence of 1,8-cineol in a high percentage, which also has a strong antifeeding action.

The research done by [58] stated that ethyl acetate and methanol extracts of *Annona squamosa* seeds caused mortalities to *T. castaneum,* and also, the seeds and fruits of *Lawsonia inermis* showed a mortality effect

[59]. Other plant species extracts, including the stem and leaves of *Launaea arborescens,* caused toxicity effects on *T. castaneum*. The results of other researchers findings [59] show that leaf extracts of *Ricinus communis, Datura stramonium, Azadirachta indica,* and *Annona reticulata* showed 60.0, 90.0, and 96.6%; 60.37 and 88.4%; 95.83%; 36.67, 100%; 33.3, 53.71%; and 33.3, 70.0% toxicities to the sixth instar after 24, 48, and 72 h, respectively, and according to the findings of [59], that seed extracts of *Raphanus raphanistrum, Ajuga iva, Aristolochia baetica,* and *Peganum harmala* showed 26, 31, 34, and 58% toxicity effects against *T. castaneum*. In the current study, when we compared the contact and residual toxicity results, *N. rustica* (77.50 and 73.74%) and *N. tabacum* (74.58 and 70.83%) showed the highest toxicity effect against *T. castaneum*. To be effective at its intended site, a biopesticide must enter the insect by one or more absorption pathways, such as the cuticle, orally by eating treated food or by breathing through the spiracles. Insect larvae are negatively impacted by active phytochemicals in phytoextracts because they can cause physical flooding of the tracheal system, chemical toxicity, interference with surface forces, or a combination of these [60]. Therefore, the active ingredients alkaloids, i.e., nicotine in Nicotiana species leaf extracts, affect *T. castaneum* once inside the tissue and cells.

In the current study, deltamethrin exhibited high efficacy against *T. castaneum* (LC_{50} (0.88, 0.93%) and LC_{90} (4.74, 3.83%)) in contact as well as residual toxicity tests after 96 h of exposure. The neurotoxic action of deltamethrin on insects is caused by the disruption of nerve impulse axonal transmission by altering the ion permeability of nerve membranes [60]. The knockdown effect of deltamethrin is well-known against a variety of insect pests, including coleopterans [61]. A similar effect of higher concentrations of deltamethrin in terms of high mortality in *T. castaneum* adults was observed in our investigation.

Consumers still perceive any infestation of packaged food as unacceptable. The current study revealed that although the insect infestation of stored grains treated with plant extracts has significantly decreased, whether using plant extracts will completely prevent infestation needs to be addressed. In general, the efficacy of plant extracts against *T. castaneum* depends on a variety of factors, such as treatment doses of the plant extract, application methods, and the insect's life cycle stage, etc. More research is required to establish the ideal formulation, dosage, method of administration, impact of environmental conditions, and content of plant extracts to use them effectively to prevent *T. castaneum* infection in stored items.

Conclusions

As the adverse effects of synthetic chemicals on the environment and human health continue to escalate, it is imperative to seek alternatives that can provide equivalent or superior results to those of synthetic chemicals. Recent studies showed the potential of using aqueous plant species extracts as an effective and sustainable alternative to synthetic insecticides for managing *T. castaneum*, a significant pest of stored grains in tropical and subtropical regions. The study evaluated the toxicity of aqueous extracts from five plant species: rhizome of *A. galanga,* seeds of *A. indica,* roots of *C. longa,* leaves of both *N. tabacum* and *N. rustica,* and deltamethrin. The results revealed that as the treatment concentrations increased, the mortality of *T. castaneum* also significantly increased. In the contact toxicity effect, deltamethrin showed a maximum toxicity effect, having an LC_{50} of 0.88 ppm and an LC_{90} of 4.74 ppm. Among the plant species aqueous extracts, *N. rustica* exhibited the highest residual effect, having the lowest LC_{50} of 1.00 g/L and an LC_{90} of 5.38 g/L, followed by *N. tabacum,* having an LC_{50} of 1.09 g/L and an LC_{90} of 6.50 g/L; *C. long*, an LC_{50} of 1.33 g/L and an LC_{90} of 6.89 g/L; and *A. indica*, an LC_{50} of 1.46 g/L and an LC_{90} of 7.43 g/L after a 96-hour exposure period. In residual toxicity tests, deltamethrin again showed the lowest LC_{50} of 0.93 ppm and LC_{90} of 3.83 ppm, indicating its strong residual effect. Among the plant species extracts, *N. rustica* had the highest residual toxicity LC_{50} of 0.79 g/L and LC_{90} of 10.37 g/L, followed by *N. tabacum* with an LC_{50} of 1.18 g/L and LC_{90} of 9.02 g/L and *A. indica* with an LC_{50} of 0.79 g/L and LC_{90} of 10.37 g/L against *T. castaneum*. Incorporating these plant extracts alongside synthetics can reduce reliance on chemicals, minimize environmental impacts, and potentially mitigate resistance issues. This integrated approach fosters a more balanced and sustainable pest management strategy, benefiting both crop protection and the environment.

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Conflict of Interest

The authors declare no conflicts of interest.

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