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RESEARCH

Insecticidal and Repellent Effects of Selected Botanicals against *Tribolium Castaneum* (Herbst) (Coleoptera: Tenebrionidae) with Reference To their Effect on Detoxifying Enzymes

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Abstract

The excessive application of various synthetic pesticides led to control difficulties, including insect resistance and environmental contamination. This study aimed to evaluate the insecticidal and repellent activities of twelve botanical powders and aqueous extracts against *Tribolium castaneum*, with a focus on acetylcholinesterase and glutathione-S-transferase detoxification enzyme activities. Toxicity tests revealed that *Azadirachta indica* dry powder was the most harmful, having the lowest LD_{50} value of 2.09% w/w, while in aqueous extract *A. indica* was the most toxic, with an LC_{50} of 2.20% after 24 h. Repellency tests demonstrated that *A. indica* exhibited the highest repellent effect in both powder and aqueous forms (86.66%). As a result of the most effective botanical application, biochemical analyses showed that acetylcholinesterase activity remained highest (6.17±0.17 U/min/g) in *A. indica*-treated insects, whereas glutathione-S-transferase enzyme activity peaked in response to *Eucalyptus tereticornis* (85.00 U/min/g), indicating a strong physiological defense response. These results indicate that plants like *A. indica*, *D. stramonium*, *E. tereticornis*, and *Ar. nilagirica* can be used as promising bio-insecticides options for controlling *T. castaneum* in stored products.

Keywords Stored product pest · Tribolium castaneum · Botanical extracts · Management · Coleoptera · Enzymes

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Introduction

Beetles are among the most predominant insect groups, comprising approximately 400,000 recognized species, about 25% of all known animal species [1, 2]. The flour beetles, *Tribolium castaneum* and *T. confusumn*, are a major threat to stored grain products, causing both quantitative and qualitative losses. Its presence results in contamination with feces, cast skins, and secreted quinones, which degrade food quality and may pose health risks to consumers. Infested products often suffer from reduced nutritional value and unpleasant odor and are rendered unfit for human consumption or trade. The cosmopolitan distribution, small size, short life cycle, and synanthropic nature of these pests have led to significant losses in the stored product industries [3–5].

Although synthetic pesticides have been effective in controlling pests since their widespread use in the mid-20th century, their extensive use, both on crop pests and in controlling stored grain pests, has led to bioaccumulation and the development of pest resistance [5–7]. In the early 1952, the production of synthetic pesticides was started in India, with 76% of the products targeting insects [8]. Today, a wide variety of chemical pesticides are produced worldwide for multiple purposes, such as enhancing crop productivity, preventing postharvest losses, controlling disease vectors, and maintaining food quality [9]. However, the overzealous application of these compounds has led to many hazards, such as the direct effect on human health [10, 11]The impact through food merchandise [12], the environmental contamination [13, 14], and the influence on the non–target organisms [15, 16]. Therefore, there is a direction to follow other alternative methods to control insect pests other than the insecticidal/acaricidal applications, such as biological control and inter–crop pest management [17–20].

Botanical insecticides offer an eco-friendly alternative to synthetic chemical control. These insecticides are derived from plant compounds such as alkaloids, terpenoids, flavonoids, and essential oils, and they are known for breaking down easily, being relatively harmless to mammals, and specifically targeting pests. Historically, civilizations such as ancient Egypt, China, Greece, and India used plant-based substances to control insect pests as early as 2,000 years ago [21, 22], Over 150 years ago in Europe and North America, have applied such plant derivatives to manage the attacks of different insects. There are four main types of plant-based insecticides: rotenone, neem, pyrethrum, and essential oils, with less common use of nicotine, sabadilla, and ryania [23].

One of the universal problems regarding the application of chemical control agents against various pests is the development of resistance, which is mainly related to the increase of different detoxifying enzyme activities inside the insect's body [24–27]. These enzymes include acetylcholinesterase (AChE), mixed function oxidases (MFOs), glutathione–S–transferases (GSTs), carboxylases (CarEs), catalases (CAT), superoxide dismutase (SOD), and glutathione peroxidases (GPXs) [28–31]. Such resistance is basically dependent on either the increasing levels of the aforementioned enzymes or the reduction of target-site sensitivity [32, 33]. One important way insects become resistant to pesticides is by producing more detoxifying enzymes, like acetylcholinesterase (AChE), glutathione-S-transferases (GSTs), carboxylesterases, and cytochrome P450 monooxygenases, among others. Reversely, the decreasing levels of detoxifying enzymes are an indicator of the effective toxicity of the applied substance and the absence of the resistance phenomenon.

In the present study, we evaluated the insecticidal and repellent effects of the dry powder and aqueous forms from twelve plant sources belonging to different botanical families against *T. castaneum* for the first time. Additionally, we measured the levels of both acetylcholinesterase (AChE) and glutathione–S–transferases (GSTs) as a result of the application of the most effective extracts.

Materials and Methods

Plant Materials

After obtaining the selected plants, they were thoroughly washed with distilled water and dried in the shade at room temperature (25–30 °C) for 7–12 days until they reached a constant weight. The dried plant parts were then ground using an electric grinder to obtain a fine powder and stored in airtight glass bottles at room temperature until next use. Dry powder and aqueous extracts of twelve plant sources, viz., *Aegle marmelos*, *Annona reticulata*, *Artemisia nilagirica*, *Azadirachta indica*, *Catharanthus roseus*, *Clausena heptaphylla*, *Datura stramonium*, *Eucalyptus tereticornis*, *Heteropanax fragrans*, *Lawsonia inermis*, *Matteuccia struthiopteris*, and *Vitex negundo*, were selected to evaluate their toxicological and repellent effects on the red flour beetle, *Tribolium castaneum* (Herbst). Details of the plants are shown in Table 1.

Insect Culture

Tribolium castaneum adults were collected from highly infested harvested crops. Rearing of the insects was performed according to Abbasipour, Rastegar, Mahmoudvand and Hosseinpour [34]. Briefly, the fresh wheat seeds were loaded into eight different containers (2 No's 29 cm×12 cm, 2 No's 32 cm×13.50, 2 No's 25 cm×12 cm, 2 No's 22 cm×11

Table 1 Details of the plant sources used in this study

Local name	Binomial name	Family	Collection site	Geographical coordinates	Part used	Extraction type
Indian Wormwood (Nilum)	*Artemisia nilagirica*	Asteraceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Madagascar periwinkle (Noyontora)	*Catharanthus roseus*	Apocynaceae	Bidyapur village, Assam, India	26° 24′ 15.912″ N; 90° 27′ 7.272″ E	Leaves	Dry/Aqueous
Ostrich Fern (Dhekia)	*Matteuccia struthiopteris*	Onocleaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Kondakarivepaku (Telugu)	*Clausena heptaphylla*	Rutaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Thorn apple (Dhutura)	*Datura stramonium*	Solanaceae	Bidyapur village, Assam, India	26° 24′ 15.912″ N; 90° 27′ 7.272″ E	Leaves	Dry/Aqueous
Custard apple (Aatloch Kothal)	*Annona reticulata*	Annonaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Chinese chaste (Pochotia)	*Vitex negundo*	Lamiaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Mehndi (Jetuka)	*Lawsonia inermis*	Lythraceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Red gum tree (Tailapatra)	*Eucalyptus tereticornis*	Myrtaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Wood apple (Bael)	*Aegle marmelos*	Rutaceae	Bidyapur village, Assam, India	26° 24′ 15.912″ N; 90° 27′ 7.272″ E	Leaves	Dry/Aqueous
Fragrant Aralia (Kecheru)	*Heteropanax fragrans*	Araliaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous
Bastard tree (Neem)	*Azadiracta indica*	Meliaceae	Jorhat, Assam, India	26° 45′ 24.984″ N; 94° 14′ 37.536″ E	Leaves	Dry/Aqueous

cm), and the insects were allowed to feed on the seeds separately under 27–30 °C and 80% RH.

Preparation of Plant Concentrations

For the insecticidal and repellent bioassays, seven concentrations of each botanical powder were prepared: 0.25, 0.50, 0.75, 1.00, 1.25, 1.75, and 2.50 g. These were thoroughly mixed with 25 g of clean, uninfested wheat grains in separate glass containers to yield final concentrations of 1, 2, 3, 4, 5, 7, and 10% (w/w), respectively. The mixing was done manually by shaking and rotating the containers for 3–5 min to ensure uniform coating and distribution of the powders over the grain surface. Treated grains were then transferred to test containers before the introduction of *T. castaneum* adults. Untreated grains served as controls.

Dry Powder Extracts and Bioassay

Leaves were collected and rinsed thoroughly to remove any contamination and then allowed to dry. Dry powder extracts were obtained and sieved by a powder grinding machine (Luohe Xingdian Electromechanical Equipment Co. Ltd.). Each botanical powder of 0.25, 0.50, 0.75, 1.00, 1.25, 1.75 and 2.50 g per 25 g seed was thoroughly mixed with corresponding to 1, 2, 3, 4, 5, 7 and 10% (w/w) [35, 36]. The treated seeds were kept with three replications for each treatment in plastic containers (7 cm × 6 cm) at room temperature, and 20 adults were released in each replication. Mortalities were recorded after 24, 48, and 72 h posttreatment, and were subjected to probit analysis to find out LD_{50} value (w/w). *A. indica* was considered as standard to evaluate the relative toxicity of selected botanicals.

Aqueous Extracts and Bioassay

Fifty grams dry powder from each plant was mixed with 500 ml distilled water and kept overnight. Filtration of the material was performed with filter paper to get 10% stock solution and kept it at 4 °C until use [37]. Stock solution was diluted to obtain the desired concentrations viz. 0.5, 1, 2, 3, 4 and 5% in distilled water. The dry film residue method was used to test mortality according to Gupta and Rawlins [38]. Briefly, thin uniform film of botanicals was prepared by taking 1 ml of botanical solution in a petri dish (9 cm), then rotated till dryness. Toxicity was observed after 24, 48, and 72 h against adult insects in four replications for each treatment, with 20 adults in each replication. After six h of exposure, the exposed adults were transferred to petri dish with 50 gm of seeds. Relative toxicity was calculated by taking LC_{50} value of *A. indica* as unity because neem is an effective, popular, and recommended botanical against various insect pests [39].

Repellency Test

Nine cm diameter petri dish was divided into three parts viz. treated (with 0.3 gm of dry powder from each plant were mixed with 3gm of wheat seed (10% w/w), untreated and without grain [40, 41] (Fig. 1). In case of aqueous extract, 3 g of wheat grain are placed in each treated chamber of petri dish and applied with 0.2 ml of 5% solution of each plant extract. Ten insects were released at the central portion of each petri dish and covered by a cover plate. Then the number of insects on each chamber was counted at hourly intervals up to sixth hour. The per cent repellency was calculated by following formula:

$$\mathrm{PR}(\%) = [(\mathrm{Nc} - \mathrm{Nt})/(\mathrm{Nc} + \mathrm{Nt})] \times 100.$$

Where: Nc = the number of weevils present in the treated petri dish. Nt = the number of weevils present in the control petri dish.

Results were analyzed by using One–way ANOVA. The average values of % repellency was then categorized according to McDonald, Guy and Speirs [42] into five classes namely 0.1–20 (I), 20.1–40 (II), 40.1–60 (III), 60.1–80 (IV), and 80.1–100 (V).

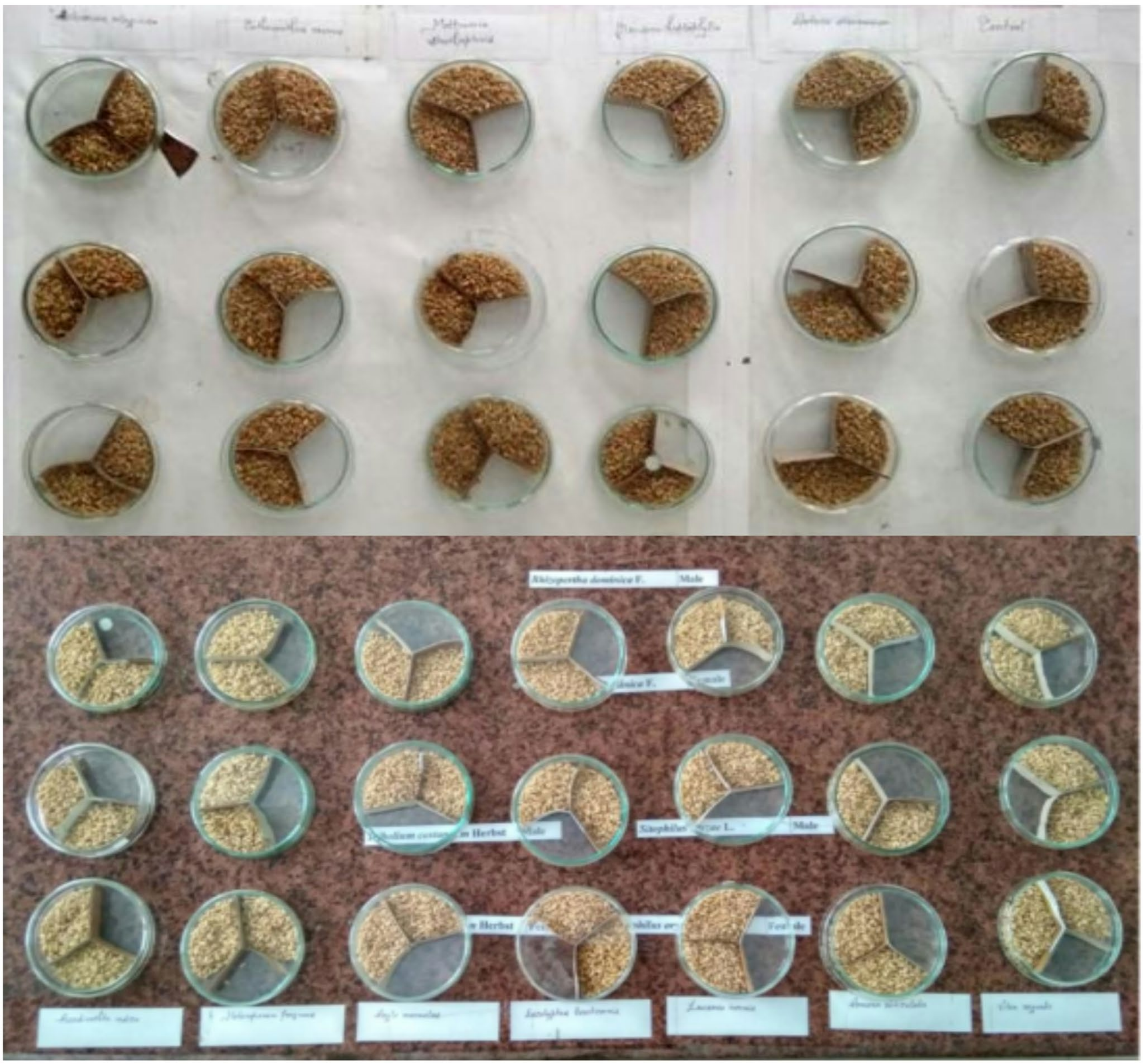

Fig. 1 Laboratory replications showing the repellency test

Enzyme Activity Assays

Acetylcholinesterase (AChE)

Adults of the treated *T. castaneum* were dissected in ice–cold Bodenstein insect ringer solution (Otto Chemie Pvt. Ltd., Mumbai, India) and their head portions were excised out. Approximately 20% (W/V) homogenates of the dissected tissues were prepared using Potter–Elvehjam tissue homogenizer (Thomas Scientific, USA) supported by Teflon coated pestle under cold condition (8 ± 1 °C). The homogenates were kept at 8 ± 1 °C for 30 min with intermittent stirring and centrifuged at 20,000 xg for 20 min. The supernatant was used as enzyme source [43].

The enzyme activity was estimated according to [44]. Briefly, 5 ml of the substrate (1.33 mM acetylcholine chloride solution) was added to 0.4 ml crude extract, incubated at 37 °C for 30 min. The reaction was terminated by the addition of 1 ml alkaline hydroxyl amine solution (prepared by mixing equal volume of 2.5 N NaOH and 1 N hydroxyl amine solution). One ml citrate buffer (1 M, pH 1.4) was added, followed by 2 ml ferric solution (0.7 M) and mixed with distilled water to 10 ml. The tubes left for 20 min at room temperature, followed by a filtration of the contents. Optical densities were recorded at 490 nm, and One unit of acetylcholinesterase activity (total and specific) is defined as one micro mole of acetyl choline hydrolyzed minute / insect head or per mg protein.

Glutathione–S–transferase (GST)

GST activity was assayed using spectrophotometer (Aquadax South Asia Pvt. Ltd., Bhubaneswar, India) at 25 °C with substrates of reduced glutathione (GSH) and 1–chloro–2, 4–dinitrobenzene (CDNB). For each assay, one ml of enzyme cocktail (980 µl PBS pH 6.5, 10 µl of 100 mM CDNB and 10 µl of 100 mM GSH) was mixed, followed by removal of 100 µl of the cocktail and the remaining 900 µl was placed in 1.5 ml cuvette. In zero spectrophotometer, one ml of distilled water was used. The blank cuvette was provided by 100 µl PBS to 900 µl of cocktail and measured absorbance at 340 nm for 3 min. In the test cuvette, 100 µl of sample to 900 µl cocktail, mixed and measured absorbance at 340 nm. GST activity was calculated using the equation:

$$\text{GST activity} = [(\text{Adjusted } \Delta 340/\min)/0.0096\ \mu\text{M}^{-1}/\text{cm}] \times (1.0\ \text{ml}/0.1\ \text{ml}) \times \text{any sample dilution} = \text{U/ml s.}$$

Statistical Analysis

The obtained data were analyzed using MedCalc statistical software v. 19.2.6 (MedCalc Software Ltd., Ostend, Belgium) based on the probit analysis [45]. It was used to do probit analyses to find the lethal concentration (LC) values and the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significance levels were set at $P < 0.05$.

Results

Efficacy of Dry Powders Extracts

The LD_{50} (w/w) values and relative toxicity levels of *Ae. marmelos*, *An. reticulata*, *Ar. nilagirica*, *A. indica*, *Ca. roseus*, *Cl. heptaphylla*, *D. stramonium*, *E. tereticornis*, *H. fragrans*, *L. inermis*, *M. struthiopteris*, and *V. negundo* after being exposed for 24 h ranged from 2.09% to 19.40%, with relative toxicity levels between 0.08 and 1.0. After 48 h, the values were between 2.01% and 14.64%, with relative toxicity levels from 0.14 to 1.0, and after 72 h, they were between 1.49% and 4.58%, with relative toxicity levels from 0.32 to 1.0 (Table 2; Fig. 2). The data showed that *A. indica* is very toxic, while *M. struthiopteris* is not very toxic after 24 h of exposure.

Efficacy of Aqueous Extracts

Ae. marmelos, *An. reticulata*, *Ar. nilagirica*, *A. indica*, *Ca. roseus*, *Cl. heptaphylla*, *D. stramonium*, *E. tereticornis*, *H. fragrans*, *L. inermis*, *M. struthiopteris*, and *V. negundo* had LC_{50} values ranging from 2.20 to 16.63% and 1.48–3.93%, while 1.36–2.88% after 24, 48, and 72 h of exposure, respectively (Table 3; Fig. 3).

Repellency Effect of Dry Powders and Aqueous Extracts against *Tribolium Castaneum*

The repellency rates of the dry powder extracts were observed highest in *A. indica* (86.66%), followed by *D. Stramonium* (73.33%), *E. tereticornis* (73.33%), *Ar. nilagirica* (66.66%), *Ae. marmelos* (66.66%) and *Cl. heptaphylla* (66.66%), *H. fragrans* (53.33%), *L. inermis* (53.33%), *Ca. roseus* (46.66%), and *An. reticulata* (40.00%) showed comparatively less repellency rate, while *V. negundo* (33.33%) and *M. struthiopteris* (26.66%) gave significantly less repellency rate. In comparison, the highest repellency rate of the aqueous extracts was observed in *A. indica* (86.66%), followed by *D. stramonium* (73.33%), *E. tereticornis* (73.33%), *Ar. nilagirica* (66.66%), and *Cl. heptaphylla* (66.66%) after 1 h of treatment. The lowest repellency rate was found in *V. negundo* (26.66%), followed by *Ae. marmelos* (33.33%), *An. reticulata* (33.33%), *M. struthiopteris* (33.33), *H. fragrans* (46.66%), and *L. inermis* (46.66%) (Table 4).

Table 2 Toxicity and relative toxicity of different dry powder extracts used against *Tribolium castaneum*

Plant species	Interval time (h)	Regression equation	Heterogeneity	LD_{50}% (w/w)	Fiducial limits L.B.	Fiducial limits U.B.	Slope ± SE	X^2	Relative toxicity	Order of toxicity
Aegle marmelos	24	Y=0.19+0.21X	11.43	7.99	4.57	57.20	2.04±0.63	1.29	0.26	V
	48	Y=0.18+0.38X	11.21	3.06	2.17	4.08	1.76±0.41	1.37	0.55	V
	72	Y=0.15+0.37X	12.07	2.51	1.65	3.35	1.42±0.66	1.78	0.59	VI
Annona reticulata	24	Y=0.21+0.22X	12.42	8.59	5.09	55.09	1.48±0.46	1.36	0.24	VII
	48	Y=0.15+0.27X	7.88	3.64	2.31	5.40	1.26±0.96	2.18	0.46	VII
	72	Y=0.19+0.41X	14.82	2.85	2.05	3.68	1.13±0.14	1.18	0.52	IX
Artemisia nilagirica	24	Y=0.11+0.20X	23.35	3.85	1.87	9.13	1.62±0.62	1.48	0.54	IV
	48	Y=0.11+0.23X	13.39	2.95	1.37	4.80	1.42±0.85	1.36	0.57	IV
	72	Y=0.10+0.40X	12.90	1.80	1.06	2.45	1.14±0.95	1.48	0.82	III
Azadirachta indica	24	Y=0.15+0.48X	25.52	2.09	1.46	2.66	1.36±0.75	1.18	1.00	I
	48	Y=0.18+0.80X	84.36	1.71	1.02	2.31	1.15±0.36	1.37	1.00	I
	72	Y=0.18+1.0X	75.49	1.49	1.03	1.91	0.92±0.75	1.29	1.00	I
Catharanthus roseus	24	Y=0.26+0.28X	7.56	8.15	5.33	22.87	1.15±0.26	1.37	0.25	VI
	48	Y=0.14+0.25X	8.25	3.82	2.33	6.61	1.01±0.921	1.66	0.44	VIII
	72	Y=0.15+0.24X	16.29	2.71	1.74	3.70	0.72±0.21	1.18	0.54	VII
Clausena heptaphylla	24	Y=0.24+0.25X	13.74	9.05	5.53	39.74	1.86±0.41	1.69	0.23	VIII
	48	Y=0.13+0.25X	6.51	3.48	2.00	5.76	1.46±0.58	1.37	0.49	VI
	72	Y=0.16+0.89X	11.10	4.44	2.78	8.94	1.30±0.48	2.16	0.33	X
Datura stramonium	24	Y=0.14+0.31X	18.54	2.97	1.85	4.22	1.91±0.59	1.55	0.70	IIIII
	48	Y=0.07+0.35X	20.30	2.01	1.34	2.57	1.64±0.55	1.62	0.85	II
	72	Y=0.16+0.89X	66.57	1.52	1.00	1.98	1.37±0.93	1.84	0.98	II
Eucalyptus tereticornis	24	Y=0.13+0.29X	26.63	2.81	1.65	4.05	1.29±0.18	1.39	0.74	II
	48	Y=0.09+0.34X	34.57	2.49	1.72	3.23	1.11±0.52	1.67	0.68	III
	72	Y=0.22+0.72X	44.35	2.02	1.45	2.53	1.00±0.48	1.27	0.73	IV
Heteropanax fragrans	24	Y=0.27+0.28X	18.32	9.27	5.89	29.63	1.96±0.84	1.95	0.22	IX
	48	Y=0.14+0.16X	18.54	7.44	4.33	74.01	1.68±0.55	1.28	0.22	X
	72	Y=0.17+0.26X	7.072	4.58	3.02	8.94	1.09±0.52	1.92	0.32	XII
Lawsonia inermis	24	Y=0.32+0.30X	9.18	11.67	7.16	40.81	2.02±0.65	1.71	0.12	X
	48	Y=0.19+0.21X	8.48	7.89	4.70	50.22	1.81±0.19	1.39	0.21	XI
	72	Y=0.09+0.26X	11.76	2.29	1.01	3.43	1.26±0.95	1.91	0.65	V
Matteuccia struthiopteris	24	Y=0.27+0.28X	7.73	19.40	592	31.48	1.36±0.18	1.81	0.08	XII
	48	Y=0.19+0.21X	15.67	14.64	9.20	39.41	1.49±0.39	1.67	0.14	XII
	72	Y=0.11+0.18X	12.45	4.45	2.15	20.83	1.35±0.18	1.25	0.33	XI
Vitex negundo	24	Y=0.25+0.23X	13.34	11.72	6.55	95.41	1.94±0.19	1.39	0.17	XI
	48	Y=0.17+0.29X	10.89	3.94	2.64	6.19	1.35±0.99	1.75	0.43	IX
	72	Y=0.13+0.29X	14.41	2.80	1.62	4.07	1.15±0.52	1.37	0.53	VIII

The data were found to be significantly heterogeneous at =0.05

Y=Probit kill, X=log dose

Mortality based on 4 replications each with 20 individuals

Enzymatic Activity

Acetylcholinesterase (AChE)

All the botanicals significantly reduced the activity of acetylcholinesterase (AChE) in comparison to control. *E. tereticornis* showed significantly the highest reduction of AChE activity (1.76±0.21U/min./g insect), whereas *A. indica* was the least (6.17±0.17 U/min./g insect) (Table 5). The decrease in AChE activity indicates that the plant extracts affect the insect's nervous system, leading to a disturbance in nerve transmission and ultimately death.

Glutathione–S–Transferase (GST)

It was observed that the activity of glutathione–s–transferase was significantly lowest in *A. indica* (11.77 U/min./g insect), whereas the highest activity was recorded in the case of *E. tereticornis* (85.00 and 84.02 U/min./g insect) (Table 5). GST enzyme activity shows how well the insect

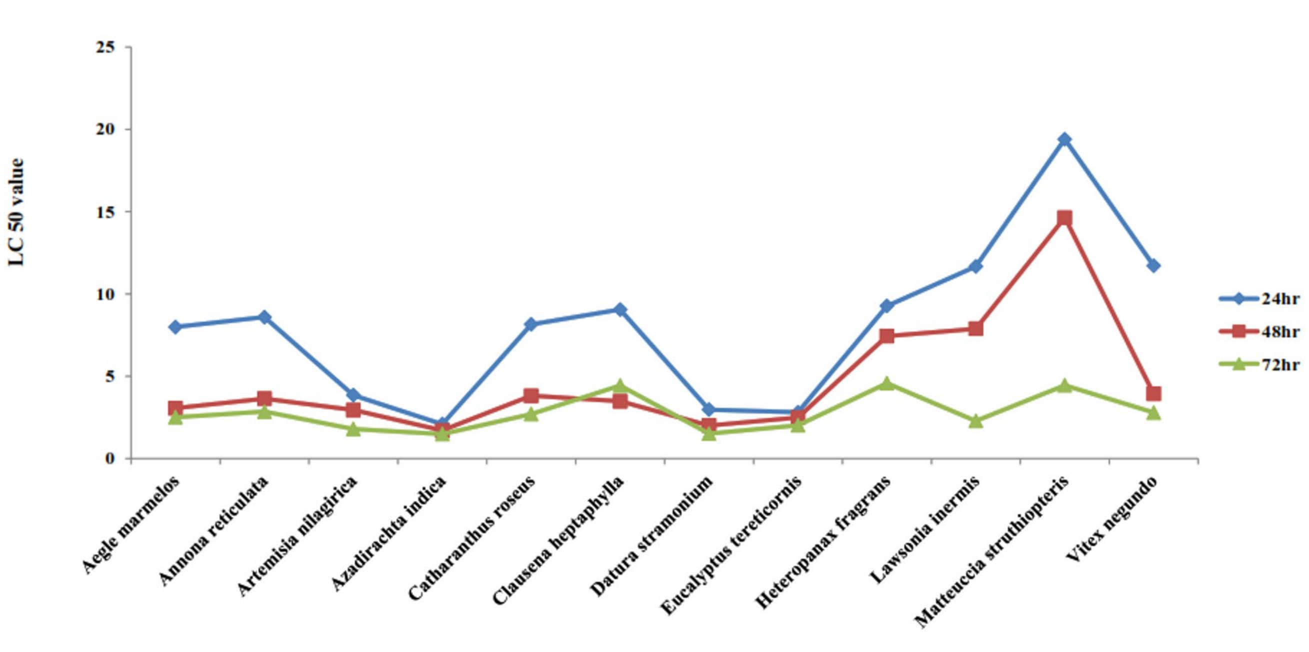

Fig. 2 LD_{50} and relative toxicity of plant powder extracts against *Tribolium castaneum* at different time intervals

can handle oxidative stress from the active compounds in the extracts; a drop in this activity means the insect is less able to detoxify.

Discussion

In addition to diseases and weeds, arthropods are considered a main threat that cause an estimated loss of 35% of the entire agricultural products, and without proper pest control it may exceed up to 50% of yield loss [46]. The increasing application of various synthetic pesticides has several drawbacks, including human and environmental issues and the development of resistance to chemicals in insect populations [47, 48]. Apparently, plant extracts are always excellent alternative to avoid all the side effects of synthetic chemicals as they were being used in the practices of agricultural pest control in ancient China, Greece, and India over two thousand years ago From stream to land: Ecosystem services provided by stream insects to agriculture [46]. Globally, there are more than 2,500 plants of 235 different families that have various biological activities against arthropods [49, 50], as they are rich by their composition of alkaloids, terpenoids, phenolic compounds, among other compounds, which have insecticidal properties and show a promising effect on insect pests.

The comparison of the twelve plants tested against *Tribolium castaneum* showed a lot of differences in how well they worked as insecticides and repellents. In the current study, *A. indica*, *D. stramonium*, *E. tereticornis* and *Ar. nilagirica* showed the highest efficacy in comparison to other tested botanicals. *A. indica* leaf powder indicated the highest toxic effects against *T. castaneum*. In context to the earlier works, a higher effectiveness of *A. indica* leaf powder and aqueous extracts against the maize weevil, *Sitophilus zeamais*, the cowpea seed beetle, *Callosobruchus maculatus*, the lesser grain borer, *Rhyzopertha dominica* [51–53] and the red flour beetle, *T. castaneum* [54, 55]. The toxicity of *A. indica* against various storage pests was correlated to the presence of triterpenoid /secondary metabolites, including azadirachtin, salanin, meliantriol in the composition of such plant [56, 57] that are responsible for its antifeedant, ovicidal, larvicidal, insect growth regulatory and repellent activity [58].

Plants like *Clausena heptaphylla*, *Annona reticulata*, and *Catharanthus roseus* showed moderate effects, indicating the presence of bioactive compounds in lower concentrations or less active forms. Conversely, *Matteuccia struthiopteris*, *Vitex negundo*, and *Lawsonia inermis* showed relatively weak insecticidal or repellent action, which may be due to poor penetration, limited interaction with the insect's detoxification systems, or the absence of major active constituents [59].

The ability of the tested plants to kill or repel insects comes from various natural chemicals they contain, like alkaloids, flavonoids, terpenoids, and phenolic compounds. *Azadirachta indica* has a mix of triterpenoids, especially azadirachtin, salanin, and meliantriol, which disturb several bodily functions in insects. Azadirachtin, the main active ingredient, disrupts the hormones that control growth and changes in insects by blocking ecdysone activity, which

Table 3 Toxicity and relative toxicity of different aqueous extracts used against *Tribolium castaneum*

Plant species	Interval time (h)	Regression equation	Heterogeneity	LC_{50}%	Fiducial limits L.B.	Fiducial limits U.B.	Slope ± SE	X^2	Relative toxicity	Order of toxicity
Aegle marmelos	24	Y = 0.09 + 0.19X	11.72	3.20	1.75	18.33	1.23 ± 0.29	1.28	0.68	VI
	48	Y = 0.08 + 0.21X	10.98	2.34	1.28	5.48	1.12 ± 0.42	1.34	0.63	VI
	72	Y = 0.07 + 0.25X	10.78	1.93	1.10	3.30	1.05 ± 0.39	1.22	0.70	VI
Annona reticulata	24	Y = 0.11 + 0.19X	9.27	3.97	2.18	38.80	1.87 ± 0.32	1.78	0.55	X
	48	Y = 0.14 + 0.30X	10.82	3.12	2.12	6.03	1.45 ± 0.31	1.37	0.47	VIII
	72	Y = 0.14 + 0.52X	88.38	1.92	1.04	7.40	1.25 ± 0.37	1.29	0.70	V
Artemisia nilagirica	24	Y = 0.09 + 0.26X	9.65	2.27	1.41	4.11	1.78 ± 0.42	1.85	0.96	II
	48	Y = 0.07 + 0.30X	17.63	1.75	1.10	3.60	1.36 ± 0.23	1.37	0.84	II
	72	Y = 0.71 + 0.43X	26.77	1.45	1.03	3.89	1.02 ± 0.75	1.77	0.93	III
Azadirachta indica	24	Y = 0.07 + 0.22X	10.13	2.20	1.18	4.82	1.76 ± 0.30	1.95	1.00	I
	48	Y = 0.76 + 0.44X	16.39	1.48	1.06	7.92	1.24 ± 0.42	2.06	1.00	I
	72	Y = 0.86 + 0.49X	24.85	1.36	1.01	9.73	1.12 ± 0.24	1.56	1.00	I
Catharanthus roseus	24	Y = 0.15 + 0.30X	10.68	3.27	2.21	6.52	1.98 ± 0.42	1.32	0.67	VIII
	48	Y = 0.08 + 0.21X	10.95	2.34	1.28	5.47	1.25 ± 0.33	1.73	0.63	V
	72	Y = 0.07 + 0.25X	9.01	2.04	1.20	4.59	1.33 ± 0.65	1.53	0.66	VII
Clausena heptaphylla	24	Y = 0.09 + 0.18X	9.93	3.26	1.75	24.89	1.24 ± 0.46	1.54	0.67	VII
	48	Y = 0.08 + 0.17X	7.40	3.14	1.57	48.49	1.03 ± 0.36	1.61	0.47	X
	72	Y = 0.07 + 0.22X	8.63	2.06	1.10	4.06	0.88 ± 0.74	1.13	0.66	VIII
Datura stramonium	24	Y = 0.09 + 0.20X	16.41	2.90	1.62	11.15	1.29 ± 0.36	1.75	0.75	IV
	48	Y = 0.08 + 0.28X	6.01	2.04	1.28	7.29	1.65 ± 0.74	1.99	0.72	III
	72	Y = 0.70 + 0.44X	14.30	1.43	1.02	8.86	1.28 ± 0.65	1.61	0.95	II
Eucalyptus tereticornis	24	Y = 0.09 + 0.02X	9.65	2.27	1.41	4.11	1.95 ± 0.13	1.47	0.96	III
	48	Y = 0.16 + 0.50X	85.64	2.10	1.17	9.98	1.86 ± 0.13	1.59	0.70	IV
	72	Y = 0.07 + 0.41X	10.85	1.55	1.09	7.06	1.36 ± 0.34	1.25	0.87	IV
Heteropanax fragrans	24	Y = 0.16 + 0.23X	14.07	5.05	2.29	27.16	2.15 ± 0.75	1.29	0.43	XI
	48	Y = 0.13 + 0.21X	9.81	3.93	2.29	19.18	1.74 ± 0.65	1.95	0.37	XII
	72	Y = 0.09 + 0.23X	11.01	2.62	1.55	6.33	1.12 ± 0.33	1.25	0.51	X
Lawsonia inermis	24	Y = 0.10 + 0.20X	5.61	3.15	1.77	14.25	2.24 ± 0.24	1.65	0.69	V
	48	Y = 0.08 + 0.17X	4.71	3.14	1.57	48.69	1.76 ± 0.51	1.48	0.47	XI
	72	Y = 0.68 + 0.17X	8.79	2.45	1.05	14.51	1.35 ± 0.32	1.29	0.55	IX
Matteuccia struthiopteris	24	Y = 0.94 + 0.18X	7.39	16.63	1.77	39.98	1.58 ± 0.45	1.58	0.13	XII
	48	Y = 0.08 + 0.18X	7.08	3.08	1.50	28.28	1.34 ± 0.98	1.28	0.48	VII
	72	Y = 0.08 + 0.17X	7.08	2.88	1.40	29.88	1.21 ± 0.52	1.33	0.47	XII
Vitex negundo	24	Y = 0.11 + 0.20X	8.52	3.83	2.17	24.69	2.00 ± 0.63	1.38	0.57	IX
	48	Y = 0.08 + 0.17X	7.49	3.13	1.56	44.90	1.75 ± 0.18	1.58	0.47	IX
	72	Y = 0.07 + 0.18X	9.00	2.63	1.26	14.95	1.62 ± 0.26	1.55	0.51	XI

The data were found to be significantly heterogeneous at = 0.05

Y = Probit kill, X = log dose

Mortality based on 4 replications each with 20 individuals

impacts their ability to shed their skin and develop into the next stage. It also acts as an antifeedant and repellent by disrupting the chemoreceptors of the insect's gustatory system [60].

'*Datura stramonium* contains chemicals like atropine and scopolamine that affect the insect's nervous system by blocking acetylcholine receptors, which can cause paralysis and eventually kill the insect [61]. *Eucalyptus tereticornis* essential oils, which are high in 1,8-cineole, can harm insects by blocking an enzyme called acetylcholinesterase (AChE). This blockage leads to too much acetylcholine building up at nerve connections, which causes the insects to become overly excited and then paralyzed. In the case of *Ar. nilagirica*, its sesquiterpene lactones and volatile oils have been shown to impair mitochondrial respiration and induce oxidative stress in insect tissues [62]. These mechanisms, coupled with enzyme inhibition observed in the current study (e.g., decreased AChE and GST activities), underline the multifaceted toxicity of these botanicals, making them promising candidates for eco-friendly pest control strategy.

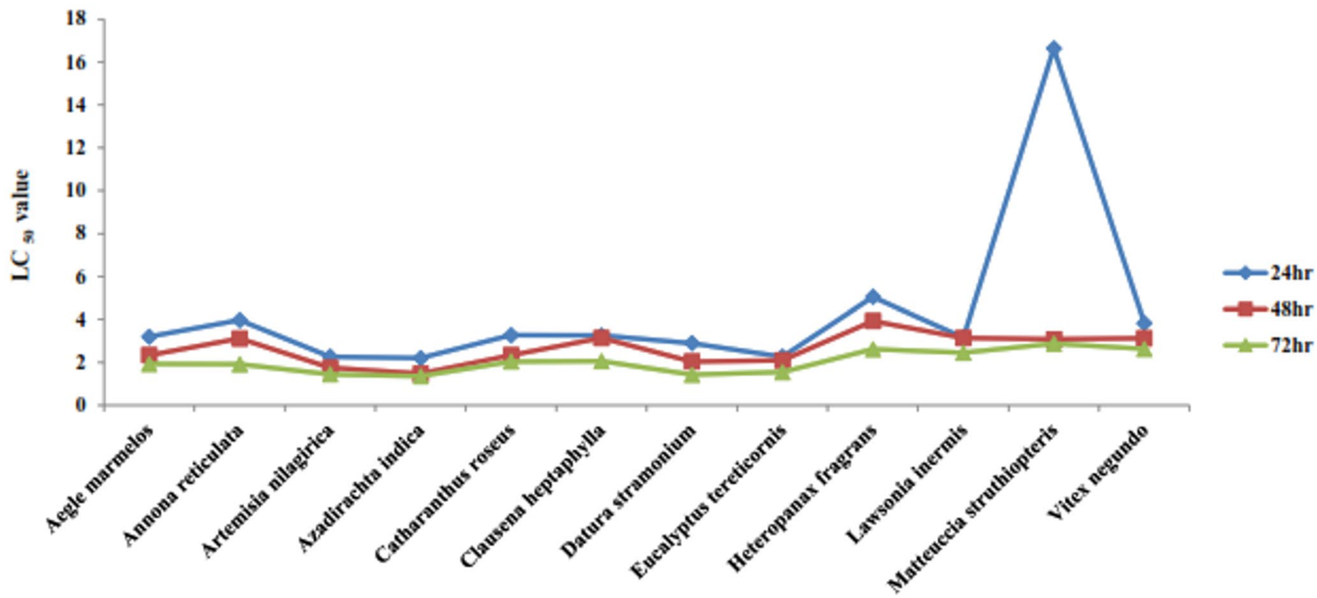

Fig. 3 LC_{50} and relative toxicity of aqueous extracts against *Tribolium castaneum* at different time intervals

Table 4 Repellency effect of different plant leaf powder against *Tribolium castaneum*

Plant species	Repellency									Mean repellency rate (%)	Repellency class
	1 h	2 h	3 h	4 h	5 h	6 h	24 h	48 h	72 h		
Aegle marmelos	66.66	40.00	60.00	73.33	27.61	20.00	40.00	33.33	26.66	43.06	III
Annona reticulata	40.00	46.66	60.00	40.00	63.33	33.33	20.00	26.66	46.66	41.84	III
Artemisia nilagirica	66.66	73.33	73.33	60.00	66.66	53.33	40.00	60.00	53.33	60.73	IV
Azadirachta indica	86.66	80.00	80.00	86.66	86.66	73.33	86.66	80.00	80.00	82.21	V
Catharanthus roseus	46.66	66.66	40.00	60.00	73.33	53.33	29.09	26.66	33.33	47.67	III
Clausena heptaphylla	66.66	70.00	66.66	66.66	60.00	66.66	60.00	60.00	60.00	64.07	IV
Datura stramonium	73.33	73.33	73.33	66.66	66.66	80.00	60.00	80.00	80.00	72.59	IV
Eucalyptus tereticornis	73.33	66.66	60.00	73.33	73.33	73.33	80.00	60.00	73.33	70.36	IV
Heteropanax fragrans	53.33	73.33	53.33	46.66	60.00	40.00	40.00	20.00	60.00	49.62	III
Lawsonia inermis	53.33	66.66	66.66	60.00	53.33	46.66	46.66	60.00	46.66	55.55	III
Matteuccia struthiopteris	26.66	26.66	33.33	26.66	33.33	26.66	40.00	40.00	40.00	32.58	II
Vitex negundo	33.33	53.33	53.33	60.00	73.33	66.66	50.00	50.00	40.00	53.33	IV
Control	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	Nil
F–value	10.60	7.79	6.57	9.35	9.24	7.61	7.11	5.57	6.64		
P>value	00.0001	00.0001	00.0001	00.0001	00.0001	00.0001	00.0001	00.0001	00.0001		
df	12, 26	12, 26	12, 26	12, 26	12, 26	12, 26	12, 26	12, 26	12, 26		

Figures within parentheses are transformed values

Data are based on 3 replications each with 10 individuals

0% values were subjected to the formula ¼ n before angular transformation (after Steel and Torrie, 1960), where n = number of insect

Although extracts of *Ae. marmelos* were reported effective as well against numerous stored product insects, such as *C. chinensis*, *Rhyzopertha dominica* and *S. oryzae* [20, 25, 63, 64], and the high insecticidal activities of *An. squamosa* and *An. reticulata* against several storage insect pests [65, 66], both *Ae. marmelos* and *An. reticulata* showed lesser toxicity our results. Further investigations to figure out such selective toxicity are needed. *Artemisia* spp. have been showing insecticidal, acaricidal and repellency activities and in many formulations, such as fumigant of *Ar. annua* against *T. castaneum* [67–69] and *Ar. tridentata* against several storage insect pests [70]. In context to previous studies performed by Jiang et al. (2012) to show the insecticidal activity of the essential oil of *Ar. eriopoda* against *S. zeamais* (LD_{50} = 24.8 µg/adult), our data showed that *Ar.*

Table 5 Effects of botanical extracts on detoxifying enzyme activities in *Tribolium castaneum* (Herbst)

Botanical	Enzyme (U/min./g insect)	
	AChE*	GST*
Artemisia nilagirica	$4.00^{b}\pm0.00$	$26.04^{d}\pm0.10$
Azadirachta indica	$6.17^{c}\pm0.17$	$11.77^{a}\pm0.27$
Datura stramonium	$4.28^{b}\pm0.20$	$79.16^{c}\pm1.04$
Eucalyptus tereticornis	$1.76^{a}\pm0.21$	$85.41^{d}\pm1.04$
Control	$7.21^{d}\pm0.01$	$138.19^{c}\pm2.62$
P < Value	$P<0.0001$	$P<0.0001$
F Value	599.47	4221.73
Df	4,5	4,10

* AChE: Acetylcholinesterase

*GST: Glutathione–S–transferase

nilagirica powder and aqueous extracts had higher repellency rate against *T. castaneum*.

Several previous studies showed the toxicological and repellency effects of *D. stramonium* extracts against *T. castaneum*, *R. dominica* and *Trogoderma granarium* [31, 34, 71]. *D. stramonium* is familiar with their poisonous and medicinal properties [72, 73] as various parts of the herb contain toxic levels of anticholinergic alkaloids (block neurotransmitters) with psychoactive effects [73]. In addition, *D. stramoniumis* is described as potential remedial medicine for various human ailments, including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises and swellings, fever, asthma and bronchitis, toothache, cancer, microbial disease [74]. We showed high toxicity and repellency effects of these plant extracts against the investigated insects.

Eucalyptus extracts were previously found to be effective against *T. castaneum* [75] and *Trogoderma granarium* [37]. Similarly, *E. globulus* registered 100% mortality [76] and 71% feeding inhibition activity [77] of *S. oryzae*. Naseem and Khan [78] showed that higher concentration (60%) of *E. camaldulensis* recorded 75.83% repellency of *T. castaneum* after three h of treatment. In this context, our data showed that *E. tereticornis* powder and aqueous extracts are effective against the concerned insect pests.

Various plants material contains many secondary metabolites viz. essential oils, alkaloids, isoterpenoids mainly mono and sesquiterpenes and volatile substances [79, 80]. Specifically targeting enzymes associated with pesticide resistance enhances the effectiveness of plant compounds as natural alternatives, contributing to reducing the use of traditional chemical pesticides and limiting the development of resistance [81, 82]. These findings are consistent with studies that have indicated that enzyme inhibitors play a pivotal role in improving insect response to pesticides [83].

Most of the secondary metabolites are highly effective against insect pests, ecofriendly, easily extractable and biodegradable, with low or no mammalian toxicity [84–86]. Most of the plant terpenoids showed repaid knockdown effect indicating neurotoxic mode of action [87]. Moreover, botanicals had fumigants activity against stored insect pests [88–91] and do not leave residues. Moreover, environmental factors such as temperature, humidity, and UV exposure significantly influence the effectiveness of plant powders by modifying the stability and enzyme activity of active compounds [92]. Therefore, it is important to conduct field studies to evaluate how these factors affect effectiveness under different environmental conditions to ensure the practical and successful application of these botanicals [93, 94].

Even though some plant extracts show good potential to kill or repel *Tribolium castaneum*, we need to recognize a few drawbacks. We conducted the bioassays under controlled laboratory conditions, which may not fully replicate the complex and fluctuating environments of real storage systems. Variability in plant chemistry due to geographic origin, seasonal changes, and extraction methods may influence the consistency and efficacy of the botanicals [23].

Nevertheless, these plant-based extracts offer significant potential for incorporation into integrated pest management (IPM) strategies, especially for small-scale or organic storage systems. Their eco-friendly nature, low mammalian toxicity, and biodegradability make them attractive alternatives to synthetic pesticides [95]. Future research should look at how stable these products are, how long they last, and how well they work with storage methods to help turn them into commercially successful biopesticide products.

Conclusion

The drawbacks of using diverse groups of synthetic pesticides to manage various harmful stored pest insects and arachnids are on the rise. In view of the promising results that the application of plant extracts shows, it appears that it could be effectively used to control destructive insects, such as *T. castaneum*. Therefore, we recommend evaluating a wide range of botanicals in this work to control the concerned insect species, promote health, and protect the environment. Further research should explore formulation techniques and field-level applications to enhance practical utility.

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