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# Assessment of *Piper longum* L. (Piperaceae) leaves toxicity on the adults of *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

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## Abstract

**Background** Numerous insect pests attack stored grains causing both qualitative and quantitative losses. The most damaging pest that infests dry stored produce is the red flour beetle, *Tribolium castaneum*, a secondary pest of stored goods. This pest, especially in its adult stage, exhibits resistance to chemical insecticides, thereby rendering the traditional pesticides ineffective in controlling it. Phyto-derivatives, which are strong insecticides and also ecologically benign, have gained interest as non-chemical solutions for controlling this pest. Hence, the objective of this study was to investigate the potential of *Piper longum* leaf extract insecticidal action as an environmentally benign insecticide for the first time against the adults of *T. castaneum*. In this study, *P. longum* leaf ethanol extract was tested against the adults of *T. castaneum* by petri dish bioassay method. Ad hoc studies to verify significant mortality for the initial confirmation of adulticidal activity were conducted for 24 h at different dosages of 62.5, 125, 250, 500 and 1000 mg/L of *P. longum* leaf ethanol extract. Thereafter, dosages set at 10, 20, 30 and 40 mg/L for the fractions of *P. longum* leaf ethanol extract were conducted. Prior to this, the leaf extract of this plant was subjected to column chromatography for fractionation. The fractions tested for adulticidal activity were subjected to gas chromatography–mass spectroscopy.

**Results** Significant adulticidal action with 100% adult mortality was observed in ethanol extract of *P. longum* leaves. Among the fourteen fractions (F0–F13) obtained tested, only fractions, F5, F10 and F13, demonstrated adulticidal activity, and the remaining fractions displayed poor activity. One hundred per cent morality was noted in *T. castaneum* adults after 96 h at 40 mg/L in F5 and F10, and in F13 at 20 mg/L, and their respective $LD_{50}$ values were 17.6, 26.6 and 10.0 mg/L. The fractions F5, F10 and F13 contained fatty acids, viz., hexadecanoic acid, dotriacontane and heptacosane in F5; tetradecanoic acid and nonadecanoic acid in F10; and octadecanoic acid, aspartame and tridecanoic acid in F13, revealed through gas chromatography–mass spectroscopy.

**Conclusions** The results of the study showed that *P. longum* ethanol leaf extract revealed significant adulticidal activity and is a promising toxic agent to the adults of *T. castaneum*. The fatty acids in the ethanolic leaf extract fractions of *P. longum* could have caused toxicity to the adults of *T. castaneum*. According to the current literature survey, this is the first research report on the adulticidal activity of *P. longum* leaf extracts against the adults of *T. castaneum*.

**Keywords** Stored grain pest, *Tribolium castaneum*, Adulticidal, *Piper longum*, Leaf extract, Fractions, Insecticidal property

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## Background

The destruction of stored grains by a variety of insect pests causes both qualitative and quantitative losses [1, 2]. Internally feeding insects consume the endosperm of grains, causing the grain to lose its weight, nutrients, quality, seed vigour and viability. Through excrement contamination, empty eggs, larval moults, empty cocoons and adult carcasses, externally fed insects cause damage to grains. In addition, insects play a key role in the spread of fungal contaminants, which can worsen by causing mycotoxin contamination, and reduce the quality of stored grains [3, 4]. This makes maintenance of grain quality during storage a constant challenge [5]. Insect pests that affect stored goods are a concern everywhere because they affect the quantity and quality of a wide range of post-harvest goods and grains [6–8]. As a result of their morphological, physiological and behavioural evolutionary adaptations [9], stored product insect pests are responsible for the majority of damage to stored grains. They are very adaptable, can survive in environments with low nutrient levels and are frequently viewed as persistent pests [10, 11]. The genera *Cryptolestes*, *Rhyzopertha*, *Sitophilus*, *Sitotroga*, *Tribolium* and *Trogoderma* contain the most dangerous insect species that attack stored goods. The red flour beetle, *Tribolium castaneum*, a notorious global pest of stored food goods and a member of the secondary grain insect pest, is a significant, widespread and global pest of stored goods that has been thoroughly studied to enhance pest management initiatives [7], since this pest can reduce grain weight by up to 40% [12].

Infestation by *T. castaneum*, a major pest of a wide range of stored foodstuffs, causes loss to stored goods in both quality and quantity [13, 14]. They primarily target milled grain products (flour and cereals) that have been stored, which results in significant loss and damage [15, 16] and causes serious damage to the processed cereals in the form of flour rather than whole grains [17–19]. It favours feeding and laying eggs on stored products with good nutritional quality [20–22]. According to Yao et al. [23], both adults and grubs cause damage, and 'vertical infestation', which occurs from top to bottom or vice versa, is the term used to describe *T. castaneum* infestation of stored grain [24]. According to Campbell and Hagstrum [25], this pest species can colonize small patches of food and sustain itself with small amounts of food, and cause damage by reducing the mass and/or volume, and also the physiological quality [26]. When this pest is prevalent, it causes the flour to become stained and greyish, and raises the temperature and moisture levels, which promote the rapid growth of moulds. A revolting taste and fragrance can occasionally be imparted to flour by the harsh, strong odour given off by the scent glands of this pest [27]. Therefore, protecting stored products from this beetle is challenging.

Chemical insecticides and pesticides are crucial for controlling this pest [28–30]. Despite variables influencing the effectiveness of using chemical pesticides to control insect pests in stored products, the prevalence of insecticide resistance remains high [31–33]. The success of pesticide-based control techniques [28, 34] is in jeopardy due to this insect pest's ability to acquire resistance to a variety of insecticides as a result of continuous pesticide treatment [35]. High emphasis on synthetic pesticides for control of *T. castaneum* has eventuated due to incidences of resistance to almost all main types of conventional synthetic insecticides, and this insect pest has become resistant to a variety of insecticide classes [36], particularly as it reaches adulthood [37]. In addition to insecticide resistance, toxicity issues derived from synthetic insecticides have made it necessary to find more effective, healthier and more environmentally friendly alternatives.

Phytoextracts are effective against the adults of *T. castaneum* [15, 38–50]. The whole plant chloroform extract of *Polygonum hydropiper* exhibited 10–43%, 17–50% and 17–55% mortality after 24, 48 and 72 h of exposure, respectively [51]. Acetonic extracts of *Cucurbita maxima* leaves, *Citrus sinensis* and *Citrus aurantium* fruit peels caused 68.0%, 67.6% and 49.4% mortality, respectively, after 72 h [52]. The same solvent leaf extract of *Ocimum sanctum* caused 70.0% mortality [53], and *Cleistanthus collinus* caused 62.5% mortality [54]. Leaves of *Jatropha curcas* petroleum ether extract caused 56.6% mortality [53], and *Cymbopogon citratus* methanolic extract exhibited 100% mortality [55]. Hexane, chloroform and ethyl acetate extracts of *Artemisia vulgaris*, *Prosopis juliflora*, *Sphaeranthus indicus* and *Tephrosia purpurea* at concentrations of 0.5, 1.0, 2.5 and 5.0% induced mortality after 72 h, by *A. vulgaris* hexane extract (58.0%), *S. indicus* chloroform extract (34.0%), and by the ethyl acetate extracts of *T. purpurea* (52.0%) and *P. juliflora* (30.0%) [56]. Hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extracts of *Rivina humilis* caused mortality ranging from 19.0 to 98.0% when tested at concentrations of 100, 200, 300, 400 and 500 ppm, and their respective lethal concentration$_{50}$ ($LC_{50}$) values were 267.7, 263.7, 260.0, 253.5 and 197.0 ppm [57]. Hexane leaf extracts of *Cassia mimosoides*, *Eucalyptus camaldulensis* and *Vepris heterophylla* at concentrations of 0.12, 0.25, 0.50 and 0.75 g/mL caused 18.7, 47.5, 88.7 and 96.2%; 26.2, 42.5, 80.0 and 87.5%; 10.0, 37.5, 72.5 and 82.5% mortality after 24 h; 28.7, 52.5, 96.2 and 98.7%; 33.7, 48.7, 95.0

and 98.7%; 13.7, 43.7, 76.2 and 91.2% mortality after 48 h; 33.7, 62.5, 98.7 and 100.0%; 38.7, 65.0, 100.0 and 100.0%; 27.5, 48.7, 88.7 and 97.5% mortality after 72 h, respectively [58].

Plants in the Piperaceae family are reported to possess insecticidal activity against stored products pests, *Callosobruchus chinensis*, *Callosobruchus maculatus*, *Sitophilus zeamais* [59], including *T. castaneum* [60–64]. *Piper longum* known as Indian long pepper and as 'Tippili' by its Tamil vernacular name is a perennial climber/herb with a delicate aroma. It is grown in the evergreen forests across the Indian subcontinent [65], in Tamil Nadu and Andhra Pradesh, and in regions with high relative humidity and strong rainfall [66]. *P. longum* possess insecticidal activity against agricultural pests, *Myzus persicae*, *Nilaparvata lugens*, *Plutella xylostella*, *Spodoptera litura* and *Tetranychus urticae*, [67, 68], and against mosquitoes too [68–74]. However, the toxicity of *P. longum* against the adults of *T. castaneum* has not been documented before this study. Hence, the objective of this study was to investigate the insecticidal property of *P. longum* leaves against the adults of *T. castaneum*, as an essential biological component to safeguard post-harvest production from insect pests of stored products.

## Methods

### Plant collection and leaf extract preparation

Mature and healthy leaves of the *P. longum* plant were collected in Marthandam, Kanyakumari, Tamil Nadu, India (8° 18′ 14.07 ″N and 77° 13′ 23.77″E). The Department of Botany of Scott Christian College, Nagercoil, Kanyakumari, Tamil Nadu, India, confirmed the plant's taxonomic identity. Thereafter, the leaves were washed in de-chlorinated water, shade dried and grounded to powder form in an electric blender. A soxhlet extraction [75] was carried using one kilogram of the powdered leaf material and three litres of ethanol, and thereafter, the solvent was distilled in a rotary vacuum evaporator (Puchi RII, Switzerland). The crude ethanolic extract concentrate was then further evaporated to complete dryness at room temperature, transferred to dark amber-coloured bottles, tightly closed and maintained at 4 °C for bioassays [74].

### Qualitative phytochemical screening

Qualitative phytochemical screening and qualitative analysis of secondary metabolites in the ethanol leaf extract of *P. longum* was carried out using standard procedures (Table 1) for the presence of alkaloids, fatty acids, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenes and terpenoids [76]. To confirm the

**Table 1** Standard procedures for qualitative phytochemical screening and analysis of secondary metabolites

| Phytochemical | Name of the test | Procedure | Inference |
|---|---|---|---|
| Alkaloid | Wagner's test | P.E. (0.5 g) + 1% aqueous hydrochloric acid (3 mL) were stirred in a test tube over a steam water bath. Mixture was filtered and the filtrate (1 mL) transferred to another test tube, to which three drops of Wagner's reagent were added | Formation of brownish red precipitate |
| Fatty acid | Saponification test | P.E. (0.5 g) + few drops of 0.5 N alcoholic potassium hydroxide + a drop of phenolphthalein. Mixture in the test tube was heated over a water bath | Formation of soapy foam |
| Flavonoid | Alkaline reagent test | P.E. acetone filtrate (1 mL) + distilled water (1 mL). Mixture was filtered; filtrate (5 mL) was transferred to another test tube, to which 2% sodium hydroxide solution (5 mL) was added | Appearance of yellow colour |
| Glycoside | Keller–Kiliani test | P.E. (0.1 g) + glacial acetic acid (1 mL) + few drops of 5% ferric chloride solution. To this mixture in the test tube, concentrated sulphuric acid (1 mL) was added | Formation of brown ring |
| Phenol | Ferric chloride test | P.E. (5 mg) + distilled water (5 mL) + few drops of 5% ferric chloride solution | Appearance of blue colour |
| Saponin | Foam test | P.E. (0.5 g) + distilled water (5 mL) were vigorously shaken and then gently warmed | Foam formation |
| Steroid | Hesse's test | P.E. (2 mL) + chloroform (2 mL) + concentrated sulphuric acid (2 mL) | Appearance of reddish brown colour |
| Tannin | Braymer's test | P.E. (0.5 g) + distilled water (1 mL). Mixture was stirred and filtered, and to the filtrate, few drops of 10% ferric chloride solution were added | Formation of bluish green colour |
| Terpene | | P.E. alcoholic solution (2 mL) + chloroform (2 mL). Mixture was evaporated to dryness; thereafter, concentrated sulphuric acid (2 mL) was added and heated (2 min) | Appearance of grey colour |
| Terpenoid | Salkowski test | P.E. (5 mL) + chloroform (2 mL) + concentrated sulphuric acid (3 mL) | Appearance of reddish brown colour |

*P.E.* Plant extract

presence of the tested phytochemical, screening was performed thrice.

#### Fractionation of crude extract by column chromatography

The residue from the crude ethanolic leaf extract of *P. longum* (26.83 g) was mixed with silica gel (60–120 mesh, 120 g) as admixture, subjected to column chromatography (silica gel, 100–200 mesh 400 g) with column length 45 cm and diameter 2 cm to obtain fractions by increasing polarity of eluents, viz. hexane and ethyl acetate in the ratio of 100:0; 90:10; 80:20; 60:40; 40:60; 20:80; 0:100, and finally ethyl acetate and acetone in the ratio of 50:50 and 0:100, respectively [77].

#### Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis for the active fractions of ethanol leaf extract of *P. longum* was performed. PerkinElmer instrument Clarus 680 GC used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m, 0.25 mm ID, 250 m df), and the components were separated using helium as the carrier gas at a constant flow of 1 mL/min. Throughout the chromatographic run, the injector temperature was maintained at 260 °C. The extract sample (1 μL) was introduced into the device with the oven temperature as follows (60 °C for 2 min; followed by 300 °C @ 10 °C $min^{-1}$ and 300 °C for 6 min). Conditions for the mass detector were transfer line and ion source temperature @ 240 °C; ionization mode electron impact at 70 eV; and scan time and scan interval of 0.2 and 0.1 s, respectively. The fragments ranged from 40 to 600 Da. Phytocompounds were interpreted by comparing spectral peaks in GC–MS chromatogram with the library database of spectra of recognized components in the GC–MS-National Institute for Standards and Technology library [78].

#### *Tribolium castaneum*

*T. castaneum* parent stock culture was purchased from the Tamil Nadu Agricultural University in Tamil Nadu, India. Adults of *T. castaneum* were raised in glass jars covered with muslin cloths at 30 ± 2 °C with a relative humidity of 75 ± 5% and 16:8 L:D photoperiod. The culture medium consisted of wheat flour and baker's yeast (*Saccharomyces cerevisiae*) in a 95:5 w/w proportion (one kilogram). Mass rearing was performed following the method of Duarte et al. [79]. A glass jar containing the culture medium (250 g) and 30 adult beetles (♀:♂ = 1:1) was covered in the open end to prevent the escape of the insect. The glass jar was stored in the culture room. Beetles were sieved out from the raising medium after 3 days, and were put into separate glass jars and nurtured under ideal growth conditions to obtain uniformly proportioned offspring for conducting bioassay studies.

#### Adulticidal bioassay

Petri dish bioassay methodology developed by Mason et al. [80] was used in this study with minor modifications. The $F_1$ generation of the culture adults, which were 2 weeks old, was employed for the bioassay. Stock solution (1%) was prepared by dissolving *P. longum* ethanolic leaf extract (0.25 g) in distilled water (25 mL). Thereafter, from the stock solution, different dosages (62.5, 125, 250, 500 and 1000 mg/L) prepared by serial dilution were tested via ad hoc studies to verify significant mortality for the initial confirmation of adulticidal activity. Each above-mentioned dose (1 mL) was dropped in the middle of a piece of Whatman filter paper Grade 1, where it was absorbed to the filter paper's outside edge. The solvent was allowed to dry and evaporate off the filter paper leaving the extract behind. The filter paper (10 cm in diameter) was placed to fit the bottom of a lower glass petri dish (10 cm in diameter), covering the entire interior surface. With the use of a Camel's soft, fine hair brush, ten unsexed *T. castaneum* adults were carefully placed on the filter paper of the lower glass petri dish and were closed with the upper glass petri dish. After the ad hoc bioassay with the crude extracts, the dosages for various fractions were set at 10, 20, 30 and 40 mg/L, and tests were conducted. Filter paper that had been exposed to distilled water only functioned as control. The experimental setup was then placed inside an incubator set at a temperature of 30 ± 2 °C and a relative humidity of 70 ± 5%. Five replicates were used for each test dosage. Adult mortality was noted after 24, 48, 72 and 96 h following the insect release, by probing it using the back of a Camel hair brush. Adult beetles were considered dead when they showed no signs of life and stopped moving fully, including their legs and antennae, in response to mild pressure. This proved their demise. Nevertheless, the treated beetles were again given light pressure after being monitored for 5 min to prevent the chance of death mimicry.

#### Data analyses

Percentage of mortality was determined, and any errors in the control mortality (5–20%) were corrected using Abbott's formula [81]. Mortality data were subjected to probit analysis in IBM SPSS statistics version 28.0 to estimate the lethal $dose_{50}$ ($LD_{50}$) values with significance set at 95% confidence [82].

## Results

Qualitative phytochemical screening of *P. longum* ethanolic leaf extract revealed the presence of alkaloids, fatty acids, glycosides, phenols, tannins and terpenoids. The phytochemical compounds present in fractions, F5, F10 and F13 revealed by GC–MS chromatogram are shown in Figs. 1, 2 and 3. The phytochemical compounds present in F5 were 3,3-dimethyl-1-(methylsulfonyl)-2-butanone-O-((methylamino)carbonyl)oxime; methyl-13C-benzene; 1-benzyloxy-3-bromopropane; 4-benzyloxy-1-bromopropane; pentadecanoic acid, 14-methyl-methyl ester; oxiraneoctanoic acid, 3-octyl-,trans-; hexadecanoic acid; 1,2,4-trioxolane-2-octanoic acid, 5-octyl-,methyl ester; dotriacontane; nonahexacontanoic acid; β-d-mannofuranose, 2,3:5,6-di-O-ethylboranediyl-1-O-(10-undecen-1-yl)- and heptacosane (Table 2). Benzene, 1-(2,2-dimethyl-1-methylenepropyl)-3-(trifluoromethyl); thienoindole; pyridoindazole; selegiline; methandriol dipropionate; α-normethadol; tetradecanoic acid; nonadecanoic acid; lucenin 2; and 2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole were present in F10 (Table 3). Octadecanoic acid, ethyl ester; oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-; aspartame; tridecanoic acid, 13-formyl-, ethyl ester; oxiranepentanoic acid, 3-undecyl-, methyl ester, cis-; methyl 8,9,13-trihydroxydocosanoate; methyl 4-hydroxyoctadecanoate; pseudojervine; lucenin 2; 2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole; and 11α-hydroxyandrosta-1,4-diene-3,17-dione were present in F13 (Table 4).

The per cent adult mortality of *T. castaneum* determined at different dosages of *P. longum* leaf ethanol extract resulted in 100% mortality after 24 h. In control, no adult mortality was observed. Among the fourteen fractions (F0–F13) tested for adulticidal activity, only fractions, F5, F10 and F13, exhibited significant activity, and the remaining fractions displayed poor activity. The per cent adult mortality of *T. castaneum* determined at different dosages of fractions, F5, F10 and F13, at different hours of exposure is shown in Fig. 4. In the control group for fractions, all of the introduced adult beetles were alive, except for 10% mortality in F5, F10 and F13 after 72, 96 and 96 h, respectively.

No mortality was noted in adults treated by F5 till 30 mg/L dosage, but thereafter caused 10% mortality at

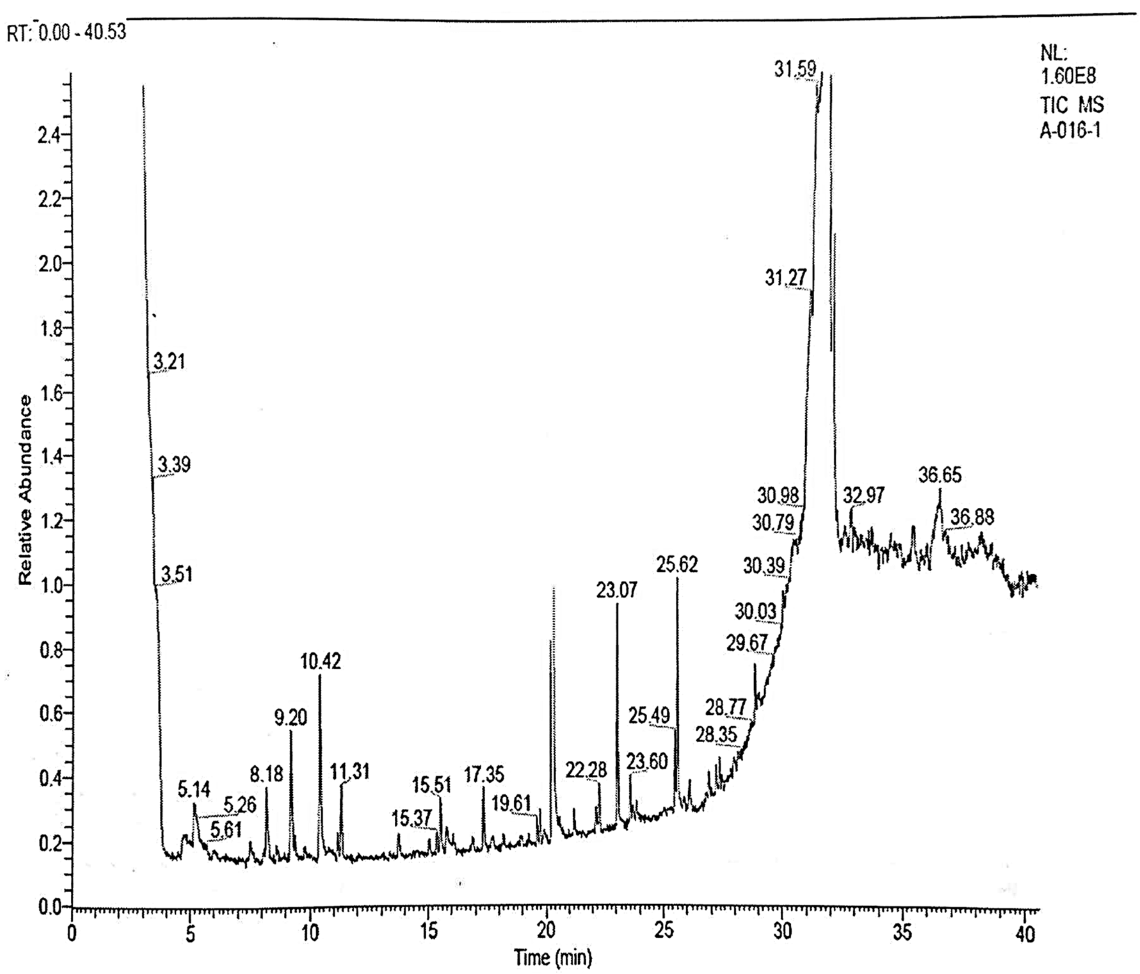



**Fig. 1** GC–MS chromatogram of F5 of *P. longum* ethanolic leaf extract

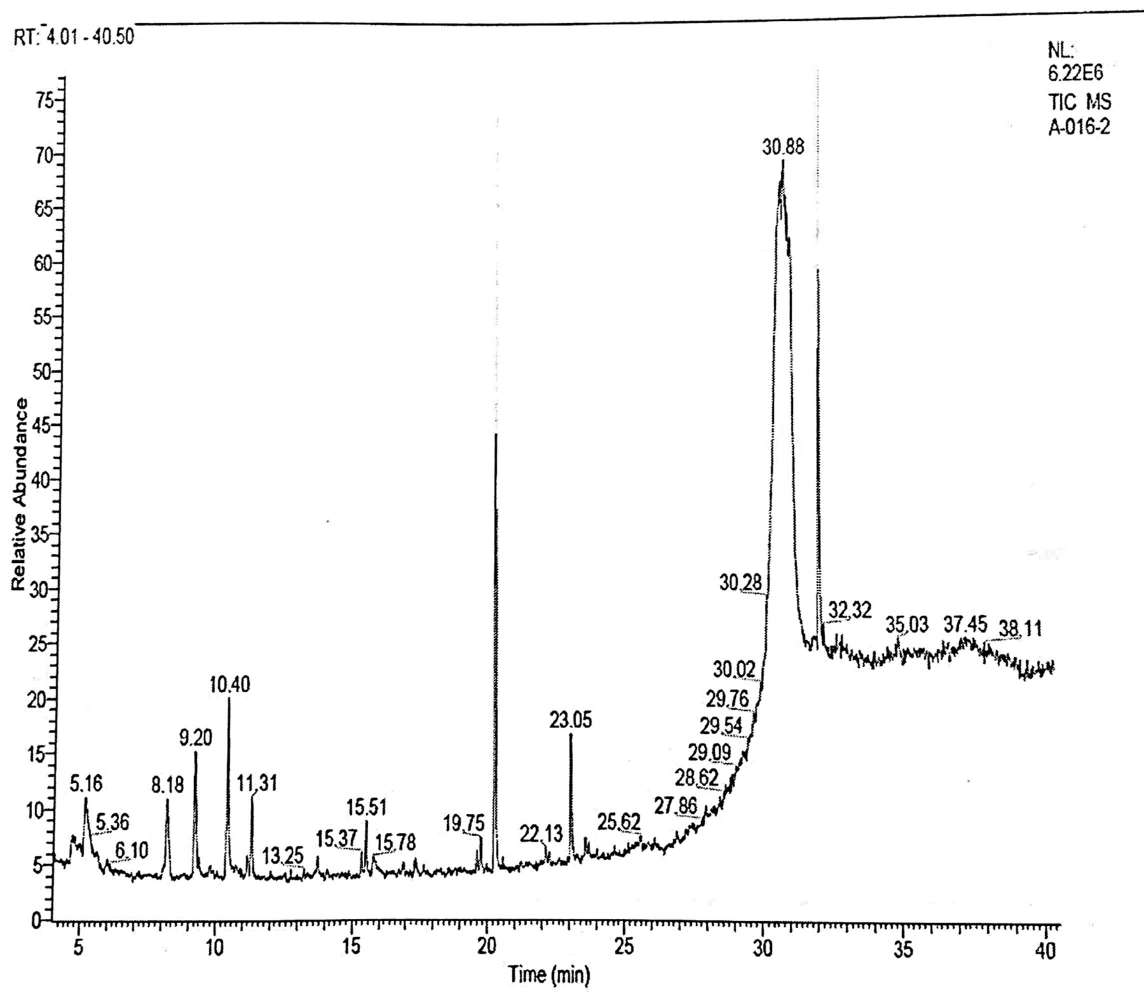



**Fig. 2** GC–MS chromatogram of F10 of *P. longum* ethanolic leaf extract

40 mg/L after 24 h of exposure. After 48 h, no adult mortality was observed till 20 mg/L, but thereafter 30 and 40 mg/L dosages caused 20% and 40% mortality, respectively. Near similar result occurred after 72 h of exposure. No adult mortality was noted till 20 mg/L, but thereafter 40% and 70% mortality were noted at dosages of 30 and 40 mg/L, respectively. After 96 h, the fraction in dosages of 20 and 30 mg/L caused 10% and 90% mortality, with 100% mortality observed at 40 mg/L. The $LD_{50}$ values (in mg/L) of F5 treatments were 71.3, 45.0, 33.3 and 17.6 for 24, 48, 72 and 96 h, respectively.

In F10, no mortality was noted in adults treated till 30 mg/L dosage, but thereafter caused 10% mortality at 40 mg/L after 24 h of exposure. After 48 h, no adult mortality was observed till 20 mg/L, but thereafter 30 and 40 mg/L dosages caused 10% and 40% mortality, respectively. Near similar result occurred after 72 h of exposure. No adult mortality was noted till 20 mg/L, but thereafter 30% and 70% mortality were noted at dosages of 30 and 40 mg/L, respectively. After 96 h, the fraction in dosages of 20 and 30 mg/L caused 10% and 60% mortality, with 100% mortality observed at 40 mg/L. The $LD_{50}$ values (in mg/L) of F10 treatments were 60.0, 43.3, 35.0 and 26.6 for 24, 48, 72 and 96 h, respectively.

In F13, no mortality was noted in treated adults at 10 mg/L dosage, but thereafter 10%, 10% and 30% mortality was noted at 20, 30 and 40 mg/L, respectively, after 24 h of exposure. After 48 h, no adult mortality was observed at 10 mg/L, but thereafter 20, 30 and 40 mg/L dosages caused 50%, 60% and 80% mortality, respectively. After 72 h of exposure, adult mortality was noted in all dosages ranging from 10 to 40 mg/L, with 20%, 80%, 90% and 100%, respectively. After 96 h, the fraction in dosage of 10 mg/L caused 50% mortality and thereafter 100% mortality till 40 mg/L. The $LD_{50}$ values (in mg/L) of F13 treatments were 63.3, 45.0, 16.1 and 10.0 for 24, 48, 72 and 96 h, respectively.

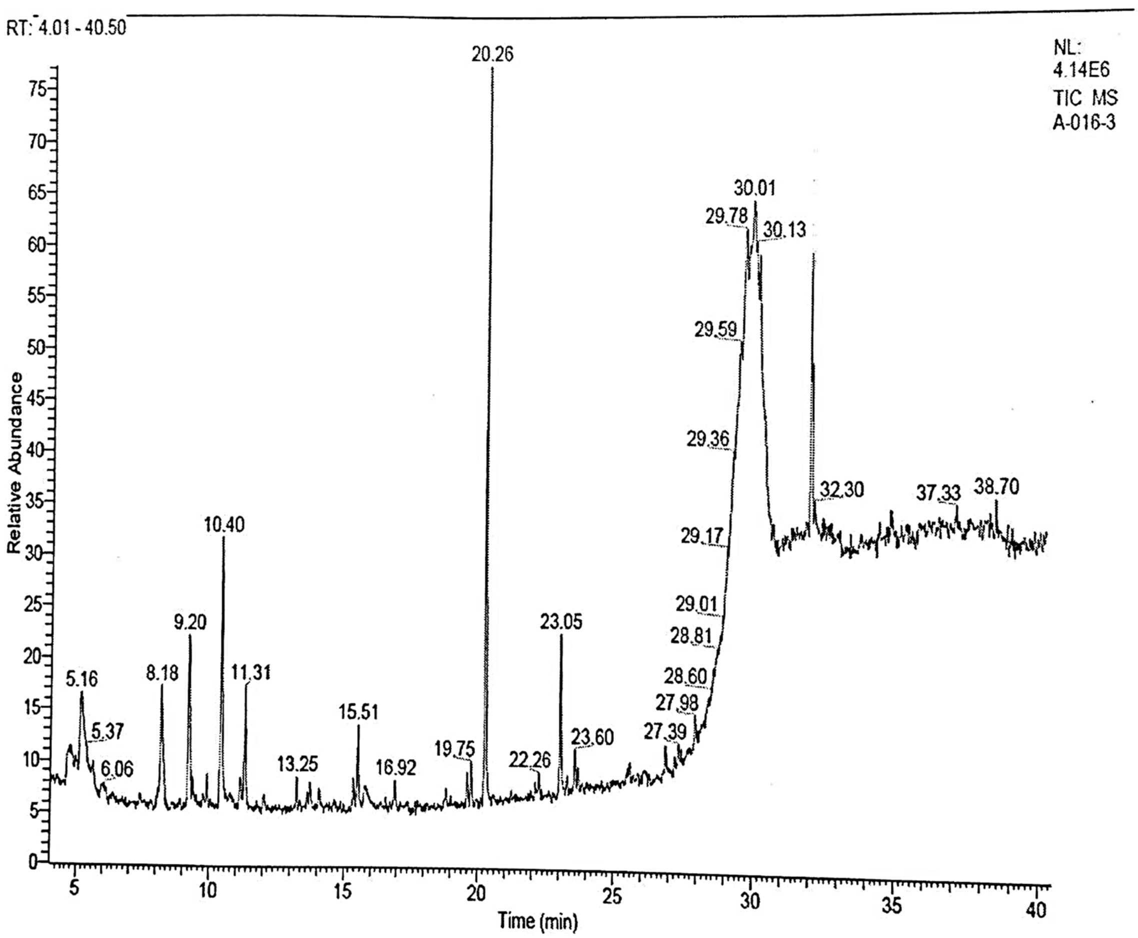



**Fig. 3** GC–MS chromatogram of F13 of *P. longum* ethanolic leaf extract

## Discussion

An essential component to safeguard post-harvest production is controlling pests that live in stored goods. Currently, the stored grain sector relies on synthetic grain protectants. Due to the negative effects of chemical insect management, it is necessary to investigate natural but equally potent substances that can be utilized to combat insect pests of stored products without significantly endangering human health or lowering grain quality. The use of botanicals to control them is stressed by increased public concern over the residual toxicity of insecticides applied to stored products, the occurrence of insecticide-resistant insect strains and the precautions needed to work with traditional chemical insecticides. Due to environmental concerns and an increase in insect populations that are resistant to traditional pesticides, interest in botanical insecticides has grown over time. Islam [83] listed the phytoderivatives deemed effective for controlling *T. castaneum*. The results of the present study when compared to the reports of the literature reported elsewhere show that the leaves of *P. longum* have strong adulticidal activity based on their per cent adult mortality and $LD_{50}$ values.

A botanical insecticide's phytotoxicity is typically influenced by the plant component and solvent extract employed [84]. Depending on the polarity of the solvent used, potential phytocompounds in the plant may seep out, and hence, the bioactivity of the extracts is influenced by the solvents used for extraction. The primary consideration for determining insecticidal activity is the phytochemical compound certainty in plant extracts. The solvent used affects the variation because it has been demonstrated that the polarity of the solvents employed affects the extraction of active phytochemical substances from plants [85]. Therefore, it is advised to employ intermediary or more polar extracts if a reasonable management of natural products is sought [86], and the same was done in the current investigation by utilizing ethanol (polarity index 5.2). The secondary metabolites, viz. alkaloids, fatty acids, flavonoids, phenols, quinines, saponins, sterols, tannins, terpenes and terpenoids that are poisonous to insects, can be extracted using ethanol [87]. As

**Table 2** Phytochemical compounds in the F5 fraction of ethanolic leaf extract of *P. longum*

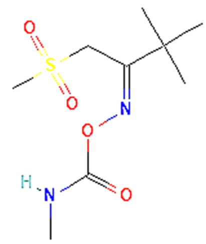
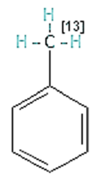
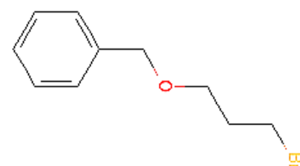
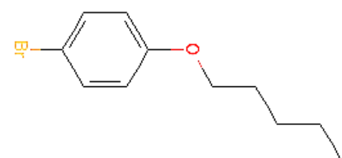
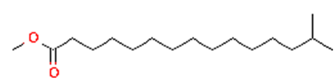
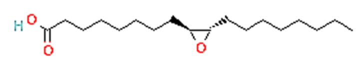
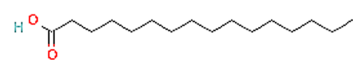
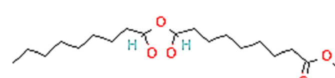
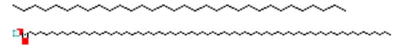
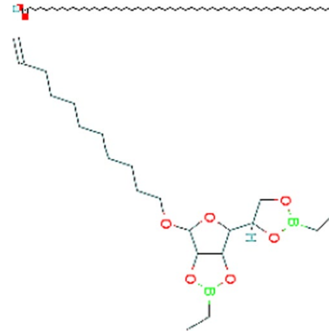
| Compound name | Retention time (in min) | Molecular weight (g/mol) | Molecular formula | Structure |
|---|---|---|---|---|
| 3,3-dimethyl-1-(methylsulfonyl)-2-butanone O-((methylamino)carbonyl)oxime | 3.37 | 250.32 | $C_9H_{18}N_2O_4S$ |  |
| Methyl-13C-benzene | 3.37 | 93.13 | $C_7H_8$ |  |
| 1-benzyloxy-3-bromopropane | 3.37 | 229.11 | $C_{10}H_{13}BrO$ |  |
| 4-benzyloxy-1-bromopropane | 3.37 | 243.14 | $C_{11}H_{15}BrO$ |  |
| Pentadecanoic acid, 14-methyl-, methyl ester | 22.28 | 270.50 | $C_{17}H_{34}O_2$ |  |
| Oxiraneoctanoic acid, 3-octyl-, trans- | 22.28 | 298.50 | $C_{18}H_{34}O_3$ |  |
| Hexadecanoic acid (Palmitic acid) | 22.28 | 256.42 | $C_{16}H_{32}O_2$ |  |
| 1,2,4-trioxolane-2-octanoic acid, 5-octyl-, methyl ester | 22.28 | 344.50 | $C_{19}H_{36}O_5$ |  |
| Dotriacontane | 32.97 | 450.90 | $C_{19}H_{66}$ |  |
| Nonahexacontanoic acid | 32.97 | 998.80 | $C_{69}H_{138}O_2$ |  |
| β-d-mannofuranose, 2,3:5,6-di-O-ethylboranediyl-1-O-(10-undecen-1-yl)- | 32.97 | 408.10 | $C_{21}H_{38}B_2O_6$ |  |
| Heptacosane | 32.97 | 380.70 | $C_{27}H_{56}$ |  |

**Table 3** Phytochemical compounds in the F10 fraction of ethanolic leaf extract of *P. longum*

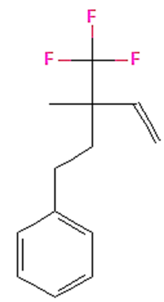
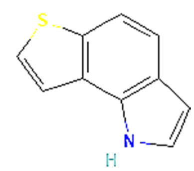
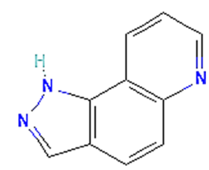
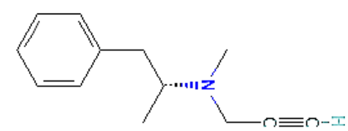
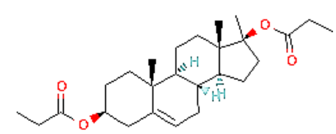
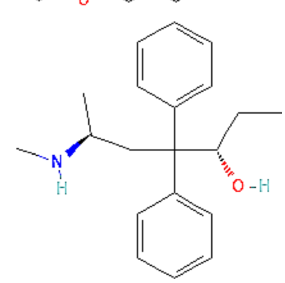
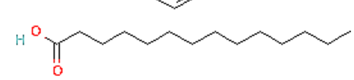
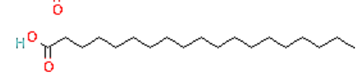
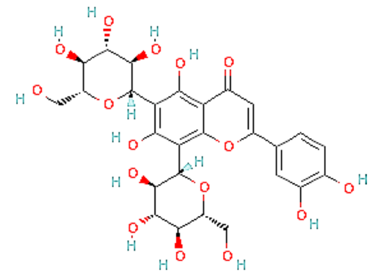
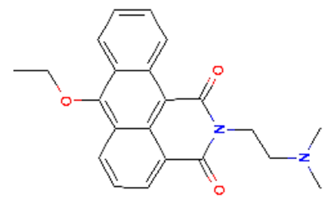
| Compound name | Retention time (in min) | Molecular weight (g/mol) | Molecular formula | Structure |
|---|---|---|---|---|
| Benzene,1-(2,2-dimethyl-1-methylenepropyl)-3-(trifluoromethyl) | 10.42 | 228.25 | $C_{13}H_{15}F_3$ |  |
| Thienoindole | 10.42 | 173.24 | $C_{10}H_7NS$ |  |
| Pyridoindazole | 10.42 | 169.18 | $C_{10}H_7N_3$ |  |
| Selegiline | 10.42 | 187.28 | $C_{13}H_{17}N$ |  |
| Methandriol dipropionate | 17.37 | 416.60 | $C_{26}H_{40}O_4$ |  |
| α-normethadol | 17.37 | 297.40 | $C_{20}H_{27}NO$ |  |
| Tetradecanoic acid (Myristic acid) | 17.37 | 228.37 | $C_{14}H_{28}O_2$ |  |
| Nonadecanoic acid (Methyl stearate) | 17.37 | 298.50 | $C_{19}H_{38}O_2$ |  |

**Table 3** (continued)

| Compound name | Retention time (in min) | Molecular weight (g/mol) | Molecular formula | Structure |
|---|---|---|---|---|
| Lucenin 2 | 27.45 | 610.50 | $C_{27}H_{30}O_{16}$ |  |
| 2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole | 27.45 | 362.43 | $C_{22}H_{22}N_2O_3$ |  |

these secondary metabolites dissolve in mid, intermediary and high polar solvents rather than those with less polarity, the active phytochemicals present in the extract may have contributed to the performance of the extract with regard to toxicity on the adults of *T. castaneum* in the present study.

Ethanol extracts of the following medicinal and aromatic plants have caused adult mortality in *T. castaneum* [88]. *P. hydropiper* whole plant caused mortality which ranged from 7–40%, 10–47% and 17–57% after 24, 48 and 72 h of exposure, respectively, and the concentrations used were 31.5, 62.5, 125.0, 250.0 and 500.0 mg/mL [51]. *Datura stramonium* caused mortality with $LC_{50}$ values of 3936 and 1954 mg/L after 24 and 48 h, respectively [89], and *Psidium guajava* leaves caused 30.0 and 50.0%; and 100.0% mortality at 5 and 10 ppm after 7 and 14 days, respectively [90]. *Cardiospermum halicacabum*, *Coriandrum sativum*, *Mentha longifolia*, *O. sanctum* and *Pongamia glabra* leaves at dose of 25, 50, 75 and 100 µg/mL caused 25.7, 45.3, 78.1 and 93.0%; 25.4, 42.6, 75.9 and 80.0%; 29.4, 55.6, 82.6 and 98.1%; 26.2, 53.4, 79.3 and 96.9%; 27.6, 57.4, 85.2 and 96.3% mortality, respectively, and their respective $LC_{50}$ values were 64.9, 52.7, 49.8, 49.7 and 49.2 µg/mL [91]. Extracts of *Curcuma longa* rhizome, and seed extracts of *Myristica fragrans* and *Piper nigrum* caused 37.5, 51.0, 71.0 and 96.6%; 40.1, 47.9, 80.0, 100.0%; 30.0, 42.6, 45.2 and 60.0% at dosage of 2, 4, 6 and 8% after 24 h, respectively [63]. *Limoniastrum guyonianum* aerials parts (phytochemicals were alkaloids, flavonoids, saponins and tannins) caused 91.6% mortality after 72 h [92], and *Melissa officinalis*, *Mentha piperita*, *Rosmarinus officinalis* and *Thymus vulgaris* leaves caused 42.6, 40.0, 58.6 and 24.0% mortality, respectively [93]. The present findings corroborated with the reports of the above-mentioned studies.

Identification of highly active fractions and chemicals separated from the crude extract during screening, on the other hand, requires insecticidal bioassay-guided fractionation, according to Shaalan et al. [87], as a complex variety of biocidal active substances can be found in the crude extract. Further, the extract be fractionated in order to identify the specific chemical component producing the deadly effect if an unusually low lethal dose is found. Thus, the goal of fractionation is to create a combinations of substances in order to decrease the number of substances that may be discovered through further analysis. Since they include various phytochemicals, fractions recovered from the same extract always have varying insecticidal activities, and this was noted in the present study, wherein out of the fourteen fractions tested, only three fractions exhibited significant adulticidal action while the remaining displayed poor activity. Thereafter, once a fraction has demonstrated its efficacy, its active phytochemical compounds are to be isolated. However, it is to be noted that though various synergistic relationships may exist in botanical preparations that may improve killing activity, some chemicals lose efficiency when separated [77].

There is a growing interest in plants of the Piperaceae family as potential sources of promising bioactive phytochemical compounds with insecticidal activity against various insect pests, and toxicity to mosquitoes [94–96]

**Table 4** Phytochemical compounds in the F13 fraction of ethanolic leaf extract of *P. longum*

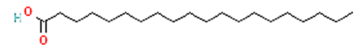
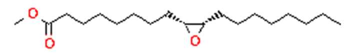
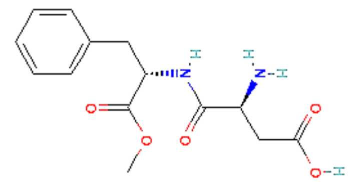
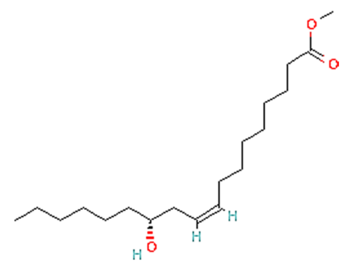
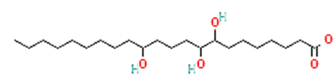
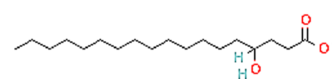
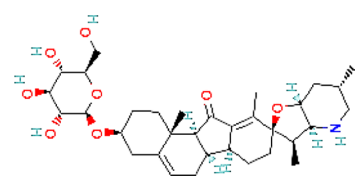
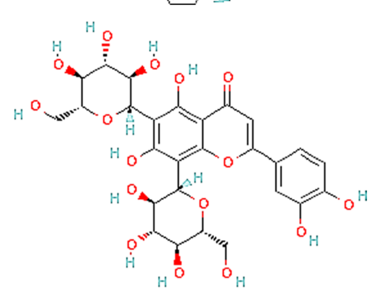
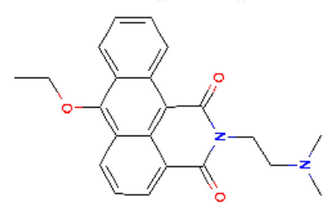
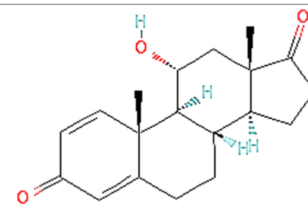
| Compound name | Retention time (in min) | Molecular weight (g/mol) | Molecular formula | Structure |
|---|---|---|---|---|
| Octadecanoic acid, ethyl ester | 23.60 | 312.53 | $C_{20}H_{40}O_2$ |  |
| Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis- | 23.60 | 312.50 | $C_{19}H_{36}O_3$ |  |
| Aspartame | 23.60 | 294.30 | $C_{14}H_{18}N_2O_5$ |  |
| Tridecanoic acid, 13-formyl-, ethyl ester | 23.60 | 270.41 | $C_{16}H_{30}O_3$ |  |
| Oxiranepentanoic acid, 3-undecyl-, methyl ester, cis- | 28.00 | 312.50 | $C_{19}H_{36}O_3$ |  |
| Methyl 8,9,13-trihydroxydocosanoate | 28.00 | 402.60 | $C_{23}H_{46}O_5$ |  |
| Methyl 4-hydroxyoctadecanoate | 28.00 | 314.50 | $C_{19}H_{38}O_3$ |  |
| Pseudojervine | 28.00 | 587.70 | $C_{33}H_{49}NO_8$ |  |
| Lucenin 2 | 32.89 | 610.50 | $C_{27}H_{30}O_{16}$ |  |
| 2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole | 32.89 | 362.43 | $C_{22}H_{22}N_2O_3$ |  |

**Table 4** (continued)

| Compound name | Retention time (in min) | Molecular weight (g/mol) | Molecular formula | Structure |
|---|---|---|---|---|
| 11α-hydroxyandrosta-1,4-diene-3,17-dione | 32.89 | 300.40 | $C_{19}H_{24}O_3$ |  |

and agricultural pests [97]. *Piper* secondary plant components have numerous mechanisms of action, including contact toxicity [98], synergism [99], repellent and antifeedant characteristics. Scott et al. [100] presented an extensive assessment of the large diversity of secondary plant chemicals in *Piper* species as prospective leads for insecticides, many of which are utilized in traditional pest control of stored harvests. *Piper* extracts have been tested for their insecticidal property against insect pests of stored products, viz. *C. maculatus* [59, 101–103], *C. chinensis* [59], *Corcyra cephalonica* [104, 105], *Plodia interpunctella* [106], *Rhyzopertha dominica* [106, 107], *Sitophilus oryzae* [104, 106, 108], *S. zeamais* [59, 101], and *T. castaneum* [61]. *P. longum* extracts are toxic to agricultural pests, viz., *M. persicae*, *N. lugens*, *P. xylostella* and *S. litura* [67], and to mosquitoes [67, 70–72, 94, 95, 109]. *P. longum* leaf (petroleum ether, chloroform, methanol and aqueous) extracts exhibited 100% ovicidal and larvicidal activity against the third instar of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* with their $LC_{50}$ values ranging from 50.81 (aqueous extract) to 395.51 ppm (methanol extract), due to the active phytochemicals, viz. flavonoids, terpenoids, tannins, alkaloids and saponins [69].

Secondary metabolites have shown insecticidal activity against stored product pests including weevils and beetles [110]. Alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenes and terpenoids are reported for toxicity against *T. castaneum* which had induced mortality in them [10, 11, 46, 58, 111–115]. Saponins possess insecticidal activity against *T. castaneum* [116, 117]. They inhibit acetylcholinesterase (AChE) [118], causing acetylcholine to accumulate at cholinergic synapses and hyperexcite cholinergic pathways in *T. castaneum* [32]. Terpenes kill insects by inhibiting the action of the AChE assay in the nervous system [119]. Terpenoids, the most abundant component in many plant species, have insecticidal properties [120], and they influence the insecticidal activities of *T. castaneum* through the degree of penetration into the insect cuticle, and the ability to migrate to and interact with an active site, according to Rice and Coats [121]. *Piper* extracts contain alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides and terpenoids that are insecticidal against *T. castaneum* [122]. Choudhary and Singh [123] provided an exhaustive list of phytochemicals obtained from *P. longum*. The leaves of this plant contain alkaloids, saponins, flavonoids, phytosterols, terpenes, phenols, tannins, steroids and terpenoids [69–71, 124–127]. Furthermore, Sindhu et al. [128] and Kavitha et al. [129] reported alkaloids, fatty acids, glycosides, phenols, tannins and terpenoids in *P. longum* ethanolic leaf extract. In the current investigation, the same were found in *P. longum* leaves, which could have been toxic to *T. castaneum* adults.

Several groups of plant-derived phytochemicals are employed for their poisonous activity against numerous insect pests of stored products [130, 131]. Phytochemicals cause phytotoxicity in insects by disrupting critical metabolic pathways resulting in rapid mortality [132, 133]. The major phytocompounds in *P. longum* ethanolic leaf extract fractions in the present study revealed by GC–MS were fatty acids, viz. hexadeacnoic acid, octadecanoic acid, tetradecanoic acid, nonadecanoic acid, dotriacontane, aspartame and heptocosane. Fatty acids are insecticidal in nature, and their insecticidal property against various insect pests is tabulated in Table 5. Fatty acids (palmitic acid, stearic acid, myristic acid) and their respective methyl esters are insecticidal in action [157, 158] and have insecticidal effects on pests of stored products due to toxicity of contact treatments [159, 160]. Fatty acids affect the neurological system of insects by entering through the cuticle, blood barrier and perineurium. They kill the basic unit of the nervous system, disrupt the insect's behaviour, locomotion and eventually lead to death [161]. According to de Melo et al. [162], fatty acids have insecticidal effect via generating cell instability in insect midgut cells and inhibiting voltage-gated potassium channels of nerve cells [163]. Fatty acid treatment has been shown to suppress stored product pests, particularly

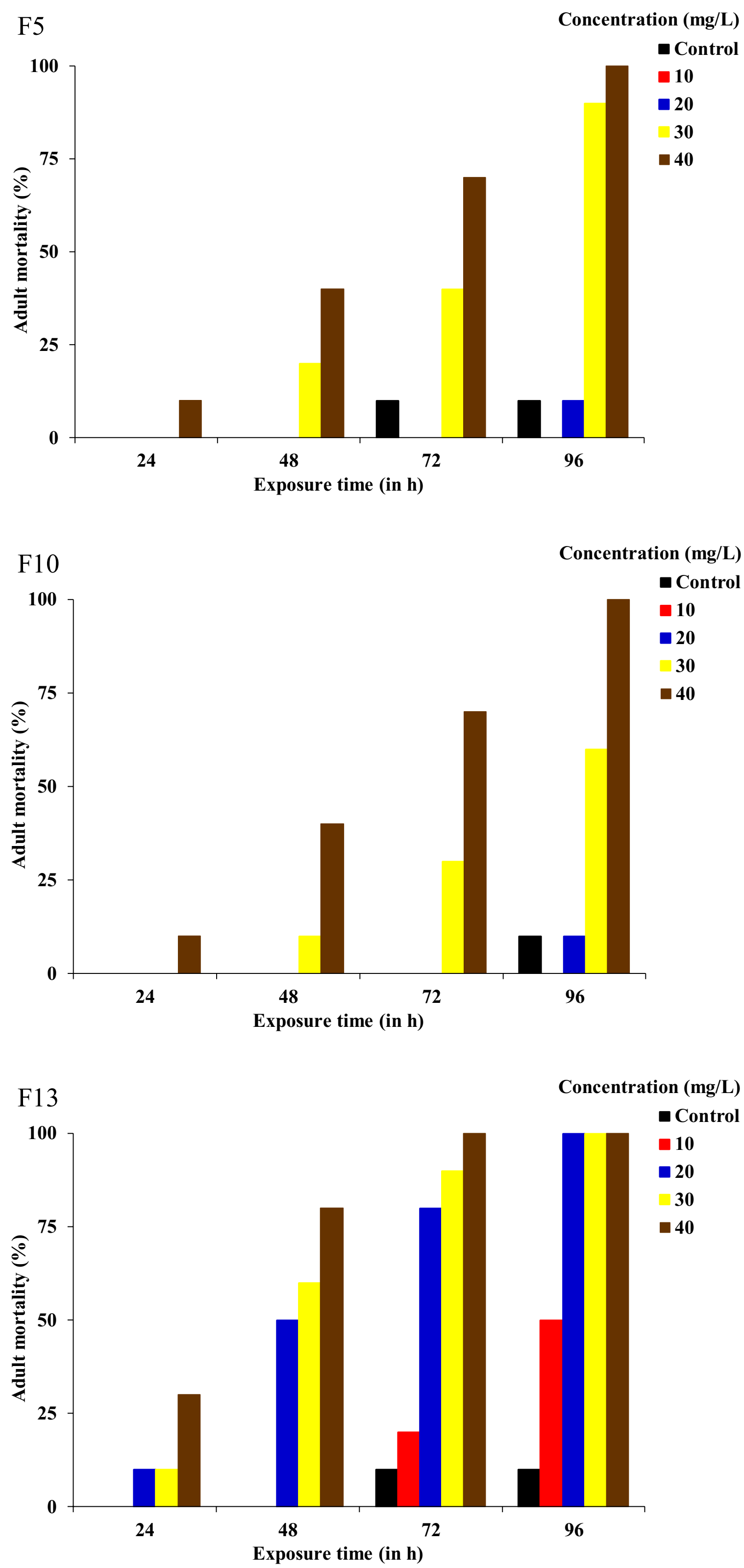



**Fig. 4** Per cent adult mortality of *T. castaneum* on exposure to fractions of *P. longum* ethanolic leaf extract

**Table 5** Phytocompounds present in the fractions of *P. longum* ethanolic leaf extract reported for insecticidal property against various insect pests

| Phytocompound | Class of insect pest | Insect pest species | Reference |
|---|---|---|---|
| Hexadecanoic acid (Palmitic acid) | Mosquitoes | *Aedes albopictus* | Ravi et al. [134] |
| | | *Cx. quinquefasciatus* | Vivekanandhan et al. [135] |
| | Agriculture | *Aphis gossypii* | Ravikumar [136], Abdullah [137] |
| | | *Helicoverpa armigera* | Satyan et al. [138], Coloma et al. [139], Praveena and Sanjayan [140] |
| | | *Mamestra brassicae* | Motukuri et al.[141] |
| | | *Spodoptera frugiperda* | Figueroa-Brito et al. [142], Coloma et al. [139], Ravikumar [136], Ramos-López et al. [143], Evangelista-Lozano et al. [144] |
| | | *Spodoptera littoralis* | Barakat et al. [145], Abdullah [137] |
| | Stored products | *R. dominica* | Neggaz et al. [146] |
| | | *Sitophilus granarius* | Abay et al. [147] |
| | | *S. oryzae* | Rajkumar et al. [148], Neggaz et al. [146] |
| | | *S. zeamais* | Lawal et al. [149] |
| | | *T. castaneum* | Rajkumar et al. [148], Mokhtar et al. [150] |
| Octadecanoic acid (Stearic acid) | Mosquitoes | *Ae. albopictus* | Ravi et al. [134] |
| | | *Cx. quinquefasciatus* | Vivekanandhan et al. [135] |
| | Agriculture | *A. gossypii* | Abdullah [137] |
| | | *H. armigera* | Praveena and Sanjayan [140] |
| | | *M. brassicae* | Motukuri et al. [141] |
| | | *S. frugiperda* | Figueroa-Brito et al. [142], Ramos-López et al. [143], Evangelista-Lozano et al. [144] |
| | | *S. littoralis* | Abdullah [137] |
| | Stored products | *R. dominica* | Neaggaz et al. [146] |
| | | *S. oryzae* | Neaggaz et al. [146] |
| | | *T. castaneum* | Mokhtar et al. [150] |
| Tetradecanoic acid (Myristic acid) | Mosquitoes | *Ae. aegypti* | Sivakumar et al. [151] |
| | | *Cx. quinquefasciatus* | Sivakumar et al. [151] |
| | Agriculture | *A. gossypii* | Abdullah [137] |
| | | *S. littoralis* | Abdullah [137] |
| | Stored products | *S. granarius* | Abay et al. [147] |
| | | *T. castaneum* | Ayaz et al. [152] |
| Nonadecanoic acid | Agriculture | *H. armigera* | Praveena and Sanjayan [140] |
| Dotriacontane | Agriculture | *A. gossypii* | Ravikumar [136] |
| | | *S. frugiperda* | Ravikumar [136] |
| Aspartame | Agriculture | *Bactrocera dorsalis* | Zheng et al. [153] |
| | Garden | *Solenopsis invicta* | Zhang et al. [154] |
| Heptocosane | Mosquitoes | *Cx. quinquefasciatus* | Vivekanandhan et al. [135] |
| | Agriculture | *H. armigera* | Satyan et al. [138], Praveena and Sanjayan [140] |
| | | *M. brassicae* | Motukuri et al. [141] |
| | Stored products | *Oryzeaphilus mercator* | Pasdaran et al. [155] |
| | | *S. oryzae* | Rajkumar et al. [148] |
| | | *Tribolium* species | Hammami et al. [156] |
| | | *T. castaneum* | Rajkumar et al. [148], Pasdaran et al. [155] |

*Tribolium* species [164]. The physicochemical properties of fatty acids make them ideal for use as contact insecticides that cause total paralysis [165, 166]. Fatty acids bind to cell membrane components, causing the membrane's integrity to deteriorate and the insect to perish, and obstruct insect breathing by blocking the spiracles. Fatty acid molecules bind to the cuticle's waxy external layer, allowing them to infiltrate into tissues, as well as within the trachea, particularly trachioles, and then penetrate into the body to cellular action sites [167]. The closeness of mortality responses in this study shows that fatty acids act in a similar manner. Hence, the fatty acids present in the fractions of the ethanolic leaf extract of *P. longum* certainly would have exhibited toxicity to the adults of *T. castaneum*. Further, toxicity synergy may also occur when two or more fatty acids with broadly comparable mechanisms of action are mixed [167].

Botanical pesticides used against coleopteran stored product pests have been linked to paralysis and electron transport blockage in insect respiratory processes, immobilization and toxicity [168]. Understanding the mode of action, which includes the physical, biological and chemical interactions between the insect and the pesticide, is critical in pest control since it determines the management plan to be used [169]. According to Fang et al. [170], *Tribolium* species are among the least susceptible insect pests of stored products and are frequently more difficult to kill than other stored product beetles; however, the order of toxicity generally varies depending on the specific insecticide. Secondary metabolites from plants have numerous routes of action that have sub-lethal effects on the target insect. AChE has a high catalytic activity and is a crucial enzyme in the nervous system that terminates nerve impulses by catalysing the hydrolysis of the neurotransmitter acetylcholine [171]. Inhibiting AChE induces acetylcholine buildup at synapses, resulting in paralysis and death of the insect, with neurotoxicity being the method of action. This was seen in adults of *T. castaneum* after exposure to an ethanolic extract of *L. guyonianum* [92]. The same mode of action could have killed *T. castaneum* adults in the present study on exposure to ethanolic fractions of *P. longum* leaves. Furthermore, adult mortality could be ascribed to contact toxicity or the production of unknown physiological alterations [172]. One of the numerous possible causes of adult mortality is the effective adherence of phytocompounds to the insect's spiracles, causing its death due to asphyxia. Contact poisoning was possible in this study since the insects had symptoms such as convulsions and tremors followed by paralysis (knockdown), which were similar to those seen in the study by Kanmani et al. [78]. This response could be related to octopaminergic receptor activation [173]. Above and beyond that, phytocompounds that function as insecticides affect insect behaviour via the olfactory sensilla of their antennae. This is because, throughout the experiment, live insect species strive to avoid coming into contact with the treated surface (filter paper), which works by creating a vapour barrier that prevents them from coming into contact with the stimulus or surface [174]. The same was observed during the experimentation phase in the present study. This could be possible as the presence of phytocompounds with strong odours could inhibit the insects' tracheal respiration, resulting in their death. Liu and Ho [175] also reported a similar observation against *S. zeamais* and *T. castaneum*.

## Conclusion

The present investigation revealed the adulticidal activity of *P. longum* ethanol leaf extract fractions on *T. castaneum*. This is the first-hand information on the toxicity of *P. longum* ethanol leaf extract fractions on *T. castaneum* adults. Isolation of active phytochemical compounds of *P. longum* ethanol leaf extract that induces insecticidal activity on *T. castaneum* adults, and their bioassays on *T. castaneum* adults merit further study.

**Abbreviations**

| | |
|---|---|
| GC–MS | Gas chromatography–mass spectrometry |
| $LD_{50}$ | Lethal $dose_{50}$ |
| $LC_{50}$ | Lethal $concentration_{50}$ |
| AChE | Acetylcholinesterase |

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Not applicable

**Author contributions**
JK and ST contributed to conception and design of the experiments. JK and GM prepared the phytoextracts, fractions and performed the bioassay experiments. JK and SA carried GC–MS analysis. ST wrote the manuscript. All authors have read and given their approval to the final version of the manuscript.

**Plant authentication**
The taxonomic identity of the plant material was confirmed and authenticated by Dr. S. Jeeva, Assistant Professor, Department of Botany, Scott Christian College, Nagercoil, Kanyakumari, Tamil Nadu, India.

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**Availability of data and materials**
Data and materials have been provided with the manuscript as figures and tables.

## Declarations

**Ethics approval and consent to participate**
Not applicable for this work.

**Consent for publication**
All authors have approved the publication of this study.

**Competing interests**
The authors declare that they have no competing interests.

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