Phytochemicals in Food and Medicine

WILEY

RESEARCH PAPER OPEN ACCESS

Bio-Insecticidal Efficacy of Phytochemicals in *Acorus calamus* Rhizome and *Piper longum* Plant Extracts as Green Alternatives to Synthetic Insecticides Against *Tribolium castaneum*

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Received: 8 January 2026 | **Revised:** 9 June 2026 | **Accepted:** 10 June 2026

Keywords: asarone | botanical insecticides | greener alternative | piperine | storage grain pests | *T. castaneum*

ABSTRACT

To meet the increasing food demands of a growing global population, agricultural productivity must rise substantially. However, the excessive reliance on synthetic pesticides has resulted in the development of resistance among stored-product insect pests, necessitating the search for sustainable and eco-friendly alternatives. The present study evaluates the bioactive potential of extracts obtained from the rhizomes of *Acorus calamus* Linn and the fruits of *Piper longum* Linn against *Tribolium castaneum* Herbst. The investigation focused on the insecticidal, phenolic, flavonoid and antioxidant properties of the extracts, along with the identification of the bioactive compounds responsible for these activities. *A. calamus* exhibited the highest repellency (98.3%) and induced complete mortality within 9 days at a concentration of 100 mg/mL, with Lethal Time 50 (LT_{50}) and Lethal Time 90 (LT_{90}) of 5.34 and 7.70 days, respectively. In contrast, *P. longum* demonstrated 92.1% repellency but did not exhibit significant contact toxicity. Gas chromatograph–mass spectrometry profiling revealed asarone (88.2%) as the predominant bioactive constituent in *A. calamus*, whereas piperine (39.5%) was identified as the major compound in *P. longum*, both of which are likely responsible for the observed insecticidal activity. Furthermore, both extracts displayed strong antioxidant potential, with *P. longum* showing higher total phenolic content (10.45 mg GAE/g) and total flavonoid content (9.17 mg QE/g) compared to *A. calamus*. Overall, the findings suggest that extracts of *A. calamus* rhizomes and *P. longum* fruits possess significant potential as sustainable plant-derived protectants for stored commodities, providing an environmentally friendly alternative to conventional synthetic insecticides.

1 | Introduction

The agriculture sector is widely regarded as the backbone of global survival, fulfiling the essential requirements of the increasing population. Cereals are a critical component of the global food chain and must be consistently available for everyone worldwide (D'cruz et al. 2023). As per the United States Food and Agricultural Organisation (FAO), around 17% of the food that is produced in the world is spoilt during storage and out of which 10% of the production is affected by insects, 7% by other factors such as rodents, mites and other diseases. Upadhyay et al. (2018) reported that nearly 100 species from the Tenebrionidae family (Order: Coleoptera) are responsible for postharvest losses in stored food products. Among these, *T. castaneum* Herbst, is a particularly destructive pest causing losses to a broad spectrum of

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Highlights

- The plant extracts of *Acorus calamus* and *Piper longum* can be used as a bio-based insecticide against storage insects.
- *A. calamus* rhizome extract and *P. longum* fruit extract has 98% and 92% repellency at 500 mg/mL concentration, respectively.
- Plant extract from *A. calamus* have 100% mortality in 9 days against *Tribolium castaneum.*

stored food products, including flour, milled rice, nuts, spices, cereals, legumes and baked goods. *T. castaneum* is widely distributed in tropical and warm climates worldwide and is capable of infesting a broad spectrum of crops such as wheat, maize, rice, legumes and oilseeds. This results in considerable food weight loss and severe economic impacts (Zhang et al. 2023). The red flour beetle is highly prolific, completing its life cycle from egg to adult in just four to 6 weeks, which contributes to its rapid multiplication and devastating effects on stored food products (Agarwal and Agashe 2020). Over decades, the indiscriminate and unregulated use of synthetic pesticides to control primary and secondary storage pests has led to significant environmental, ecological and ecotoxicological challenges (Thirugnanam et al. 2026). This overuse has been associated with a decline in ecological diversity and negatively impacts beneficial insects such as pollinators, predators and pest parasitoids (Monisha and Loganathan 2022). Given the current food and agri business relies on a limited range of approved pesticides and nontoxic control systems (Stejskal et al. 2021), there has been a growing interest in scientists to research environment friendly solutions for pest management on a global scale (Chandran and Manickam 2026). The botanical insecticides have emerged as promising alternatives demonstrating properties such as insect repellency and limited contact toxicity (Mangang et al. 2020).

A. calamus, commonly known as sweet flag and a member of the Acoraceae family, has been identified as a suitable botanical material for storage pest management. Sweet flag exhibited various insecticidal properties (El-Nahal et al. 1989) including ovicidal and oviposition deterrent, antifeedant (Koul and Isman 1990) and repellency (Aryal et al. 2023). Aryal et al. (2023) reported that 2.92% of *A. calamus* oil achieved 50% mortality in 3 h whereas 8.75% oil gave 90% mortality against *Sitophilus zeamais* during the scintillating vial bioassay test. The combined fumigant and contact effects within a closed vial environment contributed to high death rates and shorter survival duration. Additionally, Dhivya et al. (2019) observed that cowpea seeds treated with 1% of *A. calamus* nano-emulsion for 24 h exhibited 90% fatality compared to 78.33% for the microscale emulsion at the same concentration. Similarly, at 0.8% concentration, the nano-emulsion achieved an 80% fatality rate against pulse beetle (*Callosobruchus maculatus F.*) compared to 63.33% for the microemulsion.

Piper longum and *Piper retrofractum* commonly referred to as long pepper are native to southeast Asia and are widely known as 'pippali' in India. The principle components of long pepper have exhibited insecticidal, deterrent and repellent effect against a variety of pests (Isman 2006; Pumnuan et al. 2022). This highlights the potential of the plant's dual properties as a viable alternatives to chemical insecticides. Although previous studies have reported the insecticidal and repellent activities of *A. calamus* and *P. longum*, the present study provides a comparative evaluation of multiple bioactivity modes under standardised conditions. By correlating phytochemical composition and physicochemical characteristics with biological activity, this study highlights the functional differentiation of these extracts as repellent and contact toxic agents for targeted botanical pest management. Thus, this study aims to evaluate the effect of plant extracts from two botanicals, *P. longum* and *A. calamus*, on the insect repellent activity against *T. castaneum*. Additionally, the studies examine the chemical, functional, antioxidant, phenolic, and flavonoid content of the plant extracts, providing insights into their potential as an eco-friendly pest control solution.

2 | Materials and Methods

2.1 | Plant Materials

Sweet flag rhizome (*A. calamus*) and long pepper fruits (*P. longum*) are the two dried plant parts that were selected for this study against *T. castaneum* which is collected from Spice Board, India. The identification of the procured plants was done by the Spice Board, Government of India. They were then put in airtight containers and stored in dark place for further analytical purpose.

2.1.1 | Extract Preparation

The dried plant materials were thoroughly cleaned to remove foreign particles and subsequently milled using a pulveriser to obtain a fine powder. The powdered samples were transferred into airtight containers and stored in a dark environment at room temperature until further analysis.

2.1.2 | Extraction of the Botanicals

Soxhlet extraction was done by a method suggested by Kongkham et al. (2024) with a slight modification. Powdered sample of 30 g was taken in a filter paper and was extracted using 300 mL of methanol as the solvent, maintaining a solid to solvent ratio of 1:10 (w/v). The extraction was performed in soxhlet apparaturs at a temperature of ~65°C for 6 h until the solvent un the syphon tube turned colourless. Once the extraction was done, the solvent was evaporated using of rotatory evaporator at temperature of 45°C–50°C for 30 min. The obtained extract was kept in clean amber-coloured vials and stored in dark place under refrigeration condition at 4°C for further use.

2.2 | Volatile Fingerprinting of Plant Extracts Using Gas Chromatography–Mass Spectroscopy (GC–MS)

GC–MS is a quantitative as well as a qualitative technique for determining the volatile components in the sample (Mangang et al. 2020). The essential oils were extracted using methanol as the solvent and the constituents were analysed with GC–MS (Make: Agilent Model: 8890GC/5977B GC/MSD). The essential

oils of 0.5 mL were taken in a test tube with 5 mL of methanol. It was vortexed for 5 min and then kept in the shaker for 12 h overnight at 250 rpm. The extract was then filtered through Whatman filter paper No.1 with sodium sulphate in it. Sodium sulphate is used to remove any trace of moisture present in the sample. The filtrate was then collected in vials and injected into the GC–MS. The sample was injected through an auto sampler (2 μL) and was passed through the column (Rtx-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30 m × 0.25 mm ID × 0.25 μm df)) with the help of the carrier gas (1 mL per min, Split 10:1). The source temperature of the GCMS was kept at 250°C with an inlet temperature of 290°C. Electron energy of 70 eV is applied to the sample to separate the components in the sample which is then passed through the mass scanning at 50–550 amu. The delay of the solvent was for 0–3.5 min, and the total GC and MS run time was 40.50 min. After the run time, the data were recorded and the chromatogram for each of the sample was obtained with the support of NIST library version 2020.

2.3 | Functional Characteristics of the Plant Extract Using Fourier Transform Infrared Spectroscopy (FTIR)

An FTIR with attenuated total reflectance (ATR) (Shimadzu, IRTracer-100, India) was used to determine functional characteristics of the plant extract (Manickam et al. 2024). Five microlitres of the essential oil were put into the ATR cell for examination. The transmittance (%T) was measured over a range of 400–4000 cm^{-1} from 45 scans, with a wavelength resolution of 4 cm^{-1}. LabSolutions software was then used to analyse the data. DLATGS detector was used with source from IR (Ceramic Rod) and Kbr was used as a beam splitter. LabSolutions IR Software was used for the obtaining and preprocessing of the spectral data. All the transmittance spectra were baseline-corrected utilising several anchor points to guarantee data consistency and minimise baseline offsets.

2.4 | Antioxidant Activity of Plant Extract

The antioxidant activity of the EOs were assessed using DPPH (2,2-diphenyl- 1-picrylhydrazyl) method. For this purpose, a modified version of Assaggaf et al. (2024) protocol was used. One gram of sample and the standard were taken and dissolved in 100 mL of methanol, and this was taken as stock solution. Further, 200 μL aliquots of samples and standards from the stock solution were taken in various concentrations (100, 200, 300, 400 and 500 μg/mL) making it up to 1 mL using methanol and to that 1 mL of DPPH solution (0.004 mg/100 mL) was added. The resulting solutions were then placed in a dark area and incubated for 30 min. The absorbance was then measured with a UV spectrophotometer (Thermo fisher scientific, model: GENESYS 50) at 517 nm. Methanol was taken as blank, and DPPH was taken as the control. The triplicate values were noted, and the inhibition percentage was calculated.

$$\text{Inhibition Percentage} = \frac{C - S}{C} \times 100$$

where C—absorbance of control and S—absorbance of sample

2.5 | Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Plant Extract

TPC of the extracted plant extracts is assessed using Folin and Ciocalteu reagent (FC reagent), and aluminium chloride colourimetric method was employed to determine TFC (Chandra et al. 2014) with a slight modification. Further, TPC is expressed as milligrammes of gallic acid equivalents per gram of extract (mg GAE/g extract), whereas TFC was expressed as milligrammes of quercetin equivalents per gram of extract (mg QE/g extract).

2.6 | Culturing of Insects

The adult *T. castaneum* beetles were received and initially reared from the mother culture maintained at NIFTEM—Thanjavur, Tamil Nadu, India. The insects were reared in sterilised glass jars containing 100 g of whole wheat flour as the diet. The flour was pretreated (sterilised in hot air oven at 60°C–80°C for 4–6 h) to avoid any prior infestation. 30 randomly selected adults from mother culture (unsexed) were introduced into each jar and maintained under controlled conditions of 32°C–37°C temperature, 70%–80% relative humidity and ~12:12 h light:dark photoperiod. Adults were allowed to mate and oviposit for 24 h, after which they were removed using a 0.6 mm mesh sieve. Eggs were allowed to develop into larvae, pupae and adults under the same conditions. Newly emerged adults (0–3 days old) were collected and used for experiments. No sex separation was performed, and mixed-sex cohorts were used.

2.7 | Repellent Activity Against *T. castaneum*

Repellency test was done using the area preference method based (Mangang et al. 2020). Whatman No 1 filter paper of 9 cm diameter was taken and cut into two equal halves. Both the halves were kept in clean petri plates of 9 cm diameter. One half of the filter paper was coated with 0.5 mL of different concentration of the plant extract (100, 200, 300, 400 and 500 mg/mL) that was prepared using methanol. The other half was treated with methanol as control and was let to dry for a minute. Twenty adults of *T. castaneum* are introduced in the centre of the two halves and the lid was closed with a little space in-between the lid and the petri plate. The number of insects in each portion of the filter paper was recorded every hour for 24 h. The experiment was replicated 4 times for all the five concentrations, and the percentage repellency was calculated using the formula,

$$\text{Repellent activity } (\%) = \frac{(\text{NUT} - \text{NT})}{(\text{NUT} + \text{NT})} \times 100, \quad (1)$$

where NUT—no. of insects in the untreated area and NT—no. of insects in the treated area.

2.8 | Contact Toxicity Assay Against *T. castaneum*

The contact toxicity of the two plant extracts (*A. calamus* and *P. longum*) were conducted following the residual film method (Aisha et al. 2024) with a slight modification. For the test, 5 different concentrations of the plant extract (EO) were prepared at 100, 200, 300, 400 and 500 mg/mL with methanol as a solvent.

Upon preparation, clean petri plates of 9 cm diameter were taken. Whatman No 1 filter paper of 9 cm diameter was taken and fitted inside the clean petri plates. The filter paper was coated with 1 mL of the prepared concentration uniformly and let to dry for a minute. Twenty adults of *T. castaneum* were introduced on the coated filter paper kept inside the petri dish and the lid was closed. The number of dead and live insects were counted every day for 9 days and recorded. The percentage mortality was calculated using Abbott's equation (Abbott 1925). The lethal time was also calculated using probit analysis. The experiment was replicated four times. The control was coated with the solvent (methanol) alone without the oil.

$$\text{Contact toxicity } (\%) = \frac{C - T}{C} \times 100, \quad (2)$$

where *C*—percentage of insects alive in the control plate and *T*—percentage of insects alive in the treated plate

2.9 | Fumigant Toxicity Against *T. castaneum*

Fumigant toxicity of the plant extracts against *T. castaneum* were done based on the method suggested by Aisha et al. (2024) with a minor modification. An airtight plastic container of 100 mL volume was used for the experiments. The circular discs of 3.5 cm diameter was cut out from whatman No.1 filter paper. The filter paper was coated with different concentrations of the plant extracts 100, 200, 300, 400 and 500 mg/mL using a micropipette and let to dry for a minute. Ten adults of *T. castaneum* were introduced into the container with 2 g of feed (wheat flour). The coated filter paper was strung and suspended from the lid of the container without coming in contact with the insects at the bottom and the lid was closed tightly. Each concentration was replicated four times to reduce the error. The mortality of the insects was recorded every 12 hours and calculated using the formula,

$$\text{Fumigant toxicity } (\%) = \frac{C - T}{C} \times 100, \quad (3)$$

where *C*—percentage of insects alive in the control container and *T*—percentage of insects alive in the treated container.

2.10 | Statistical Analysis

The mortality assessment of *T. castaneum* (LT_{50} and LT_{90}) was done using probit analysis statistical approach using Polo plus 2.0 (LeOra software, Petaluma, CA, USA) (Loganathan et al. 2011). To ascertain the significance of the treatments, all the data sets were subjected to IBM SPSS software version 2023 and grouped using the one-way ANOVA and Tukey's test (Aisha et al. 2024). The level of significance was assessed at $p < 0.05$. The FTIR graph was drawn using Origin pro version 2024b.

3 | Results and Discussion

3.1 | Determination of Volatile Components of Plant Extracts Using GC–MS

The Soxhlet extraction method was employed to extract plant extracts from the botanicals *A. calamus* and *P. longum*. The moisture content of the powdered raw material used for extraction was 8.94% ± 0.07% for *A. calamus* and 9.02% ± 0.04% for *P. longum*. The yield of plant extract per 100g of powdered sample was 14.7 ± 0.08 mL for *A. calamus* rhizome and 13.4 ± 0.04 mL for *P. longum* when methanol was used as the extraction solvent. The GC–MS analysis identified 12 active components in *A. calamus* plant extract and 68 active components in *P. longum* plant extract. Among these, eight constituents in *A. calamus* and 24 in *P. longum* exhibited prominent peak areas. The major compounds found in *A. calamus* were asarone (88.21%), followed by its isomers β-asarone (3.48%) and γ-asarone (1.78%) (Table 1). Asarone (2,4,5-trimethoxy phenylpropanoid) is a phenylpropanoid characterised by a propen-1-yl group is substituted at position 5 and methoxy groups are attached at positions 1, 2 and 4 of the benzene ring. It functions as a plant metabolite and belongs to the alkenyl benzene and methoxybenzene groups.

Piperine, an alkaloid, responsible for the characteristic pungency of Piperaceae family was the primary compound identified in *P. longum* plant extract (Derosa et al. 2016). The retention times for the major components of *A. calamus* are 11.0832 min for asarone, 11.7184 min for β-asarone and 10.4252 min for γ-asarone. The retention times for the components of *P. longum* are found to be 28.2263 min for piperine, 24.2781 min for Iso di hydro

TABLE 1 | GC–MS analysis of *A. calamus* plant extract.

RT	Compound name	Formula	Molecular weight	Peak area (%)
11.0832	Asarone	$C_{12}H_{16}O_3$	208.1	88.21
11.7184	β—Asarone	$C_{12}H_{16}O_3$	208.1	3.48
10.4252	γ—Asarone	$C_{12}H_{16}O_3$	208.1	1.78
11.7985	Muurola-4,10(14)-dien-1beta-ol	$C_{15}H_{24}O$	220.2	1.02
16.7252	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.3	1.01
12.7369	Coumarin-6-ol,3,4-dihydro-4,4,7,8 tetramethyl-	$C_{13}H_{16}O_3$	220.1	0.99
9.4696	Cedrol	$C_{15}H_{26}O$	222.2	0.79
14.5222	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	0.48
9.7386	Caryophyllene oxide	$C_{15}H_{24}O$	220.2	0.75
18.8802	Benzene, 1,2-dimethoxy-4-propenyl-, (Z)-	$C_{11}H_{14}O_2$	178.1	0.55
12.1075	Ylangenol	$C_{15}H_{24}O$	220.2	0.49
12.5996	Isocalamenediol	$C_{15}H_{26}O_2$	238.2	0.45

piperlonguminine and 32.7754 min for pipernonaline. In the current study, the primary compounds of *P. longum* were piperine (39.50%), followed by Iso di hydro piperlonguminine (4.65%) and Pipernonaline (4.68%) (Table 2). Rawat et al. (2022) reported the identification of 48 components with piperine (54.46%) in the chloroform extract as the major eluted constituent. In a separate study by Liu et al. (2013), *A. calamus* plant extract was found to contain 50.09% of α-asarone and 3.51% of β—asarone among 32 identified components. Similarly, R. Kumar et al. (2016) reported the presence of 18 components with β—asarone as the predominant component eluted. Their study highlighted the geographical variation in β—asarone concentration which ranged from 31.56% to 91.27%, influenced by differences in ploidy levels and environmental conditions. Elshikh et al. (2022) emphasises the potential use of asarone and its derivatives in integrated pest management for their repellent and contact toxicity effects. The observed variation in the chemical composition of the plant extracts may primarily be attributed to differences in plant variety, regional origin and climatic and seasonal conditions as well as storage duration, all of which are known to significantly influence the volatile components present (Liu et al. 2013).

3.2 | Analysis of the Plant Extracts With Fourier Transform Infrared Spectroscopy

The FTIR analysis was carried out to determine the structural composition and functional groups present in the extracts of *A. calamus* (Figure 1) and *P. longum* (Figure 2). The obtained spectra predominantly revealed the presence of aldehydes, alkenes, vinyl ethers, alcohols and related functional groups, which were largely consistent with previously reported studies. For *A. calamus*, the present study identified major absorption peaks comparable to those reported in earlier investigations. A prominent peak observed at 2929.87 cm^{-1} corresponding to C–H stretching and aldehydic functional groups was in close agreement with the peaks reported by Thakkar et al. (2024) at 2993.63 and 2936.14 cm^{-1} associated with $-CH_3$ stretching vibrations. Similarly, the strong absorption peak at 1203.58 cm^{-1} observed in the current study, indicative of C–O stretching of vinyl ether groups, closely corresponded with the peak at 1208.02 cm^{-1}

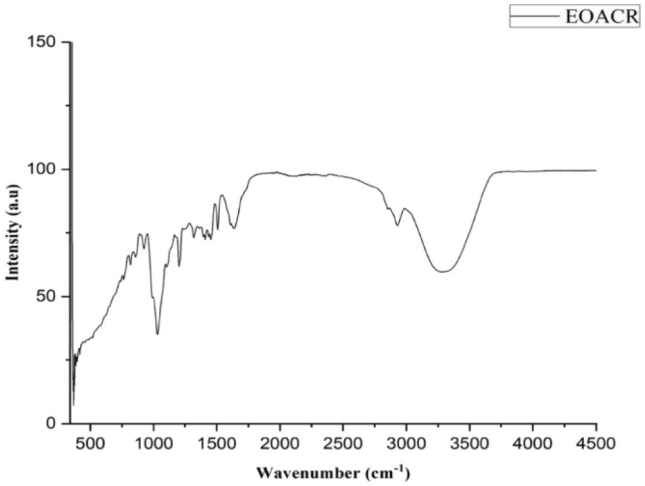


FIGURE 1 | FTIR spectrum of *A. calamus* plant extract indicating the presence of functional groups.

TABLE 2 | GC–MS analysis of *P. longum* plant extract.

RT	Compound name	Formula	Molecular weight	Peak area (%)
6.2308	Benzene propanoic acid, methyl ester	$C_{10}H_{12}O_2$	164.1	0.14
7.1749	Piperonal	$C_8H_6O_3$	150	0.22
7.8330	Propanamide, N-(2,6-dimethylphenyl)-3-(1-piperidinyl)-	$C_{16}H_{24}N_2O$	260.2	0.23
8.4967	Caryophyllene	$C_{15}H_{24}$	204.2	0.87
9.4237	Hexadecane, 1-chloro-	$C_{16}H_{33}Cl$	260.2	0.74
11.0144	Asarone	$C_{12}H_{16}O_3$	208.1	1.85
11.7640	8-Heptadecene	$C_{17}H_{34}$	238.3	1.07
13.2174	Acorenone B	$C_{15}H_{24}O$	220.2	0.19
14.6880	2,4-Decadienamide, N-isobutyl-, (E, E)-	$C_{14}H_{25}NO$	223.2	1.35
16.5305	2,4-Pentadienoic acid, 5-(1,3-benzodioxol-5-yl)-, methyl ester	$C_{13}H_{12}O_4$	232.1	0.97
17.4288	(2E,4E)-1-(piperidin-1-yl) deca-2,4-dien-1-one	$C_{15}H_{25}NO$	235.2	0.18
20.0896	Pipataline	$C_{19}H_{28}O_2$	288.2	0.24
22.3956	1-Chloroeicosane	$C_{20}H_{41}Cl$	316.3	0.18
23.3569	(2E,4E)-N-isobutylhexadeca-2,4-dienamide	$C_{20}H_{37}NO$	307.3	0.56
24.2781	Iso di hydro piperlonguminine	$C_{16}H_{21}NO_3$	275.2	4.65
27.6827	(E)-1-(piperidin-1-yl) icos-2-en-1-one	$C_{25}H_{47}NO$	377.4	0.20
28.2263	Piperine	$C_{17}H_{19}NO_3$	285.1	39.50
29.2506	Piperundecalidine (isomer 2)	$C_{23}H_{29}NO_3$	367.2	0.15
31.3563	(E)-9-(Benzo[d] [1,3] dioxol-5-yl)-1-(piperidin-1-yl) non-8-en-1-one	$C_{21}H_{29}NO_3$	343.2	0.14
32.7754	Pipernonaline	$C_{21}H_{27}NO_3$	341.2	4.68
33.8912	Stigmasterol	$C_{29}H_{48}O$	412.4	0.33
35.2072	γ—Sitosterol	$C_{24}H_{33}NO_3$	383.2	0.34

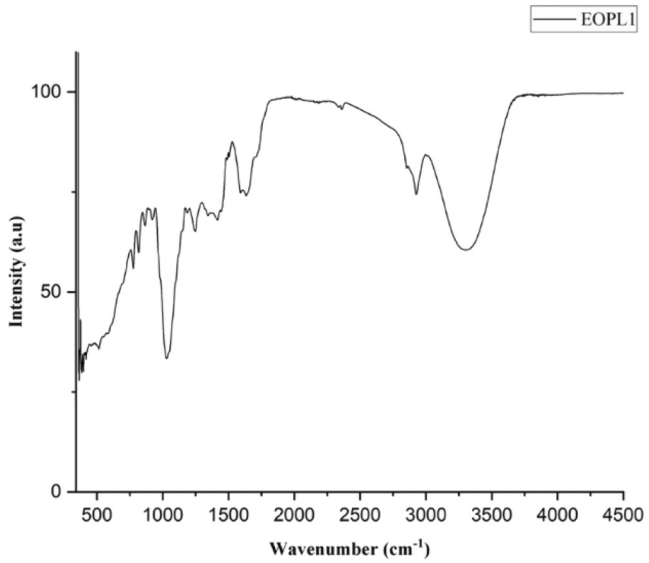


FIGURE 2 | FTIR spectrum of *P. longum* plant extract indicating the presence of functional groups.

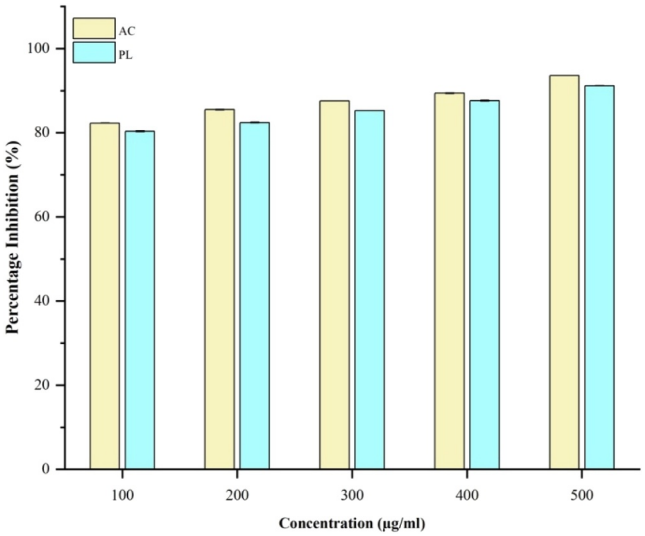


FIGURE 3 | DPPH radical scavenging activity of *A. calamus* rhizome and *P. longum* fruit extracts at different concentrations.

reported by Krishnan et al. (2022), which was associated with the presence of asarone. Additional peaks observed at 763.8, 815.89 and 858.32 cm^{-1} corresponding to substituted aromatic C–H bending patterns also aligned with the characteristic spectral features previously reported for *A. calamus* extracts. These similarities indicate that the functional groups identified in the present study are in accordance with the established results of *A. calamus*.

Similarly, the FTIR spectrum of *P. longum* fruit extract demonstrated spectral patterns highly comparable with earlier reports. The peak observed at 2924.09 cm^{-1} in the present study closely matched the peaks at 2928 cm^{-1} reported by Reddy et al. (2014) and 2923 cm^{-1} reported by Nakkala et al. (2016), both corresponding to C–H stretching vibrations. The peak observed around 1610 cm^{-1} was also comparable to the C=C stretching peak reported at 1598 cm^{-1} in previous studies. Furthermore, the absorption peak at 1035.77 cm^{-1} corresponding to C–O stretching closely agreed with the peak at 1041 cm^{-1} previously documented. Overall, the FTIR findings obtained in the present study showed strong agreement with earlier published reports, thereby confirming the consistency of the identified functional groups and spectral constituents in both plant extracts.

3.3 | Antioxidant Activity of Plant Extract

The *A. calamus* exhibited an inhibition rate of 93.58%, whereas *P. longum* showed an inhibition of 91.172% with both results being statistically significant ($p < 0.05$). These findings indicate that plant extracts are rich in antioxidants (Figure 3). The antioxidant activity exhibited a dose-dependent response, with percentage inhibition increasing linearly as the concentration of the sample increased. The previous studies have reported comparable results. Akbar et al. (2014) reported a 76.86% inhibition in *P. longum* ethanolic extract at 500 µg/mL, whereas Reddy et al. (2014) reported a higher inhibition concentration of 87% in *P. longum* fruit aqueous extract. Devi and Ganjewala (2011) observed a concentration-dependent antioxidant activity, with 84% inhibition observed in rhizome extract. Additionally, Loying et al. (2019) reported 96% inhibition at 48 µg/mL concentration. The antioxidant activity of plant extracts from *A. calamus* and *P. longum* was visually indicated by the colour change from violet to yellow in the DPPH assay, signifying effective scavenging if free radicals. The high inhibition percentages suggest that *A. calamus* and *P. longum* are effective in neutralising free radicals. The higher percentage inhibition of *A. calamus* also has a positive correlation with the insecticidal activity against *T. castaneum*.

3.4 | TPC of the Plant Extract

The results revealed that *A. calamus* rhizome plant extract contained 8.51 mg GAE/g of extract of phenolics. Bahukhandi et al. (2013) reported that the TPC of methanolic extract of *A. calamus* varied depending on the geographical location within Uttarakhand, ranging from 4.58 mg GAE/g of dry weight in Metalla to 9.25 mg GAE/g of dry weight of sample in Jyoli. Their study also emphasised that the solvent used during extraction significantly influences the TPC of the plant (Figure 4). In a subsequent study, Bahukhandi et al. (2021) indicated the impact of seasonal variation in the TPC content of *A. calamus*. Samples collected from the same location showed variations from 5.87 mg GAE/g of dry weight in June (vegetative growth phase) to 9.11 mg GAE/g of dry weight of sample in October (preparation for senescence period). In contrast, Devi and Ganjewala (2011) reported a subsequently higher TPC of about 27 mg GAE/g of dry weight of sample in the methanolic extract of *A. calamus* rhizome grown in an herbal garden. The phenolic content of *P. longum* was found to be 10.45 mg GAE/g of dry weight of sample. A study conducted by Thapa et al. (2024) revealed that the methanolic extract of *P. longum* fruit contained 10.55 mg GAE/g of dry weight, and the crude extract exhibited a higher phenolic content of 391.74 mg GAE/g of sample. The study also noted that phenolic compounds, known for their antioxidant property, effectively scavenge free radicals by interrupting chain reactions.

3.5 | TFC of the Plant Extract

The TFC values were calculated using a standard curve prepared using quercetin. The analysis revealed that the *P. longum* plant extract exhibited a (Figure 4) higher TFC (9.17 mg/QE/g of sample) than the *A. calamus* plant extract (7.04 mg QE/g of sample). These findings are in accordance with the previous studies, although slight variations in flavonoid content is mainly due to plant origin, extraction method, and solvent utilized. Devi and Ganjewala (2011) reported a higher flavonoid content of 11.1 mg QE/g in *A. calamus* rhizome extract, while Susanah et al. (2018) reported significantly lower flavonoid content of 1.904 mg QE/g of sample in *A. calamus* extract. Regarding *P. longum*, Thapa et al. (2024) revealed TFC of 10.55 mg QE/g of dry weight in the methanolic fraction, whereas the crude extract showed a higher content of 113.94 mg QE/g of dry weight. Additionally, a study indicated that the ethanol extract of *P. longum* grown in Bangladesh had a flavonoid content of 41.65 mg QE/g of extract (Akbar et al. 2014). The observed variations in results can be attributed to several factors, including differences in the solvent used for extraction, growth conditions and the geographical location of the botanical (Bahukhandi et al. 2021).

3.6 | Repellency Activity Against *T. castaneum*

The repellent activity of plant extracts from *A. calamus* and *P. longum* was evaluated against adult *T. castaneum* beetles showing significant efficacy (Table 3). *A. calamus* rhizome plant extract exhibited 90%–98% ($p < 0.05$) repellency, whereas *P. longum* showed 63%–92% ($p < 0.05$) against adult *T. castaneum*. This study observed a dose and time dependent repellent activity, with the active components asarone in *A. calamus* and piperine in *P. longum*, their derivatives contributing to the insecticidal effects. The repellent effect was directly proportional to the concentration with higher concentrations leading to greater repellency. At 100 mg/mL, *Acorus calamus* achieved a repellency of 90.21% ± 10.46%, significantly higher than that of *P. longum* (63.54% ± 15.53%). Further increasing the concentration to 500 mg/mL resulted in near-complete repellency, reaching 98.33% ± 5.56% and 92.08% ± 11.78% for *A. calamus* and *P. longum*, respectively. Chen et al. (2015) reported that a 10% essential oil (*A. calamus)* achieved up to 98% repellent efficacy against *T. castaneum*. Similarly, Aryal et al. (2023) found that *A. calamus* essential oil exhibited significant repellency against *S. zeamais*, with a 10% concentration achieving up to 98.75% repellency, and even the lowest concentration (1.25%) showing approximately 77.5% repellency. Additionally, α-asarone displayed insecticidal activity against grain storage pests such as *Sitophilus oryzae* and *Callosobruchus chinensis*. Miao et al. (2016) demonstrated that with sweet flag oil treatment for 60 h, the repellent activity against *T. castaneum, Rhyzopertha dominica, Tribolium confusum and Oryzaephilus surinamensis* exceeded 90%. J. Kumar et al. (2024) reported that the leaf extract of *P. longum* exhibited significant toxicity against *T. castaneum* eggs and larvae. Furthermore, Sharmah et al. (2018) noted that the methanol extract of *P. longum* fruits exhibited insecticidal activity against *Myzus persicae, Spodoptera litura and Tetranychus urticae,* achieving a 100% control rate at a concentration of 1000 ppm. The differences in repellent activity may be due to the concentration of active components, secondary metabolites, climatic growth conditions of the botanicals, the extraction method employed, the type of solvent used for the extraction of the plant extract and the target pest for the extracted botanical. Thus, indicating that *A. calamus* has a better repellent activity against *T. castaneum* in comparison to *P. longum.*

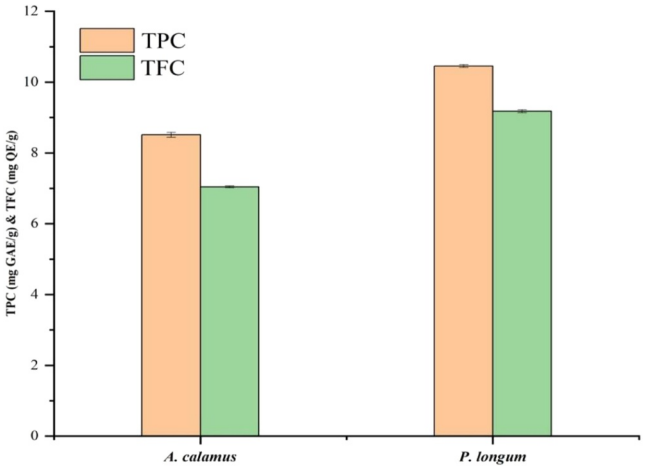


FIGURE 4 | Total phenolic and flavonoid content of *A. calamus* and *P. longum*.

3.7 | Contact Toxicity Against *T. castaneum*

The contact toxicity *A. calamus* and *P. longum* plant extracts were assessed by observing the insect mortality rates. Results revealed that the mortality increased with higher dosages and longer incubation times. Notably, *A. calamus* rhizome plant extract demonstrated a significant toxic effect against *T. castaneum*. The lethal time for all the five concentrations were calculated and summarised (Table 4). Prolonged exposure of the

TABLE 3 | Repellent activity (mean ± SD) of plant extracts of *A. calamus* and *P. longum*.

Target insect	Concentration (mg/mL)	Repellency (%)	
		A. calamus	***P. longum***
T. castaneum adults	100	90.210 ± 10.460^{d}	63.54 ± 15.53^{d}
	200	91.040 ± 10.410^{cd}	72.50 ± 15.42^{c}
	300	94.250 ± 9.138^{bc}	73.33 ± 15.54^{c}
	400	96.167 ± 7.847^{ab}	84.38 ± 12.38^{b}
	500	98.333 ± 5.557^{a}	92.08 ± 11.78^{a}

Note: Within each column, mean values bearing different superscript letters are significantly different at $p < 0.05$, whereas values sharing at least one common letter are not significantly different.

TABLE 4 | LT_{50} and LT_{90} of *A. calamus* rhizome plant extract.

Concentration (mg/mL)	Lethal time	LT (range)	Slope	χ^2(df)
100	LT_{50}	5.336 (5.126–5.543)	8.050 ± 0.558	25.349 (34)
	LT_{90}	7.698 (7.309–8.205)		
200	LT_{50}	5.299 (4.966–5.640)	6.155 ± 0.422	59.381 (34)
	LT_{90}	8.559 (7.824–9.659)		
300	LT_{50}	4.438 (4.194–4680)	5.132 ± 0.345	30.859 (34)
	LT_{90}	7.887 (7.319–8.642)		
400	LT_{50}	3.987 (3.722–4.246)	5.175 ± 0.341	42.008 (34)
	LT_{90}	7.052 (6.498–7.809)		
500	LT_{50}	3.390 (3.008–3.765)	4.252 ± 0.269	83.565 (34)
	LT_{90}	6.786 (5.957–8.079)		

Note: LT_{50} and LT_{90} are expressed in days.

plant extract resulted in increased mortality rates. At a 100 mg/mL (*A. calamus*) concentration, the LT_{50} and LT_{90} values were 5.336 and 7.698 days, respectively, whereas at 500 mg/mL were observed to be 3.390 and 6.786 days, respectively. Probit analysis indicated parallel effects across different concentration with varying intercepts reflecting normal distribution data. However, the hypothesis of equality and parallelism were rejected ($p < 0.05$). No mortality was spotted in the control which was treated with methanol. The percentage mortality results (Figure 5) further demonstrated that at 100 mg/mL, 100% mortality was achieved by the 9th day, whereas at 500 mg/mL, 100% was attained more rapidly between 7th and 8th day. A study by El-Nahal et al. (1989) investigating the effect of *A. calamus* oil against *Sitophilus granarius* showed that the percentage fatalities across all five doses was not directly dose—dependent but increased over the duration of exposure. This indicates that, at the tested levels, the duration of exposure plays a more crucial role than the dosage itself. Similarly, Adfa et al. (2015) although studying the termiticidal activity of *A. calamus*, concluded that Lethal Time (LT) is more appropriate metric than lethal concentration (LC) for evaluating the effectiveness of *A. calamus* oil vapours. In this study, although *A. calamus* showed significant contact toxicity, *P. longum* showed no contact toxicity toward *T. castaneum* adults. However, J. Kumar et al. (2024) reported that leaves of *P. longum* exhibited larvicidal and ovicidal activity against *T. castaneum.* In the petri dish bioassay, ethyl acetate and methanol extracts of *P. longum leaves* exhibited notable ovicidal effects between 300 and 500 µg/dL, resulting in 100% egg mortality at ages '0′, '2′ and '4′ days. Ethyl acetate extract achieved complete mortality of fourth instar larvae at concentrations of 10, 9 and 8 µg/dL, and fifth instar larvae at 10, 8 and 7 µg/dL after 48, 72 and 96 h, respectively. The methanol extract produced similar results for fourth instar larvae at the same intervals (Figure 6), whereas fifth instar larvae exhibited 100% mortality at concentrations of 9, 8 and 7 µg/dL, respectively. Pumnuan et al. (2022) indicated that with a 24 h exposure time, their study reported that the hexane extract of long pepper showed an LC_{50} and LC_{90} values ranging from 5.57–6.75 $\mu g \cdot cm^{-2}$ and 9.30–11.56 $\mu g \cdot cm^{-2}$ against *C. maculatus* and *C. chinensis*, respectively. Although the leaf and the fruit demonstrated efficacy against storage pest, our findings indicate that the plant extracts of fruits of *P. longum* are ineffective against *T. castaneum.* These findings underscore the potent contact toxicity of *A. calamus* and highlighting its potential as an effect as an insect repellent.

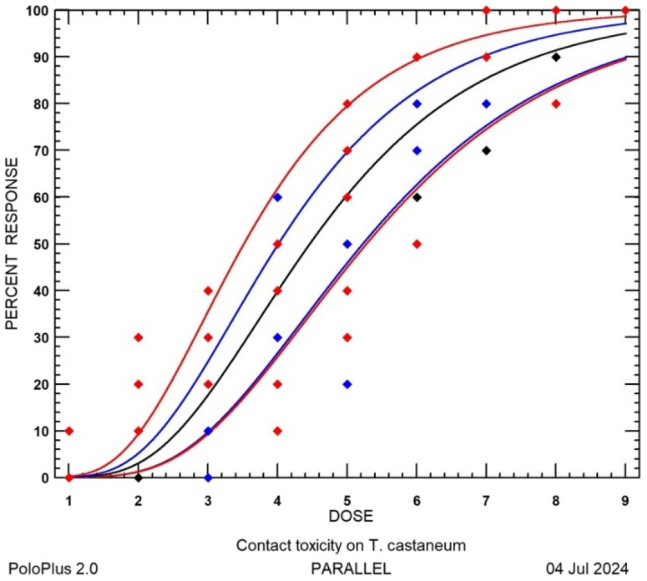


FIGURE 5 | Dose—response curve for contact toxicity of *T. castaneum* Dose—Time (days): Response—Percentage mortality of *T. castaneum*.

3.8 | Fumigant Toxicity Against *T. castaneum*

The fumigant toxicity of *A. calamus* and *P. longum* was evaluated over a period of 10 days across five different concentrations. It results indicated that neither botanicals exhibited fumigant effects against *T. castaneum* adults at any tested concentrations. In contrast, Liu et al. (2013) reported that *A. calamus* possesses fumigant toxicity toward *Liposcelis bostrychophila* with an LD_{50} value of 392.13 µg/L air. Similarly, Shukla et al. (2016) indicated that the essential oil of *A. calamus* exhibited fumigant toxicity, achieving 100% mortality at 0.1 µg/mL after 12 h of treatment against *C. chinensis.* To the best of our knowledge, there have been no reports documenting the fumigant toxicity of *P. longum*. However, our study showed that neither *A. calamus* nor *P. longum* demonstrated fumigant toxicity against *T. castaneum* adults under the tested conditions.

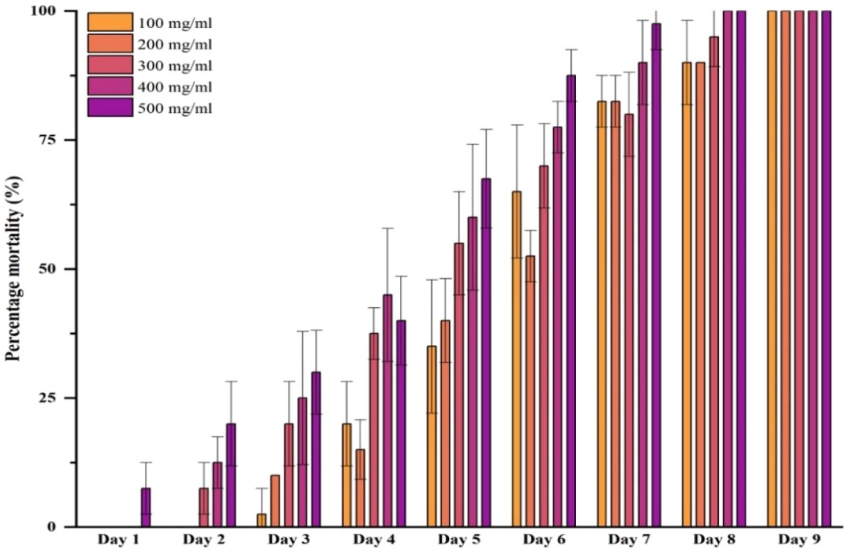


FIGURE 6 | Time and concentration dependent contact toxicity of *A. calamus* rhizome extract against *T. castaneum*.

3.9 | Insecticidal Activity

The strong repellency and comparatively low contact toxicity exhibited by *P. longum* fruit may be associated with the behavioural mode of action of piperine and related bioactive compounds present. These compounds are reported to interact primarily with insect olfactory and chemoreceptor pathways, influencing odour perception and behavioural responses rather than inducing acute toxicity (Venthur and Zhou 2018; Wicher and Miazzi 2021). The low volatility and moderate lipophilicity of piperine may further limit its penetration through the insect integument, thereby reducing its effectiveness in contact and fumigant toxicity assays whilst still producing strong repellent activity through stimulation of olfactory receptor neurons (Chandran and Loganathan 2025; Cassau and Krieger 2021). This suggests that *P. longum* may function more effectively as a botanical repellent for preventing pest infestation rather than directly eliminating insect populations. In contrast, *A. calamus* demonstrated stronger contact toxicity, which may be attributed to the presence of β-asarone and related phenylpropanoid compounds. Previous studies have reported that β-asarone exhibits insecticidal and neurotoxic effects by interfering with detoxification mechanisms, acetylcholinesterase activity and ion channel functions in insects (Xu et al. 2019; Lynn et al. 2019). Furthermore, the comparatively higher lipophilicity of asarone may facilitate enhanced penetration through the insect cuticle, resulting in greater contact toxicity upon direct exposure, whereas its relatively low vapour pressure may account for the limited fumigant activity observed. These findings indicate a functional differentiation between the two botanicals, where *P. longum* acts predominantly as a repellent, whereas *A. calamus* exhibits greater potential as a contact insecticide against *T. castaneum*.

3.10 | Correlation Between Antioxidant Activity and Repellent Efficacy of *A. calamus* and *P. longum*

The Pearson correlation analysis revealed strong positive relationships among concentration, antioxidant activity (DPPH inhibition) and repellency activity for both *A.calamus* (AC) and *P. longum* (PL) (r = 0.95–1.00), indicating that the biological activities of the extracts were concentration dependent (Figure 7). The near perfect correlation between concentration and DPPH inhibition (r = 0.99–1.00) confirms that increasing extract concentration enhanced free radical scavenging capacity, which is consistent with previous reports attributing antioxidant activity to the higher availability of phenolic and flavonoid compounds at elevated doses (Salehi et al. 2018). A strong positive correlation was also observed between antioxidant activity and repellency efficacy for both plant extracts (AC inhibition vs. AC repellency, r = 0.97; PL inhibition vs. PL repellency, r = 0.98). This finding suggests that phytochemicals contributing to antioxidant activity may also play a role in insect repellency. Phenolic compounds, flavonoids and volatile secondary metabolites have been reported to exert dual functionality by scavenging reactive oxygen species and disrupting insect olfactory and behavioural responses (Benelli and Pavela 2018; Isman 2020). Therefore, observed relationship supports the hypothesis that bioactive constituents responsible for antioxidant activity may contribute synergistically to repellency against *T. castaneum*. Furthermore, the high correlation between the repellency responses of *A. calamus* and *P. longum* (r = 0.95) indicates that both extracts exhibited a similar concentration-dependent repellent pattern despite differences in their absolute efficacy. Similarly, the strong association between the DPPH inhibition of the two plant extract (r = 0.99) suggests comparable antioxidant response trends across the tested concentration range. Such strong correlations imply that the underlying mechanisms governing antioxidant and repellent activities may be influenced by related classes of phytochemicals, although their abundance and composition may vary between species. Thus, the Pearson correlation analysis demonstrates that increasing extract concentration significantly enhanced both antioxidant and repellency activities and revealed a close association between these biological properties.

4 | Conclusion

In modern integrated pest management practices, synthetic and chemical insecticides are commonly used to control storage pests, specifically *T. castaneum*. Although effective, these methods raise serious concerns regarding their impact on human health and

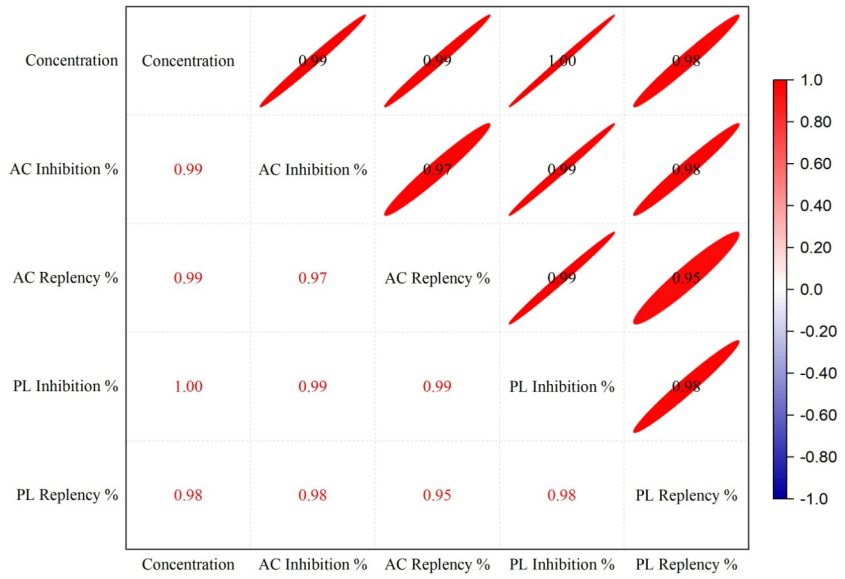


FIGURE 7 | Pearson correlation matrix showing the relationships among concentration, DPPH inhibition and repellency activities of *Acorus calamus* and *Piper longum* against *T. castaneum*.

environmental. This study explores an eco-friendly and sustainable alternative using plant extracts of *A. calamus* and *P. longum*. Both the botanicals exhibited repellent activity, with *A. calamus* showing notable contact toxicity. The primary bioactive component responsible for the insecticidal properties were identified as asarone in *A. calamus* and piperine in *P. longum*. Overall, *A. calamus* plant extract demonstrated a superior insecticidal efficacy against *T. castaneum* and displayed higher antioxidant activity, making it a promising alternative to chemical insecticides. Further research can focus on practical applications of these plant extracts such as incorporating them into packaging films, developing insect repellent pellets or as a coating material in gunny bags.

Author Contributions

Poornachandrika Viswanathan: conceptualisation, investigation, methodology, validation, formal analysis, software, data curation, writing – original draft, visualisation, resources. **Monisha Chandran:** conceptualisation, investigation, writing – original draft, writing – review and editing, methodology, software, data curation, validation, formal analysis, supervision, visualisation, project administration. **Loganathan Manickam:** conceptualisation, methodology, software, investigation, validation, supervision, visualisation, project administration, resources, writing – review and editing.

Acknowledgements

The authors express gratitude to the National Institute of Food Technology, Entrepreneurship and Management, Thanjavur (NIFTEM-T) for providing laboratory facility to this research work.

Funding Statement

This research received no specific grant from any funding sources. The authors have nothing to report.

Ethics Statement

This is to certify that all the procedures outlined in the manuscript have been performed in compliance with out institutional guidelines and policies research ethics.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from corresponding author upon reasonable request.

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