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POTENTIAL OF *PIPER CHABA* FOR THE MANAGEMENT OF THE STORED-PRODUCT PEST (*CALLOSOBRUCHUS CHINENSIS*)

ABHISHEK MUKHERJEE*, **ROHAN DAS and DEBOJOTI CHAKRABORTY**

Department of Zoology, Maulana Azad College, Kolkata-700001, West Bengal

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ABSTRACT

The present study investigated the insect-repellent potential of *Piper chaba* (Piperaceae) stem in multiple formulations-dry powder, fume and water extract-against the pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae), a major pest of stored grains. With the increasing concern over pesticide resistance, toxicity to non-target organisms and residue hazards associated with synthetic insecticides, this research emphasises plant-based alternatives for sustainable pest management. Bioassays were conducted in I-tube (wind tunnel) to evaluate repellency using various concentrations of each formulation, and the results were statistically analysed using chi-square tests and regression analysis. The dry powder of *P. chaba*stem showed a clear dose-dependent repellent response, with 1.75 g dry powder yielding 96.66% repellency ($\chi^2 = 29.36$, $p < 0.001$). Similarly, the fume exhibited full repellency at higher release rates (2–4 ml/min) compared to 60% at 0.5 ml/min ($\chi^2 = 88.71$, $p < 0.001$). Water extracts followed the same trend, with 100% extract achieving complete repellency and diluted forms showing reduced effectiveness ($\chi^2 = 66.25$, $p < 0.001$). Phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenoids, and phenols compounds previously associated with insecticidal activity. Infrared spectroscopy (IR) further validated the presence of functional groups such as O–H, C–H, and C=O, indicative of bioactive molecules. The findings establish *P. chaba* as a potent natural repellent with formulation-specific and concentration-dependent efficacy. Notably, the fume exhibits the highest repellency and richest phytochemical profile. This highlights *P. chaba*'s promise in eco-friendly pest control strategies, although further research is needed to assess toxicity on non-target species, residue safety, and field-level applicability.

Keywords: *Callosobruchus chinensis,* I-tube, *Piper chaba,* Piperine, Repellency, Stored grain pest, Sustainable pest management

INTRODUCTION

Managing pestsis a challenging aspect of crop production. Numerous species worldwide are recognized for causing significant yield losses, both quantitatively and qualitatively. In India, insect infestations alone cause 20–30% reduction in crop output. Among these, *Callosobruchus chinensis*(L) is a particularly destructive pest responsible for severe damage to stored legumes. Itis the most prevalent and widely distributed pulse beetle across tropical and subtropical regions,

*Corresponding Author Email: *2007.mukherjee@gmail.com

especially in India, Bangladesh, and Pakistan (Singh *et al.,* 2021).

Excessive use of synthetic pesticides causes environmental pollution, loss of beneficial organisms and also creates pesticide resistant pests. Repeated applications of commonly used insecticides-such as organophosphates, pyrethroids and methyl bromide fumigants-disrupt natural enemy populations and trigger secondary pest outbreaks. These concerns have led to increased interest worldwide in safer plant-derived alternatives. Plant extracts and essential oils have shown effective insect control (de Oliveira *et al*., 2020).

Piper chaba(Hunter), a culturally important yet scientifically underexplored spice, commonly known as Chui Jhal in Bangladesh and Chavya in India. It is widely consumed in southeastern Bangladesh, Tripura, West Bengal, and parts of southern India, including Tamil Nadu, Kerala and Karnataka. Beyond its culinary use, the plant is used in Ayurvedic medicine, where it is a key component of Panchakolachurna, traditionally prescribed for conditions including psoriasis (Agalcha *et al*., 2025), epilepsy (Adiga *et al*., 2024), abdominal disorders, and heartburn.Modern research has identified that *P. chaba* contains bioactive compounds such as chabamide and piperine, which are associated with antimicrobial, immune modulatory, antimalarial, antioxidant, and anticancer activities (Al-Mamun *et al., 2024). P.chaba* is a promising yet under explored botanical candidate which may have role in stored-product pest management.

MATERIAL AND METHODS

Mass Culture of *C.chinensis*

Adults of *C. chinensis* were obtained from local markets. Then a laboratory culture was established and maintained on chickpeas (*Cicer arietinum* L.), an annual legume widely used for pulse beetle rearing (Banik *et al.,* 2024). For mass culturing, 100 adult beetles of mixed sexes were introduced into a 1000 mL container containing 250 g of chickpeas. The container was lined with fine steel mesh gauze (35-mesh) to ensure adequate aeration and maintain optimal conditions for colony development. The insects were maintained at 27 ± 1 °C, 65 ± 10% relative humidity, and a 12L:12D photoperiod in a Biological Oxygen Demand (BOD) incubator. After three generations of laboratory rearing, newly emerged adults from the fourth generation (to ensure a homogeneous and laboratory acclimatized insect population for reliable bioassays) were separated by sex. Equal numbers of males and females were used in behavioral assays, with 90 individuals per bioassay.

Preparation of dry powder, water extract and fume of *P. chaba* stem

Dry powder and water extract

Shade dried maturestems of *P. chaba* were cut into small pieces and then ground into dry powder by blender. For aqueous extraction, 100 g of powder was cold-macerated in distilled water for five days at 25 ± 0.2 °C in darkness with periodic agitation. The extract was filtered with filter paper (Whatman No. 1) to obtain the crude aqueous extract.Thenthe extract was stored at 4 °C for future use.

Fume preparation

Dried stems of *P. chaba* (100 g) were coarsely powdered and subjected to dry heating in a sealed 500-mL twin-neck round-bottom flask maintained at 160 ± 10 °C using an electric mantle. Volatile fumes generated during pyrolysis were directed into a 500-mL gas-wash bottle containing layers of glass wool and silica gel to remove ash particles and moisture, respectively. The purified fumes were channeled into a 2 L glass vacuum desiccator,

Table 1. Phytochemical screening procedure

Sl.No.	Phyto-chemicals	Nameof thetest	Combinations of solutions	Out come
1	Carbohydrates	Molish's test	2 mL filtrate+ 2 drops of alcoholic α–naphthol+1 mL conc.H_2SO_4(along the sides of test tube)	A violet ring appeared
2	Proteins	Biuret test	2 ml of extract with a few drops of 2% copper sulphate solution, add 1ml of ethanol,followed by excess of potassium hydroxide pellets	Formation of pink colour in the extract layer indicates presenceof protein
3	Alkaloids	Mayer's test	2 ml of extract+few drops of 1 HCl.Take 1mL of this mixture and add 6 drops of Mayer's reagent.	Yellow cream of precipitate.
4	Glycosides	Keller-killani test	5 ml of extract+2 ml of glacial acetic acid is contained in one drop of ferric chloride solution	Appearance of brown ring
5	Phenol	Ferric chloride test	Extract aqueous solution+ a few drops 5% ferric chloride sol	Dark green / bluish black colour
6	Flavonoid	Shinoda test	1 ml of extract + three pieces of magnesium chips, and add a few drops of concent rated HCl.	Appearance of anorange, pink orred to purple colour.
7	Tannin	Ferric chloride test	10% Ferric chloride + 2 mL test solution	Darkblue/ green precipitate
8	Terpenoid	Salkowski test	Dissolve plant extract in chloroform followed by adding concentrated Sulfuric acid	Reddish brown colour of interface

which served as the fumigation chamber for bioassays. Humidity (75%) inside the chamber was regulated using a 200 mL beaker containing saturated sodium chloride solution. The setup followed the procedure of Rohitha Prasantha (2020).

Bioassays using dry powder, water extract and fume of *P. chaba* stem

Repellency test with dry powder

The repellent activity of *P. chaba* stem powder was assessed against 30 adult *C. chinensis* using varying quantities of powder (0.75–1.75 g). An I-tube (wind tunnel) apparatus was used, consisting of a rectangular chamber with removable ends and a small entry port for releasing insects.

Powdered stem material was placed at one end of the I-tube, which was sealed before introducing the beetles through the entry port. The I-tube measured 51 cm in length, 15.5 cm in width, and 15.6 cm in height, with a total internal volume of 12,331.8 cm^3 (Mukherjee & Makal,2021). Repellency was evaluated by observing insect movement and avoidance behavior.

Repellency test with fumes

Volatile fumes generated from heated *P. chaba* stem were introduced into the airtight fumigation chamber, maintained at 25 ± 1 °C. The repellency effect was assessed using adult *C. chinensis* at four different fume intensities (0.5 mL/min, 1 mL/min, 2 mL/min and 4 mL/min). After each test, the chamber was thoroughly ventilated, and fumes were discarded following the protocol described by Rohitha Prasantha (2020).

Repellency test with water extract

Repellency bioassays using the aqueous extract followed the same procedure as test with dry powder except that blotting paper strips impregnated with different concentrations of extract were used (100%, 50%, 25%, and 12.5%,). After air-drying, each treated strip was placed inside the I-tube, and adult beetles were introduced to evaluate concentration-dependent repellency.

Qualitative phytochemical screening

Qualitative phytochemical screening

Preliminary phytochemical screening of *P. chaba* extracts was conducted following the standard procedures of Harborne (1984). These qualitative assays enabled the detection of key classes of phytochemicals, including alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, terpenoids, and glycosides. Tests were based on characteristic colour changes following the addition of specific reagents (Table 1).

Infrared Spectroscopy (IR) analysis

Infrared spectroscopy was used to identify functional groups and molecular structures of the bioactive constituents in *P. chaba*. Samples were prepared usingKBr pellet method: powdered plant material ground with dry KBr and compressed into a transparent pellet.A background spectrum was recorded prior to sample analysis to account for instrument optics and atmospheric CO, /H, O absorption. The sample spectra were processed using Fourier Transform algorithms to obtain plots of intensity versus wave number (Remina *et al.*, 2022).

Statistical analysis

Repellency data from dry powder, aqueous extract, and fume bioassays were analysed separately using contingency table chi-square (χ^2) tests to determine the significance of *P. chaba* effects on *C. chinensis*. Regression curves were generated for all three types of assays to evaluate dose-dependency. The insect which does not show any response (classified as “no result”) were excluded from analysis.

RESULTS AND DISCUSSION

Effect of dry powder of *P. chaba* stem on *C. chinensis*

When dry powder of stem were used to test the repellency of insects it showed repellencyincreased with increasing weight of stem dry powder of *P. chaba*. The highest repellency was observed at 1.75 g (96.66%), whereas the lowest repellency was recorded at 0.75 g (80.00%) in I-tube (wind tunnel) apparatus. A contingency table chi-square (χ^2) test showed a highly significant association between stem powder weight and insect response (χ^2= 29.36, $p < 0.001$). These results

Table 2. Effect of drypowder of *P.chaba* stem on *C.chinensis*

Weight (Grams)	Repellency Percentage	Non Repellency Percentage	No result (%)
0.75	80	16.66	3.33
1	93.33	3.33	3.33
1.25	93.33	6.66	3.33
1.5	93.33	3.33	0
1.75	96.66	3.33	0

indicate that increasing stem powder weight significantly enhanced the repellency effect against the test insects (Table 2).

Linear regression analysis revealed a strong positive relationship between stem powder weight and repellency percentage ($R = 0.811$, $R^2 = 0.658$) which indicates that approximately 65.8% of the variation in repellency could be explained by the amount of stem powder applied.

Effect of fume of *P. chaba*stem on *C. chinensis*

While testing effect of *P. chaba*stem fume,it showed that increasing fumigation rate increases repellency, reaching 100% at 2 and 4 ml min^{-1}. Contingency table chi-square (χ^2 analysis based on the repellent and non-repellent insects revealed a highly significant association between fumigation rate and insect response ($\chi^2 = 88.71$, $p < 0.001$). These results demonstrate that increasing fumigation rate significantly enhanced insect repellency (Table 3).

Linear regression analysis revealed a strong positive relationship between fumigation rate and repellency percentage ($R = 0.840$, $R^2 = 0.705$), indicating that approximately 70.5% of the variation in repellency was explained by fumigation rate.

Effect of water extract of *P.chaba* stem on *C.chinensis*

In water extract of *P. chaba* stem experiment also,the repellency increased progressively with increasing water extract concentration and reached 100% at the highest concentration (100% extract) tested. Contingency table chi-square (χ^2) analysis based on the repellency and non repellency responses revealed a highly significant association between extract concentration and insect response ($\chi^2 = 66.25$, $p < 0.001$).These results demonstrate that increasing extract concentration significantly enhanced the biological response of the test insects (Table 4).

Linear regression analysis revealed a very strong positive relationship between

Table 3. Effect of fume of *P.chaba* stem on *C.chinensis*

Rate (ml/min)	Repellency Percentage	Non Repellency Percentage
0.5	60	40
1	66.67	33.33
2	100.00	0.00
4	100.00	0.00

Table 4. Effect of water extract of *P.chaba* stem on *C.chinensis*

Concentration	Repellency(%)	Non Repellency(%)	Noresult(%)
12.5% extract	50.00	50.00	9.00
25% extract	80.00	20.00	2.00
50% extract	90.00	10.00	2.00
100% extract	100.00	0.00	0.00

extract concentration and positive response percentage ($R = 0.948$, $R^2 = 0.899$), indicating that approximately 89.9% of the variation in response was explained by extract concentration.

Phytochemical Screening Analysis

Phytochemical screening of *P. chaba* stem (fume and extract) revealed substantial differences in chemical composition (Table 5). The fume exhibited strong and immediate positive reactions for most phytochemical groups, particularly alkaloids, flavonoids, glycosides, carbohydrates, and proteins. In contrast, the crude extract showed fewer positive reactions, though terpenoids were strongly present, and carbohydrates, proteins, and alkaloids showed moderate positivity.These findings indicate that thermal volatilization during fumigation may liberate or transform several bioactive compounds not readily extractable in the crude aqueous extract.

Infrared Spectroscopy

The IR spectrum of *P. chaba* dry stem powder (Fig. 1) revealed several diagnostic peaks corresponding to characteristic functional groups. Such as 3417.77 cm^{-1} (44.25%T): Broad O–H stretching, indicating alcohols, phenols, or carboxylic acids, 2922.28 cm^{-1} (56.24%T): C–H stretching of aliphatic hydrocarbons, 1621.75 cm^{-1} (51.12%T): C=C or C=O stretching, consistent with alkenes, aromatic rings, or carbonyl compounds, 1514.53 cm^{-1} (59.52%T): Aromatic C=C stretching.1423.98 cm^{-1} (56.22%T): C–H bending of methyl or methylene groups, 1384.32 cm^{-1} (56.34%T): Symmetric bending of –CHƒ groups or aldehydic C–H bending, 1318.67 cm^{-1} (55.70%T): Possible C–H bending or C–O stretching,1251.00 cm^{-1} (58.32%T): C–O stretching of alcohols, phenols, ethers, or esters, 1030.20 cm^{-1} (47.09%T): Strong C–O stretching typical of

Table 5. Phytochemical screening of *P.chaba*

Phytochemicals & their tests	Fume	Extract
Alkaloid	++	+
Flavonoid	++	-
Tannin	+	-
Phenol	+	-
Glycoside	+	-
Terpenoid	+	++
Carbohydrate	++	+
Protein	+	+

{++*denotes instantaneous results: A strong and immediate positive observation, suggesting a significant presence, + denotes A clear positive observation, - denotes* No pre sence detected}

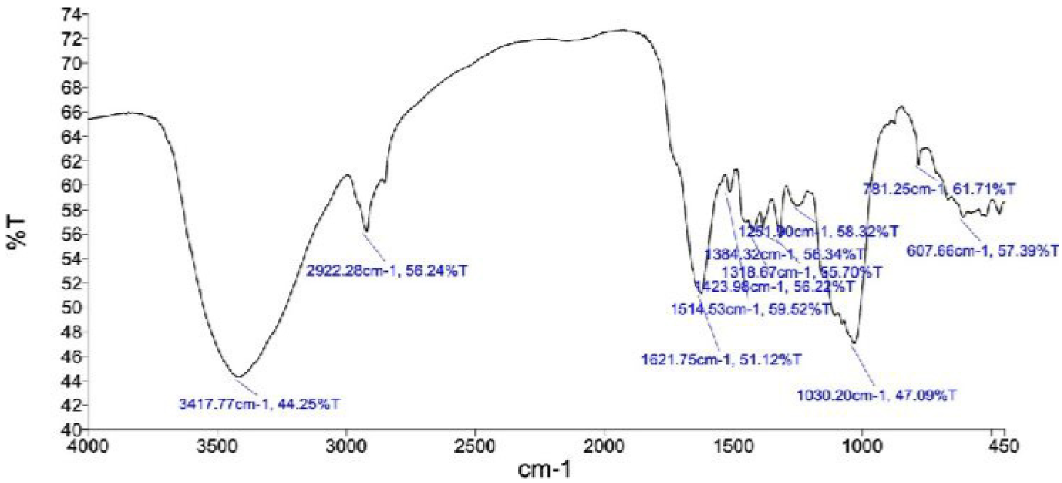

Fig.1. The diagnostic and the fingerprint region of a dry stem powder of *P.chaba* I R spectrum

primary /secondary alcohols or ethers.781.25 cm-¹ (61.71%T) and607.66 cm-¹ (57.39%T): Aromatic or alkene C–H out-of-plane bending and skeletal vibrations.

Collectively, these spectral features indicate the presence of hydroxylated compounds, saturated and unsaturated hydrocarbons, aromatic structures, and various oxygenated functional groups, consistent with the complex phytochemical profile observed in screening analyses.

This study demonstrates that the dry stem powder of *P.chaba* exhibits dose-dependent repellency against *C.chinensis*. Higher quantities consistently resulted in higher repellency levels, aligning with previous findings for other *P.* species. For example, Ojiako *et al.* (2018) reported that *P.guineense* seed powder significantly suppressed adult survival, oviposition, progeny emergence, and grain damage in *C. maculatus*.

Whereas the fume generated from dry *P. chaba* stems showed even more pronounced repellent effects, with a dose dependent manner. This observation is supported by earlier work demonstrating insecticidal and repellent properties of fumes from plants such as *Azadirachta indica* (L.), *Cinnamomum zey lanicum* (Presl), *Ocimum sanctum* (L.), and *Lantana camara* (L.) (Rohitha Prasantha, 2020). Heating plant material releases bioactive volatile compounds capable of repelling or killing stored grain pests. Our results similarly indicate that fumigation with *P. chaba* stems likely releases potent volatile phytochemicals that merit further characterization.

Aqueous extracts of numerous plant species also have been widely evaluated for their insect repellent, ovicidal, developmental, and antifeedant effects (Singh *et al.*, 2021). Consistent with these findings, the aqueous dry stem extract of *P. chaba* demonstrated strong repellency against *C. chinensis,* with efficacy decreasing gradually upon dilution. This trend parallels earlier observations from studies using extracts of *Ajuga parviflora* (Benth), *Cymbopogon citratus* (DC.) Stapf, *Nigella sativa* (L.), and *Mesua ferrea* (L.) (Shah *et al*., 2008).

Several studies have attributed toxic or behavioural effects on pests to compounds such as fatty acids, isobutylamides, alkanes, tannins, steroids, flavonoids, phenols, and glycosides (Kumar *et al.*, 2024; Pumnuan *et al.*, 2022). In the current study, phytochemical screening of *P. chaba* stem revealed the presence of a broad spectrum of these compounds, including in the fume fraction. Alkaloids, flavonoids, terpenoids, tannins, phenols, glycosides, carbohydrates, and proteins were all detected in the fumes, whereas the aqueous extract contained fewer compound classes. These differences provide the importance of fumigation as a delivery method, as heat may facilitate volatilization or transformation of bioactive constituents.

Infrared spectroscopy further confirmed the presence of functional groups corresponding to alkanes, alkenes, alcohols, aldehydes, ketones, phenols, and amides—findings consistent with earlier analyses of plantextracts (Kumar *et al*., 2024; Srikanta *et al.*, 2023). The diversity of functional groups suggests that *P. chaba* possesses a complex chemical profile potentially responsible for the observed repellency.

CONCLUSION

This study demonstrated that *P.chaba* possesses significant repellent properties against *C.chinensis* across multiple formulations. Among the tested forms, dry stem fumes exhibited the highest repellency and contained the greatest diversity of phytochemicals. The presence of alkaloids and other bioactive compounds likely underpins this strong repellent effect. However, the efficacy of *P. chaba*is highly dependent on concentration. Future research should therefore prioritize the validation through large-scale field trials to assess practical applicability, persistence, and safety in real storage conditions.With appropriate refinement and safety evaluation, *P. chaba* has the potential to serve as a sustainable botanical alternative for the management of stored grain pests.

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