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Chemical Composition, Toxicity and Biochemical Efficacy of *Phyllantus fraternus* against Major Three Stored Grain Pests

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ABSTRACT

The chemical composition, toxicity and biochemical efficacy of the Phyllanthus fraternus against three major stored grain pests was studied in the present study. The GC-MS revealed 4-Methoxy-1,3-butanediol, 2,4,6-trimethyloctane, 2,6-diisopropylnaphthalene, 2-pentadecanone, 6,10,14-trimethyl, methyl palmitate and stearic acid were found to be major components of the active fraction from ethyl acetate extract of P. fraternus. In fumigant bioassay, it was proved that adults of stored grain insect pest are susceptible to active fraction and also showed potent grain protectant properties. Further, active fractions inhibited acetylcholinesterase enzyme activity in Sitophilus oryzae. In addition, a significant impairment in the antioxidative defense system, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in test insects were observed. The results indicate that the toxicity of active fraction might be associated with inhibition of AChE activity and oxidative imbalance. In view of the strong fumigant toxicity of active fraction from P. fraternus extract as desirable tools for stored food grains against major stored grain pests and promoting eco-friendly pest control strategies.

Keywords: *Phyllanthus fraternus*, Biofumigants, Stored-product beetles, Acetylcholinesterase, Antioxidant system

INTRODUCTION

The heavy post-harvest losses and quality deterioration caused by stored grain insect pests is a major problem in agriculture [1,2] which further contributes to the inability of achieving food security especially in developing countries [3-5]. Currently synthetic insecticides are widely used to control insect infestation and awareness regarding the food safety has increased the demand for organically produced food, which necessitates evaluating the performance of biopesticides as safer alternatives to conventional insecticides [6]. In response to the high costs of pesticides and their undesirable side effects, there is a need for a paradigm shift to the development of non-chemical technologies which may eliminate the use of chemical insecticides and could have economic and health benefits to the applicators, consumers and the environment. In this regard, botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management [7].

Consequently, synthetic insecticides caused frequent pest outbreaks, pest resurgence, pesticide resistance issues which created a new avenue for the search of botanicals as an alternative measure for pest management [8]. For instance, plant-derived substances with insecticidal properties are eco-friendly in nature [9-11].

Phyllanthus fraternus Sch. et Thonn is a medicinal herb widely distributed in most tropical and subtropical countries [12]. It represents Euphorbiaceae family and has been used in folk medicine for treating different ailment such as genitourinary disorder, asthma, jaundice, bronchial infection, antiviral activity against chronic and acute hepatitis-B

[13]. However, there was no report of this plant as a biofumigant against stored grain insects. Keeping this view in mind, the present study explores the chemical composition and fumigant toxicity of active fractions of ethyl acetate extract from the leaves of *P. fraternus* Sch. et Thonn (Euphorbiaceae) along with the use of these biofumigant molecules against stored grains insect pest. Further, the possible mechanism of action was studied in terms of its on acetylcholinesterase enzyme activity and antioxidant enzyme system.

MATERIALS AND METHODS

Chemicals

AChE, acetylthiocholine iodide (ATCI), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), pyrogallol, ethylenediamine tetraacetic acid (EDTA) and Folin's reagent were procured from Sigma chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), hydrogen peroxide, sodium di-hydrogen phosphate, sodium hydroxide, sodium azide and sodium carbonate were procured from Sisco Research Laboratory, Mumbai, India.

Collection and preparation of plant materials

The matured leaves of *P. fraternus* was collected from Owo, Ondo State, Nigeria, washed in clean water and shade dried for 5 d; there after milled to powder using hammer mill and packed in air tight container for further use.

Preparation of extracts and isolation of active fraction

Hundred grams of powder was sequentially extracted with a series of solvents of increasing polarity viz., hexane, ethyl acetate, acetone and methanol, in a soxhlet apparatus for 8-9 h. The solvent from the extract was evaporated in a flash evaporator (Heidolph, Laborota 4000). The extracts were concentrated on rotary evaporator (Rotavapor R-IIA (Buchi) Switzerland) under low pressure, below 60°C to make it solvent free and the residue dissolved in a known volume of methanol, after then the solution was assayed for insecticidal activity by the fumigant toxicity [14]. Active extract (ethyl acetate), which showed maximum activity shown in Figure 1), was selected for the isolation of the insecticide compounds. The active extract (14.8 g) was subjected to column chromatography using a glass column (length, 50 cm; diameter, 3 cm) packed with silica gel (60-120 mesh) and eluted with hexane and ethyl acetate (100:0, I; 50:50, II; in the ratio) and followed by a stepwise gradient of ethyl acetate and methanol (100:0, III; 50:50, IV; 0:100, V; in the ratio). Five fractions of 300 ml each were collected, concentrated under reduced pressure, and assayed for insecticide activity. Fractions showing insecticide activity were pooled into active fractions. The most active fraction (III) showing insecticide activity and purity of the active fraction was analyzed by using GC-MS.

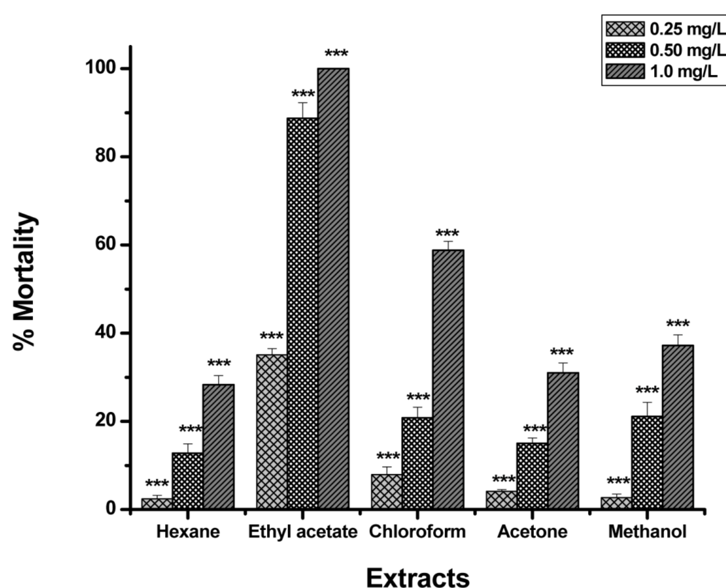

Figure 1: Insecticidal activity of the solvent extracts of *P. fraternus* to *S. oryzae* in the fumigant bioassay. The extracts were applied at 0.25-1.0 mg/L (In each group, n=4, error bars, s.e.m.), One-way ANOVA, ***P<0.001

Gas chromatography-mass spectrometry

The active fractions from plant extracts was analyzed on a gas chromatograph (HP6890; Agilent Technologies UK Ltd., South Queensferry, West Lothian EH30 9TG) directly linked to a HP5973 mass selective detector (Agilent Technologies) operated in electron impact mode (source temperature 230°C; transfer line 250°C). HP-5MS, 5% phenylmethylsiloxane capillary column (30 m × 250 μm i.d. × 0.25 μm) was used, 350°C max (Agilent 19091S-433) and the carrier gas is helium of 99.999% purity (1 ml min^{-1}). The column temperature was held at 70°C for 2 min, raised to 240°C at a temp. of 4°C/min, held for 10 min; raised to 270°C at a temperature. of 6°C/min, held for 12 min, again raised to 290°C at temp. of 8°C/min held for 14 min and finally raised to 310°C at temperature. of 10°C/min and held for 16 min.

MS parameters

The sample was analyzed in the full scan mode with a scan speed of 10,000 amu/s, a mass range of 40-300 *m/z* and a sampling frequency of 25 spectra/s; interface and ion source temperatures were 250°C and 200°C, respectively. MS ionization mode: electron ionization; detector voltage: 1.0 kV. Data were collected by the GC-MS solution software (MSD CHEM STATION G 1701 DAD 01.02.16). Mass spectra were compared with those in the Wiley 275 L mass spectral library.

Preparation of mixed-age-cultures

Rust red flour beetle, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) cultures were reared on whole wheat flour fortified with 5% dried yeast, whereas rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) were reared on whole wheat (*Triticium aestivum*). *R. dominica* cultures were maintained at (30 ± 1°C, 70% R.H.) and cultures of other insects were maintained at (25 ± 1°C, 65 ± 10% R.H.). From these cultures, adults (1-2 weeks old) were taken for preparation of mixed-age cultures. About 300 adults of *T. castaneum* was released into 1 kg of respective culture medium in 2 L glass jars. Similarly, 300 adults of *S. oryzae* and *R. dominica* were allowed to breed separately in 1 kg of wheat in 2 L glass jars. After one week, the adults were shifted from the cultures. A series of cultures of the respective species were thus maintained continuously. The insect culture jars were maintained at temperature (30 ± 1°C) for *R. dominica* and (25 ± 1°C) for other insects. Cultures in six successive weeks (containing 5-6, 4-5, 3-4, 2-3, 1-2 and 0-1 week old insects, the life span of all three insects nearly six weeks) were pooled such that the pooled populations contained all developmental stages of the respective species. The pooled mixture of individual species served as mixed-age cultures for toxicity tests.

Fumigation

The insecticidal activity of extracts of *P. fraternus* against adults of *S. oryzae*, was studied by fumigation. Forty numbers and known age of (adults) insects were released into 0.85-l L desiccators that served as the fumigation chambers. In each desiccator, a Whatman No. 1 filter circle (9 cm size) was placed to serve an evaporating surface for injecting active extract (dissolved with known volume of solvent). For each species (*S. oryzae*), there were four replicates for each dose of the active extract, with equal number of untreated control replicates.

The insecticidal activity of the active fractions was tested by fumigation on several stored-product insects (*R. dominica, S. oryzae* and *T. castaneum*). Forty insects for each treatment were used for all the species. The adult insects were exposed to a range of doses of active fractions (5-40 μg/L) for 24 h at (26 ± 2°C). For each species, there were 5 replicates per dose with an equal number of untreated control replicates. Gas-tight micro syringes were used for injecting the fumigant. Four replicates were used for each dosage and LC_{50} were determined from dose response data using probit analysis [15].

In an another experiment, rearing media containing mixed-age cultures of individual species was weighed separately in 50 g aliquots and transferred into cloth bags of size (20 cm × 14 cm). These cloth bags were placed individually in 0.85 L desiccators that served as the fumigation chambers. The desiccators were provided with holes sealed by rubber

septa for injecting active fractions (dissolved in known volume of solvent) of *P. fraternus*. At the end of exposure, the test insect bags were taken out of the desiccators. The contents of the bags were transferred to individual bottles (12 cm × 5 cm size) and kept at the rearing temperature and humidity conditions for 8 weeks. The insects, which emerged from wheat (*R. dominica* and *S. oryzae*) or survived as adults (*T. castaneum*) in their respective media were checked at weekly intervals for 8 weeks. Similarly, counts were made in untreated control batches every week. Percentage kill was determined by taking the survival/emergence in the controls as 100%. In each bioassay mortality were recorded and those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead.

Acetylcholinesterase activity (AChE)

Effect of active fraction from ethyl acetate extract of *P. fraternus* on Acetylcholinesterase (AChE extracted from whole insect) was estimated by ELISA microplate reader following the method of Galgani and Bocquene [16] which is based on the method of Ellman et al. [17]. The reaction was carried out in a 96 vial micro plate and reaction mixture containing the suitable amount supernatant (whole insect homogenates treated with or without) and DTNB in Tris-HCl buffer (100 mM, pH 8.0) was prepared. Thereafter, acetylcholine iodide (ATCI) (10 µl of 0.1 M solution) was added to reaction mixture and change in absorbance was monitored over 3 min in a microplate reader at 405 nm and at 25°C. The primary AChE inhibition assay was replicated at least four times. The percentage inhibition of the bioactive compound was calculated according to the following formula:

% of AChE Inhibition: 100 - [(V_0 of compound treatment/V_0 of control treatment) × 100]

Superoxide dismutase activity (SOD)

Superoxide dismutase (SOD) activity was measured using pyrogallol (2 mM) auto-oxidation as described by Marklund and Marklund [18]. The reaction mixture contained pyrogallol in 0.1 M tris buffer (pH 8.2) and the enzyme (whole insect homogenate). The reaction was started by adding the substrate and the absorbance was read at 420 nm for 3 min at an interval of 1 min and results were expressed as unit/mg protein. The amount of the enzyme that inhibits auto-oxidation by 50% is referred as one unit of enzyme activity.

Reduced glutathione (GSH)

Glutathione (GSH) content was estimated by the method of Ellman [19]. 10% whole insect homogenates were prepared in 5% w/v TCA, centrifuged at 2000 rpm for 10 min and supernatant (GSH) was mixed with 10 mM DTNB in 0.1 M phosphate buffer (pH 8.0). The mixture was incubated for 10 min at room temperature and the color was read at 412 nm. Glutathione content was calculated by a standard curve and expressed as µg/mg protein.

Catalase activity (CAT)

Catalase (CAT) activity was assayed by the method of Aebi [20]. The reaction mixture contained 3% H_2O_2 in 0.05 M phosphate buffer (pH 7.0). The reaction was started by the addition of 100 µl of enzyme (whole insect homogenates) and the change in absorbance was read at 240 nm for 3 min and activity was expressed as n mole H_2O_2/min/mg protein.

Glutathione peroxidase

Glutathione peroxidase (GPx) activity was measured by the modified method of Rotruck et al. [21]. The reaction mixture contained 10 mM sodium azide, 4 mM reduced GSH, 0.042% (w/w) H_2O_2 in 0.05 M sodium phosphate buffer (pH 7.4) made up to 2 ml distilled water. The reaction mixture was incubated for 10 min at 37°C and was terminated by the addition of 0.5 ml, 10% TCA and centrifuged at 5000 rpm for 15 min. 200 µl of supernatant was added to 3 ml of 0.3 M disodium hydrogen phosphate and 50 µl of 10 mM DTNB reagent and the color developed was read at 412 nm. Enzyme activity was calculated by the amount of GSH utilized during the reaction.

Glutathione-S-transferase

Glutathione-S-transferase (GST) activity was assayed by the method of Warholm et al. [22]. The reaction mixture contained 20 mM GSH and the 20 µl supernatant (whole insect homogenate treated with or without active fraction) in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA. The reaction was started by adding 30 mM CDNB and

change in the absorbance was measured at 344 nm. The enzyme activity was expressed as μmole CDNB conjugate/ min/mg protein.

Protein content was measured by the method of Lowry et al. [23] using BSA as the standard.

Statistical analysis

The results so obtained were subjected to statistical analysis, where LC_{50} were determined by Probit analysis and the data were analyzed using One-Way ANOVA ($p<0.05$) by Newman-Keuls test using Statplus 2007 software and computer program SAS (version 6.12, SAS Institute Inc. Cory, NC, USA).

RESULTS

Fumigant toxicity of extract

Figure 1 showed the insecticidal activity of different organic extracts from leaves of *P. fraternus* against adult *S. oryzae.* Among the different solvent extracts, the ethyl acetate extract showed potent fumigant activity against adult of *S. oryzae.*

Chemical composition of active fraction

The ethyl acetate extracts were subjected into silica gel column chromatography for isolation of active fraction. The yield of active fractions from *P. fraternus* was 6.83% (V/W). The chemical composition of the active fractions was summarized in Table 1. A total of 7 components of the active fractions were identified. The principal compound in the active sub fractions was methyl palmitate (81.2%) followed by 2,6-diisopropylnaphthalene (4.3%), 2,4,6-trimethyloctane (1.3%), 2-pentadecanone, 6,10,14-trimethyl (2.1%), 4-methoxy-1,3-butanediol (2.1%), stearic acid (8.2 %), and ethanol, 2-(-2-ethyoxy) (0.8%).

Table 1: Chemical composition of the active fraction* from leaves of *P. fraternus*

Peak No.	Compounds	RI	Relative Content (%)	% Match
1	4-Methoxy-1, 3-butanediol	1390	2.1	97
2	Ethanol, 2-(-2-ethyoxy)	1012	0.8	92
3	2,4,6-Trimethyloctane	9221	1.3	90
4	2,6-Diisopropylnaphthalene	1727	4.3	96
5	2-Pentadecanone,6,10.14-trimethyl	1754	2.1	92
6	Methyl palmitate	1878	81.2	99
7	Stearic acid	2167	8.2	95

*Active fraction (III, 100: 0; ethyl acetate:methanol)

Fumigant toxicity of the active fraction from P. fraternus

Among the 6 sub fractions, active fractions III exhibit strongest fumigant toxicity against all major stored grain pests discussed in Table 2. The LC_{50} values in the range between 16-23 µg/L of adults of all major stored grain pests. Further, in another experiment, the active fraction was highly toxic to mixed age cultures *S. oryzae, T. castaneum* and *R. dominica* recorded 80-90.6% mortality at dose of 100 µg/L in 24 h exposure, whereas 100% mortality was achieved at dose of 100 µg/L in 48 h exposure respectively as summarized in Table 3. Generally, an extended exposure period of 48 h increased mortality in all three species. The results clearly indicate that the active fraction from *P. fraternus* possessed good grain protectant properties.

Table 2: Insecticidal activity of active fraction (III) from leaves of *P. fraternus* against adults of stored- product insects by fumigant toxicity

Insects	LC_{50} [a, b]	LC_{99}	Slope ± SE	Degrees of freedom	Chi square (X^2)
T. castaneum	**23** (16-30)	45.3	2.171 ± 0.030	4	14.58
S. oryzae	**17.5** (14-23)	34.5	2.853 ± 0.081	4	21.12
R. dominica	**19.2** (18-22)	38.03	2.58 ± 0.115	4	18.9

[a]LC_{50} and LC_{90}=µg/L; [b]Values in parenthesis represent confidence limits

Table 3: Mortality (%) of mixed-age cultures of stored-product insects exposed for 24 h and 48 h to active fraction (III) from leaves of *P. fraternus*

Dosage (µg/L)	% Mortality (Mean ± SE)*					
	R. dominica		*S. oryzae*		*T. castaneum*	
	24 h	48 h	24 h	48 h	24 h	48 h
10	3.9 ± 1.1[a]	4.9 ± 1.2[a]	4.7 ± 0.6[a]	5.3 ± 0.2[a]	2.5 ± 0.5[a]	5.2 ± 0.8[a]
20	13.7 ± 2.3[b]	22.3 ± 2.6[b]	15.7 ± 1.4[b]	27.3 ± 2.6[b]	18.5 ± 1.5[b]	28.2 ± 2.2[b]
40	30.7 ± 4.1[c]	40.3 ± 2.4[c]	31.7 ± 3.1[c]	48.3 ± 3.6[c]	33.5 ± 3.6[c]	44.2 ± 2.8[c]
60	54.5 ± 3.7[d]	70.9 ± 4.7[d]	52.3 ± 2.4[d]	71.9 ± 4.8[d]	58.8 ± 1.9[d]	78.1 ± 2.6[d]
80	80.7 ± 4.6[e]	92.3 ± 2.6[e]	70.7 ± 2.1[e]	90.3 ± 1.6[e]	70.5 ± 4.5[e]	86.2 ± 2.8[e]
100	90.6 ± 3.5[f]	100[f]	89.5 ±1.8[f]	100[f]	80.8 ± 3.9[f]	100[f]
Control°	407.4 ± 3.8	404.8 ± 5	639.6 ± 5.9	648 ± 1.3	238.2 ± 3.5	240.5 ± 4.8

* There were 5 replicates per dose and in untreated controls (50 g infested media per replicate tested). In untreated controls, the total numbers of survivors per five replicate given. Different letters within a column indicate that means are significantly different (P<0.05) by Newman-Keuls test

Acetylcholinesterase activity (AChE)

AChE activity was significantly decreased in treating both the insects with increasing concentration of active fractions of *P. fraternus* following 3, 6 and 12 h exposure. At dosage 20 µg/L, maximum inhibition of AChE activity about more than 80.3% and 82.3% to the control was observed following 12 h exposure period for *S. oryzae* and *R. dominica* respectively depicted in Figures 2a and 2b.

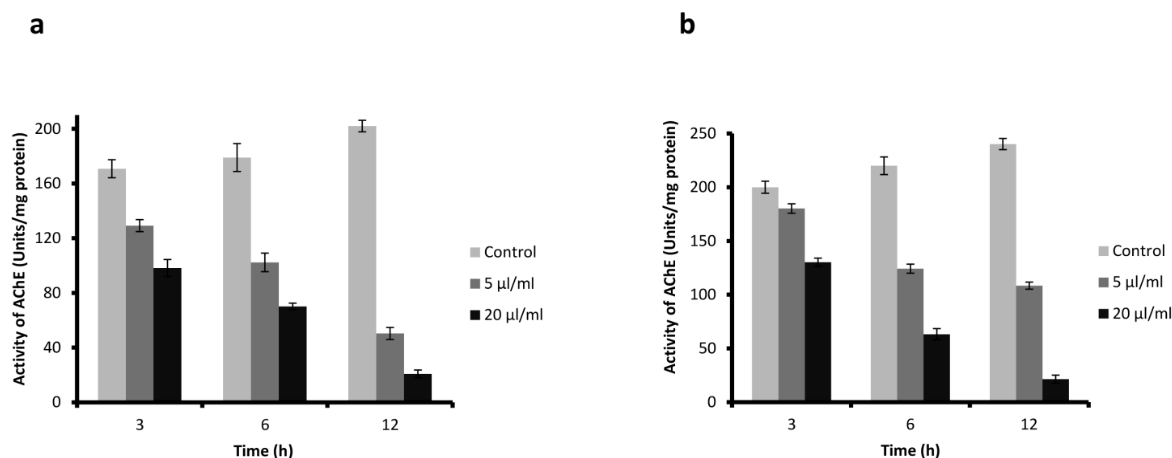

Figure 2: Effect of different concentrations of active fraction from *P. fraternus* on AChE activity (a) *S. oryzae* (b) *R. dominica* (n=4, error bars, s.e.m.)

Effect on insect antioxidant enzyme system

The effect of active fractions from *P. fraternus* on the antioxidant enzyme system was marked and showed in distinct variations in treated *S. oryzae*. The results of SOD activity showed variation in observation pattern of insect treated with different concentration of active fractions of *P. fraternus*. SOD activity was significant increase with increasing the dose of active fractions and exposure period. Similar trend was observed for GSH and CAT activity exposed to active fractions. GSH and CAT activity was significantly elevated in treated both insect with increasing concentration of active fractions of *P. fraternus*. Further, we observed parallel trend for GPx and GST activity exposed to active fractions of *P. fraternus*. GPx and GST activity was significantly decreased in *S. oryzae* following 3, 6 and 12 h exposure shown in Figure 3.

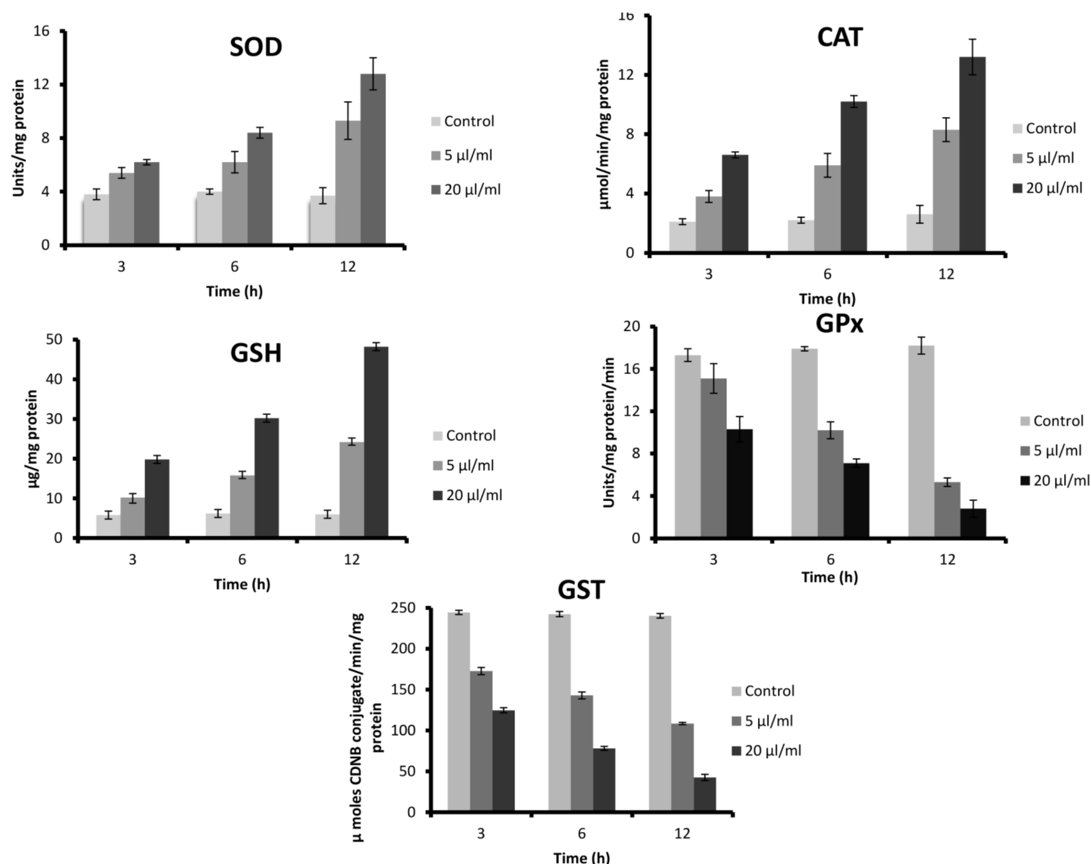

Figure 3: Effect of different concentrations of active fraction from *P. fraternus* on SOD, CAT, GPx, GST activity and GSH content in *S. oryzae* (n=4, error bars, s.e.m.)

DISCUSSION

The plant volatiles are known to posses the insecticidal activity and are eco-friendly in nature and non toxic to non target organisms so they can be efficient as an alternative to synthetic insecticides [8,24]. In the present study, we evaluated the fumigant applications of extracts/fractions from aerial parts of *P. fraternus* and which can be used successfully for the control of major stored grain insect pests.

Chromatographical analysis revealed that the active fraction contains methyl palmitate (81.2%) followed by 2,6-diisopropylnaphthalene (4.3%), 2,4,6-trimethyloctane (1.3), 2-pentadecanone, 6,10,14-trimethyl (2.1%), 4-methoxy-1, 3-butanediol (2.1%), stearic acid (8.2%) and ethanol, 2 -(-2-ethyoxy) (0.8%). It has been reported that methyl palmitate (MP), has strong acaricidal activity at 1 mg/ml at 24 h and possess larvicidal activity against *Anopheles stephensi* Liston similarly, the stearic acid toxic against to mosquito larvae (*Aedes albopictus*) [25,26].

The active fraction of *P. fraternus* showed strong fumigant activity against adults of all major stored grain pests with LC_{50} valve in the range of 16-23 µg/L summarixed in Table 2. Compared with the other essential oils/fractions from different plant origin, the essential oil of *Agastache foetniculum* was toxic to (LC_{50}=82.1 mg/L) *Lessioderma sericorne* [27]. *Nardostachys jatamansi* oil possessed fumigant toxicity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 82.13 and 53.29 mg/L, respectively [28]. The strong fumigant toxicity of essential oil *Carum copticum* against adults of *T. castaneum* with LC_{50} values is 33.14 µl/L [29]. The commercial available grain fumigant, methyl bromide (MeBr) was reported to have fumigant activity against *S. zeamais* and *T. castaneum* adults with LC_{50} values of 0.67 mg/L and 1.75 mg/L, respectively [30]. Thus the active fraction was more toxic to stored grain pests. However, fumigant activity of the active fraction of *P. fraternus* against major three stored grain pest adults is quite promising because the most effective fumigants (e.g. phosphine and MeBr) are highly toxic to humans and other non-target organisms.

Further, it is very important to know the mode of action of active fraction from *P. fraternus* because it may provide newer ideas for herbal/plant based insecticides. Therefore, the present study to investigate the effect of test active fraction on AChE and associated with antioxidant enzyme system. The active fraction from *P. fraternus* significantly inhibited acetlycholinesterase activity and is a dose-dependent with increasing exposure period shown in Figure 2. The AChE enzyme hydrolyzes the acetylcholine to acetate and choline molecules at the neuromuscular junctions, thus ensuring that signaling is rapidly terminated at neuron junctions. Due to inhibition of AChE activity, which lead to excessive neuroexcitation occurs because of the prolonged binding of acetylcholine to its post-synaptic receptor. The signs of intoxication include restlessness, hyper-excitability, tremors, convulsions, and paralysis finally lead to death. Similar symptoms observed in the synthetic insecticides such as organophosphate and carbamates [31,32].

In addition, there were several research articles reported that organophosphates induced oxidative stress in non mammalian system mainly insects [33,34]. Therefore, the present findings reveals that the effect of active fraction of *P. fraternus* on antioxidant system of stored grain pests were studied in the different concentration and exposure period. The investigation clearly showed that active fraction caused significant impairment in the antioxidant enzyme system (SOD, CAT, GSH, GPx and GST) of *S. oryzae* which is depicted in Figure 3. The enzymes, SOD, CAT & GPx are the major cellular antioxidant defenses. Our results have shown increased antioxidant enzyme (SOD, CAT) activities in the insect homogenate by active fractions, whereas in the case of GPx activities, it significantly decreased in the treated insects. The active fraction also significantly alters the enzyme activities when treated to insects, indicating induction of the enzymes that would contribute to the enhanced antioxidant defenses of the insects. Overall, our study shows that the high level of oxidative stress induced by active fractions from *P. fraternus*. Further, GSH (glutathione) is a major non-enzymatic antioxidant. It is a tripeptide (γ-glutamylcysteinylglycine) ubiquitously found in cells. The antioxidant activity of glutathione protects the cells against oxidative damage by free radicals and represents an important cellular defence mechanism. GSH pool is maintained in the cells by the restoration of oxidized form of glutathione (GSSH) [35]. Our findings showed GSH increased intreated insects with both the doses indicating higher oxidative stress brought about by active fraction from *P. fraternus*. GST plays an important role in protecting cells against ROS mediated injury by detoxicification of lipid hydroperoxides formed due to oxidative damage [36]. Our results showed drastic decrease in the activity of GST in all treated insects which could compromise the biochemical antioxidant defenses of the insects. Marked depletion of GSH and its dependent enzymes in the insects by active fractions indicates a major deleterious effect on the insects. Thus findings revealed that the oxidative imbalance may be involved in the toxic effects of active fraction from *P. fraternus*.

The currently used fumigants are synthetic insecticides and the most effective (e.g. phosphine, methyl bromide and sulfuryl fluoride), but repeated use of these fumigants lead to environmental hazards, highly toxic to humans and other non target organisms. The active fraction III from aerial parts of *P. fraternus* show potential efficacy against stored grain insect pests and can be used as natural biofumigant molecules. However, further effort to isolate bioactive molecule with insecticidal activity and studies are required on the mammalian safety of the active fractions and on development of formulations.

CONCLUSION

These studies provide relative investigation on the potential insecticidal activity; chemical composition and toxicity of active fraction of *P. fraternus* which might be associated with inhibition of AChE activity and oxidative imbalance. From the present research findings it can be said that *P. fraternus* can be used as grain protectants by local farmers in small farm holding because of their potent fumigant toxicity of active fraction and also they are easily available, and are environmentally friendly. Thus, these species might be ideal candidates for further investigation in developing novel botanical insecticide and can be used as component in an Integrated Pest Management strategy.

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