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RESEARCH ARTICLE

Effect of cypermethrin on worker and soldier termites of subterranean termites *Odontotermes brunneus* (Hagen) (Termitidae: Isoptera)

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Abstract The termite *Odontotermes brunneus* is an economically important species causing damage to cellulose containing wooden material and agricultural crops in India. Insecticide application is an effective strategy in termite control. In the present study the effect of cypermethrin was tested for workers and soldiers termite using filter paper dip method. After 24 h treatment the lethal concentration (LC_{50}) was increased to 9.7 ppm in workers and 1.8 ppm in soldiers respectively. The detoxification enzyme activities of esterase, glutathione S-transferase was increased in worker termites 23 µmol, 9 µmol/min/mg of protein compared to soldiers 15 µmol, 7 µmol/min/mg of protein respectively ($p < 0.05$). The activity of mixed-function oxidase was found very less in both samples. Further nPAGE analysis revealed that increased esterase band in workers than soldier and control sample. The data of this study revealed that possible mechanism of esterase and glutathione S-transferase mediated cypermethrin detoxification that leads to reduce the sensitivity in worker termites of *O. brunneus.*

Keywords Terrestrial insect · Synthetic pyrethriod · Toxicity · Detoxification enzymes · Electrophoresis

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Introduction

The termite or white ant belongs to the order Isopteran can inhabit in different region of the world (Smeathman 1781; Krishna and Weesner 1970; Pearce 1997). Among them, the member of Termitidae *Odontotermes* represents the most dominant economically important pest of sugarcane crop and also notorious pest of other agricultural crops and trees in Tamil Nadu, Karnataka, Andhra Pradesh (Rajagopal 2002). It is estimated that up to 90–100% of losses have been recorded in sugarcane due to damages by subterranean termites at the stage of germination (Sattar and Salihah 2001).

Today many safe and simplified practices of termite management systems have been proposed but sudden outbreak of insects belongs to stored product implies on insecticide application like organochlorine, organophosphate and synthetic pyrethroid (Ahmed and Qasim 2011). Among insecticides pyrethroids have been most widely used because of their safe, cheap, effective and long-lasting nature (Butler 2011). However, the widespread development of insecticide resistance due to detoxification mechanism, especially in pyrethroids may leads to resistance developed and also confers cross-resistance to other insecticides has become an immense practical problem challenging the control of agricultural pest (Zaim and Guillet 2002; Fragoso et al. 2007). Characterization of detoxification in insects that leads to development of resistance would help us to design novel strategies for pest control and also minimize the over use of insecticide applications. In most cases metabolic resistance mechanisms involves an array of detoxification enzymes like monooxygenase, esterase and glutathione S-transferase (Enayati et al. 2005; Ishaaya 1993).

The esterase and mixed function oxidase (or) cytochromeP450 s are mainly involved in resistance through detoxification of pyrethriod and chlordane insecticides in many insect species including termites Ahmad et al. 2007; Taskin et al. 2007). Esterase is another group of enzymes involved in the metabolism of organophosphates and pyrethroids by hydrolysis and cleavage of functional groups of toxicants (Dauterman 1985). Resistance to pyrethroid owing to elevated activity of esterase is reported in Indian strains of *Spodoptera litura, Amsacta albistriga* etc. (Muthusamy et al. 2013, 2014). Microsomal mixed function oxidase is another important metabolic enzyme involved in the detoxification of wide classes of chemical insecticides including pyrethroids and organophosphate (Liu et al. 2015). Mixed function oxidases are a complex family of heme containing enzymes, which catalyze a variety of oxidative reaction with a significant diversity of chemical substrates such as pesticides etc., (Scott 2001).

Few studies have been carried out on termites of agricultural importance and losses due to their attack. (Hussain 1935; Patel and Patel 1954; Chottani 1997). Though toxicity of insecticides to different termite has been reported, very less or no evidence revealed the detoxification mechanism of pesticide in termite. Hence the present study was focused on assessment of cypermethrin toxicity and its detoxification enzymes in workers and soldiers of *O. brunneus*.

Materials and Methods

Termites

Foraging workers and solders of *O. brunneus* were collected from field colonies in City Park and agricultural land were 5 inch round boxes placed in the ground containing rolled cardboard near MGR College. The rolls of cardboard were collected thrice weekly and brought back to the laboratory and the termites were separated from the debris. The termites were maintained in the room temperature on moistened blotting paper in plastic containers and used within a week of collection.

Chemicals

Commercial formulation of Cypermethrin (10%EC G.I.D.C Gujarat) Acrylamide, bovine serum albumin, reduced-glutathione (GSH), α-naphthyl-acetate (α-NA), 2-4dichloro-nitrobenzene (DCNB), fast blue BB salt, tetramethyl benzidine (TMBZ), Triton X-100, hydrogen-peroxide (H_2O_2) and sodium- dodecyl-sulfate (SDS), Folin–Ciocalteau reagent solution (1 N), Coomassie Brilliant Blue R-250, Bromophenol blue, 3,5- dinitrosalicylic acid (DNS), ethylene-diamine tetra acetic acid (EDTA), N,N′-methylene bis (acrylamide), sodium bicarbonate, Acetic acid, HCl, dibasic sodium phosphate, monobasic sodium phosphate, NaCl, and Ammonium persulphate, sodium acetate, NaOH, N,N,N′,N′-tetra methyl ethylene diamine (TEMED), were purchased from Hi Media (Mumbai). All reagents were of analytical grade.

Toxicity Assay

A no-choice feeding bioassay was performed to determine the mortality of *O. brunneus.* Five to six concentrations (causing mortalities at $> 0\%$ and $< 100\%$) were prepared by serial dilution in distilled water. One ml of an insecticide concentration was spread on the Whatman No. 1 filter paper placed on the bottom of a Petri dish (8 cm diameter by 1.0 cm high). Filter paper moistened with same quantity of distilled water was served as control. Then 20-30 workers and soldiers of at least the third instars were introduced into the dish. Each concentration was repeated 3 times with a total of 18 dishes per concentration. The dishes were then placed in a room at 25 ± 2 °C and $70 \pm 5\%$ RH. A filter paper underneath the glass cover of the dish was wetted with distilled water daily during treatment. The mortality was recorded after three days of exposure. The mortality was recorded after 24 h of exposure.

Termite Homogenates

The method was adopted from Muthusamy and Shivakumar (2015a, b). The test insect from each dose a group of morbid workers and soldiers were immobilized by placing them in a freezer at − 20 °C for 10–15 min. The whole body from each caste was homogenized in a vial (containing 0.1 M phosphate buffer pH 7.2) by placing on ice. The homogenates were then centrifuged at 10,000 rpm at 4 °C for 15 min. The collected supernatants were used for evaluation of enzyme activity.

Detoxification Enzyme Assay

Esterase Assay

The activity of esterase was measured with slight modification according to Muthusamy and Shivakumar (2015a, b) method using 1-napthyl acetate as substrate. The reaction mixture contains 0.1 ml of supernatant (10 µl of enzyme sample from each caste with 99 µl of 40 mM PBS pH 6.8) was added to the 15 ml clean test tube, containing 5 ml of substrate (5 mg of 30 mM α-napthyl acetate/ml of acetone dissolved in 99 ml of 40 mM PBS), tubes were incubated in dark for 15 min at 30 °C. Then 1 ml of staining solution

(5% SDS and 1% fast blue BB salt dissolved in 16 ml of sodium phosphate buffer pH 6.8) were added to the reaction tube in the ratio of 1:5. The esterase activity was measured continuously at 590 nm for 5 min. The esterase activity was calculated as µmole/min/mg protein using 1-napthyl as the standard.

Mixed Function Oxidase Assay

The assay used for MFO detects the elevation in the amount of heme, which is then converted into equivalent units (EU) of cytochrome P450. Cytochrome P450 was titrated using the heme-peroxidase assay according to Brogdon (1989) with slight modifications. 500 µl of 0.05 M potassium phosphate buffer (pH 7.0) were added to 100 µl microfuged supernatant and 1000 µl tetramethyl benzidine solution (0.05% 3,3′,5,5′ Tetramethyl Benzidine, i.e. TMBZ + 5 ml methanol + 15 ml sodium acetate buffer 0.25 M pH 5.0). 400 µl of 3% hydrogen peroxide were added and the mixture was incubated for 30 min at room temperature. Absorbance was read at 630 nm and values calculated from a standard curve of cytochrome C.

Glutathione S-transferase Assay

Glutathione S-transferase assay were performed according to the method of Muthusamy and Shivakumar (2015a, b) using 1-chloro 2, 4-dinitrobenzene and reduced glutathione (GSH) as substrate. The total reaction solution contained 2.79 ml of Phosphate buffer saline 0.1 M pH − 6.5, 10 µl of diluted enzyme supernatant (the stock solution was diluted tenfold with 0.1 M pH − 6.5, sodium phosphate buffer), 50 µl of 50 mM CDNB (dissolved in the 0.1% (v/v) ethanol), and 150 µl of reduced glutathione in Tris–HCL (0.05 M, pH 7.5). The changes in absorbance were measured continuously for 5 min at 340 nm using the time scan mode of UV–Visible spectrophotometer.

Protein Measurement

Protein contents of supernatant were determined using the folins phenol reagent method as described by Lowry et al. (1951) and bovine serum albumin (BSA) was used as the standard protein. The protein was measured at 660 nm absorbance in a spectrophotometer.

Polyacrylamide Gel Electrophoresis

Non-denaturing PAGE was carried out following the method of Muthusamy and Shivakumar (2015a, b) using 10% separating gel and 4% stacking gel with continuous Trisglycine running buffer system (50 mM, pH 8.3). Ten microliters of sample from worker, soldier and control (water treatment) containing 15 µg protein was diluted with 2 µl of 1 × sample buffer (1.5 M Tris HCl, pH 6.8, 30% v/v glycerol, 0.02% bromophenol blue). Electrophoresis was carried out at a constant current of 75 V at 5–7 °C for approximately 1.5–2 h. Gel was stained by incubating for 30 min in a 2% 1-napthyl acetate (in 40 mM phosphate buffer pH 6.8), which contain 1% acetone, and then placed the gels in 1% (w/v) fast blue BB salt for 1 h.

Data Analysis

The mortality for cypermethrin against workers and soldiers *O. brunneus* were corrected using Abbott's formula (1925), if the mortality in the control was more than 5%. Data were analyzed by probit analysis using SPSS software (Version 10.0 for windows, SPSS Inc., Chicago, USA) to determine median lethal concentrations (LC_{50}). The enzyme activities were repeated three times, each time using one of the pools extracts. Each repetition was performed in triplicate. Standard deviations (SD) were calculated using GraphPad Prism version 16.0 for Windows (GraphPad Software) and data were expressed as a mean of replicates ± SD. Significant differences between enzyme activity were analyzed by using Tukey's-multiple comparison test at $p < 0.05$.

Results and Discussion

In the present study the effect of cypermethrin was tested against workers and soldier's termites of *O. brunneus*. The results showed that cypermethrin were more toxic to soldiers compared to worker termites (Table 1). The LC50 was 9.7 ppm and 1.8 ppm for workers and soldiers respectively. Similar results were reported by previous studies where deltamethrin exposed *C. formosanus* workers had tolerance ration of 16X, whereas less tolerance found in soldiers 11.6X (Osbrink and Lax 2003). Another study reported that, at high concentration of cypermethrin (100 ppm) was effective against subterranean termites (Kuriachan and Gold 1998). The variation in cypermethrin dose between termite cast of this present study may due to

Table 1 Toxicity of cypermethrin on *O. brunneus* workers and soldiers after 24 h post treatment

O. brunneus	n^a	Slope (± SE)	LC_{50} (95% CI ppm)	χ^2	*df*
Workers	120	0.30 (±0.073)	9.712 (09.21–13.40)	0.47	2
Soldiers	120	(±0.051)	1.831 (1.20–3.36)	0.51	2

n^a, number of animals; LC_{50}, lethal concentration that kills 50% of the exposed; CI, confidence interval; χ^2, Chi square; df, degrees of freedom

the nature of worker foraging behaviour. Scheffrahn and others reported that the toxicity of chemical treatment in termites may influenced by their behaviour and gallery system architecture (Scheffrahn et al. 1997).

The detection of detoxification enzymes involved in susceptibility variations between the termite casts was performed. Various toxicity studies revealed involvement of detoxification enzymes in many insects that confer variations in the susceptibility to insecticides (Su et al. 2012). Esterase, P450/MFO and GST are the three principal enzymes that facilitate the insects to metabolize different kind of toxins (Kranthi 2005; Lee 2002). In this study the detoxification activities of metabolic enzymes were studied in control (water treatment), live worker and soldiers of *O. brunneus* (exposed to cypermethrin). With respect to toxicity bioassay esterase activity increased in worker termites (23 µmol/protein/min) compared with soldier 15 µmol and control activity 11 µmol ($p < 0.05$ Fig. 1). Similar results were reported in cypermethrin resistance Brazilian populations of *Sitophilus zeamais* (Ribeiro et al. 2003).

The MFO assay shows no significant activity in treatments and control sample. Whereas slightly decreased activity was found in workers (Fig. 2). Next GST shows high activity in soldier (9 µmol/protein/min) compared with workers (5 µmol/protein/min) and control (7 µmol/protein/min) Fig. 3. This suggests that, possible mechanism of esterase, and GST mediated detoxification in *O. brunneus* workers. The variations in the toxicity of insecticides may arise due to the differential rate of insecticide penetration and metabolic detoxification occurred between termite casts (Osbrink et al. 2001). Esterases and glutathione S-transferases are known to involve in insecticides detoxification in many arthropods (Matsumura 1985; Strange et al. 2001). Ahmed and Gunning reported that the involvement of esterase in deltamethrin resistance in *S. litura* and *H. armigera* (Ahmad et al. 2007; Gunning et al. 1991). Additionally native polyacrylamide gel electrophoresis (nPAGE) revealed high activity of esterase band in workers compared with soldier and control, suggesting that esterase mediated hydrolysis of cypermethrin may involved in workers (Fig. 4). The findings of this study correlated with the findings of Muthusamy et al. (2013) found that esterase mediated detoxification in *A. albistriga*. Similar findings were reported in pyrethroid resistance population of *H. armigera* (Srinivas et al. 2004). In summary, the present data suggest that cypermethrin was more toxic to soldiers than workers. Further this study revealed that esterase and GST mediated cypermethrin detoxification may occur in *O. brunneus* workers.

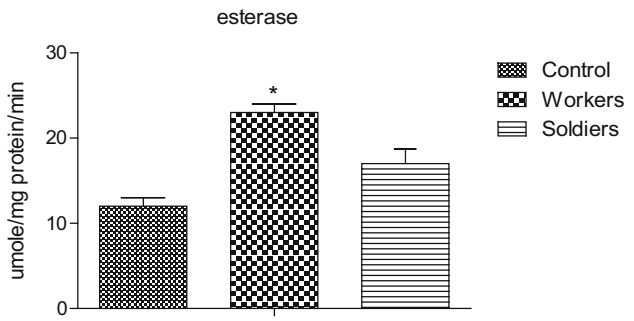

Fig. 1 Esterase activity in *O. brunneus* workers and soldiers exposed to cypermethrin are shown as mean ± SD. Asterisk (*) denotes significant activity at $p < 0.05$

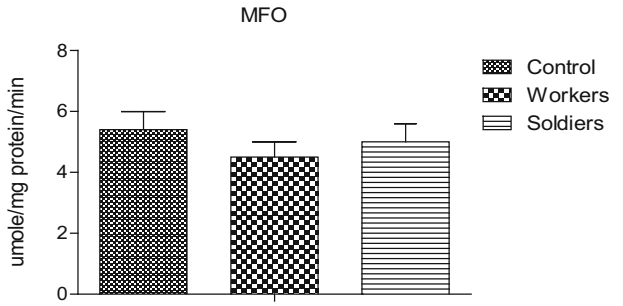

Fig. 2 Mixed function oxidase activity in *O. brunneus* workers and soldiers exposed to cypermethrin are shown as mean ± SD

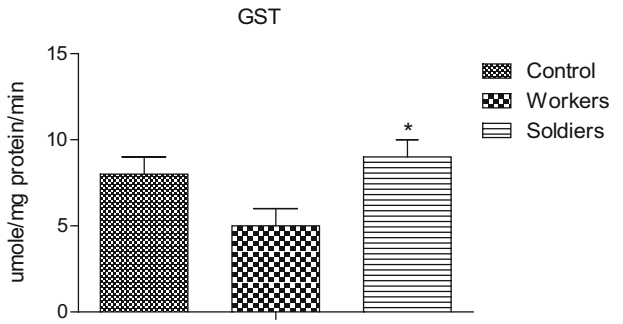

Fig. 3 Glutathione S-transferase activity in *O. brunneus* workers and soldiers exposed to cypermethrin are shown as ± SD. Asterisk (*) denotes significant activity at $p < 0.05$

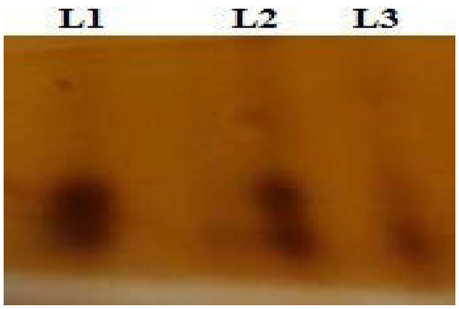

Fig. 4 Native polyacrylamide gel electrophoresis (nPAGE) of esterase from worker, soldier termite of *O. brunneus* exposed to cypermethrin and control (water treatment) sample

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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