



Termiticidal effects of *Capparis decidua* on biochemical and enzymatic parameters of *Odontotermes obesus* (Isoptera: Termitidae)

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Manuscript received 25.08.2010; revised 28.08.2010, accepted; 31.08.2010

Abstract. In the present investigation termiticidal effects of *Capparis decidua* and its combinatorial mixtures on biochemical and enzymatic parameters of *Odontotermes obesus* (Isoptera: Termitidae) were observed. *C. decidua* has shown very high termiticidal activity to *O. obesus* (Rambur) when termites were exposed with 40% and 80% of LD₅₀. Its aqueous extract and combinatorial mixtures significantly ($p < 0.05$) inhibited the level of glycogen, amino acid, lipid, DNA, RNA and protein in termites. Besides this, both single and combinatorial mixtures of *C. decidua* significantly ($p < 0.05$) decreased the level of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase enzymes at very low concentrations after 8 h treatment. More specifically, aqueous extract and combinatorial mixtures significantly inhibited the AChE activity, which confirms the presence of neurotoxic compounds. However, it can be concluded that *C. decidua* active ingredients can easily kill field termites if used in the poison baits and as fumigant in storehouses.

Keywords: *Odontotermes obesus*, biorganic termiticides, lipid, protein, glycogen, enzymes

1 Introduction

Termites are highly destructive polyphagous insect pests, which damage cereal crops, forest products and are serious problems to the farmers, tree growers and builders throughout the world. In the past, synthetic pesticides were massively used for the fast and effective control of termite population but that has led to pesticide resistance. But, such pesticides are highly toxic to non-target organisms and contaminate the environment. Hence, their alternatives are under exploration to have more potent herbal pesticides. There is a great need of novel pesticidal compositions containing no synthetic pesticide to be used against termites. In addition, there is a need for effective termiticide, which can suppress the regenerative activity in termites at a very mild dose. More specifically, plant species having strong anti-termiteic properties have been searched [1]. In this regard, few plants such as *Polygonal hydro piper* and *Progesterone parviflorus* caused significant mortality and repellent activity in termite, *R. herperus* and *O. assamensis*. Similar termiticidal activity are reported in aqueous and solvent extracts of *Geranium*, *Morus*, *Artemisia*, *Diospyros*, *Crataegus*, *Curcuma*, *Rubia*, *Polygonal*, *Gardenia*, *Cornus*, *Uncaria*, *Rheum*, *Terminalia* and *Saussurea* [2]. Besides this, monoterpenoids isolated from *Flourensia cernua* effectively control termites [3]. Moreover, Alaska yellow and red cedar (*Chamaecyparis nootkatensis*) and redwood (*Sequoia sempervirens*) have shown high antifeedant and toxic activities against termites [4].

Few other plant derivatives such as 2-methyl-anthraquinone, plumbagin, diosindgo, diospyrin, isodiospyrin and microphyllone isolated from *Diospyros sylvatica* root [5]. and Cedrol [6] isolated from *Juniperus procera* exhibited very high mortality in *O. obesus*. Similarly 2, 2': 5', 2''-Terthiophene and 5'-(3-buten-1-ynyl)-2,2'-bithiophene showed 100% mortality in *C. formosanus* [7]. Similarly, flavanoids, geninstein, biochantin A, apigenin, querceptin and glyceollin reduce fecundity and food consumption Formosan subterranean termites [8]. Moreover, Sesquiterpenes, (4S)-2, 6, 10-bisaboratrien-4-ol-1-one, 1, 8-epoxy-1(6), 2, 4, 10-bisaborpenta-en-4-ol (2), and 1-methoxy-4-cadinene (3) isolated from the black heartwood of *Cryptomeria japonica* [9, 10].

Capparis decidua is a native plant commonly known as 'Kureel' in Hindi, belongs to family Capparidaceae. It is a densely branched shrub with scanty, small, caduceus leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough and asthma. In the present study, termiticidal effects of *C. decidua* and its mixtures were observed on biochemical and enzymatic parameters of *Odontotermes obesus*.

2 Material and methods

Collection of termites and plant material

Termite, *Odontotermes obesus* (Rambur) workers were collected from the University garden and temporary culture was maintained in the laboratory at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ at 80% RH by providing green leaves as food material. Termite culture was protected from light illumination, by using black paper sheets wrapped around the glass containers (12×9 inch). *Capparis decidua* collected from semi arid regions of Rajasthan. Standard taxonomic key was applied for the proper identification of plant.

Preparation of extracts

C. decidua stem were chopped in small pieces and milled to make powder, weighed and solubilized in the water. The solubilized extracts were filtered with Whatmann paper. No 1 and were concentrated under vacuum (30°C). After evaporation of water, it was weighed and solubilized in known volume of distilled water.

Capparis decidua and other ingredients used in preparation of combinatorial mixtures

Combinatorial ingredients added mixtures	
C-ST	<i>Capparis decidua</i> stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Sulphur (11) + Water (15 liter)
C-BT	<i>Capparis decidua</i> stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Borate (11) + Water (15 liter)
C-CoT	<i>Capparis decidua</i> stem powder (180 gm) + Coconut oil (50ml) + Terpene oil (50ml) + Glycerol (50 ml) + Copper (11) + Water (15 liter)
PCU	Photoactivated cow urine (48 h) ($10 \mu\text{g}/\mu\text{l}$)
C-CuT	<i>Capparis decidua</i> stem powder (180 gm) + Photoactivated Cow urine (15 liter)
<i>Capparis</i>	<i>Capparis decidua</i> stem powder 6gm/l of water aqueous extract

Toxicity determination

Toxicity bioassays were conducted in the laboratory and LD₅₀ of each mixture was determined separately. Toxicity experiments were conducted by using increasing concentration of each mixture i.e. 24 µg, 48 µg, 96 µg, 192 µg, 384 µg, 768 µg, and 1536 µg. The treatment mixtures were then coated on cellulose paper (size 1X1 cm²), air dried and kept in the central area of Petri dish. Treatment and controls were tested in triplicate for each mixture. In each Petri dishes 125 termites (1 gm weight) were released and termite mortality and survival were observed at different periods. Dead termites were separated from the alive on the basis of body movement. LD₅₀ values were determined by Probit method [11]. LD₅₀ values were calculated in µg/gm body weight of termites 16 h after treatment.

Determination of biomolecular parameters

Termite workers were treated with 40% and 80% of LD₅₀ (22.68 µg/gm, 13.00 µg/gm, 1.56 µg/gm, 19.20 µg/gm, 15.68 µg/gm and 26 µg/gm body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. decidua* respectively). Termites were sacrificed, homogenized and centrifuged to prepare their whole body extracts for biomolecular estimation. Changes in the level of various biomolecules were measured after various time intervals i.e. 4, 8, 12 and 16h. Few important biomolecules such as glycogen, total free amino acids, total lipid, nucleic acids (DNA and RNA) and total protein were determined.

Determination of glycogen

Glycogen contents were measured according to method of Dubois et al. [12]. For this purpose 500 mg of termites were homogenized in 2ml of 5% Trichloro acetic acid with the help of glass-glass homogenizer and centrifuged. Optical density of the reactant was read at 530nm. Glycogen contents in unknown (supernatant) were calculated by using standard curve drawn with known amount of glucose. The blank was set by taking 0.50ml of 5% TCA and 6 ml of concentrate H₂SO₄. The amount of glycogen was expressed in gm/100gm of body weight of termites. Three treatments were performed at three trials. Data obtained was statistically analyzed by using ANOVA method.

Determination of total free amino acid

Level of free amino acids was determined following Spies et al. [13]. A total 500 mg of termites were homogenized in 2 ml of 95% ethyl alcohol. Homogenate was centrifuged at $15,000 \times g$ for 20 minutes and supernatant was separated. For estimation of total free amino acids 0.1 ml of supernatant was taken and to it 0.1 ml of distilled water and 2.0 ml Ninhydrin reagent were mixed. The reaction mixture was kept in boiling water for 15 minutes. A total of 2 ml of 5.0 % ethyl alcohol was added to the above boiled mixture. A violet color was developed in the reaction mixture which was measured at 575 nm. For calculating the total free amino acid content standard curve was prepared by using known amount of glycine and was expressed in gm/100 gm body weight of termites. Three replicates were used and data is statistically analyzed by ANOVA method.

Determination of total Lipid

Level of total lipid in whole body extracts of termite was estimated according to method of Floch et al. [14]. A total of 500 mg of termite workers were homogenized in 5 ml of chloroform and methanol mixture (2:1 v/v). Total lipid contents were weighted at the end and expressed in gm/100 gm body weight of termites. Three replicates were set and data was statistically analyzed by ANOVA method.

Determination of nucleic acids

Level of nucleic acids in the whole body extracts of termites was estimated according to method of Scheidner et al. [14]. For this purpose a total 500 mg of termite workers were fed with 40% and 80% of LD₅₀ (22.68 $\mu\text{g/gm}$, 13.00 $\mu\text{g/gm}$, 1.56 $\mu\text{g/gm}$, 19.20 $\mu\text{g/gm}$, 15.68 $\mu\text{g/gm}$ and 26 $\mu\text{g/gm}$ body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. deciddua* respectively) separately. Insects were scarified and homogenized in 5%TCA with glass-glass homogenizer at $15,000 \times g$ for 25 minutes.

DNA estimation

For DNA estimation, 0.2 ml of supernatant was taken and it was diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 gm of diphenylamine, 100 glacial acetic acid and 2.5 ml of conc. H₂SO₄) were added to it. The mixtures were kept in boiling water bath for 10 minutes. A blue color was developed in the solution which is measured at 595 nm (O.D.).

RNA estimation

For RNA estimation 0.2 ml of supernatant was taken and it was diluted by adding 4.8ml of distilled water. Now 2ml of orcinol reagent (1 gm orcinol, 100 ml conc. HCl and 0.5 gm ferric acid) was added to it. The solution was kept in boiling water bath for 10 minutes, a green color was developed, which was measured at 660nm. In both cases three replicates were set and data obtained was statically analyzed by ANOVA method.

Determination of total protein

Total proteins of termites were estimated according to Lowry et al. [16]. For this purpose 500mg of termite workers were treated with 40% and 80% of LD₅₀ (22.68 μ g/gm, 13.00 μ g/gm, 1.56 μ g/gm, 19.20 μ g/gm, 15.68 μ g/gm and 26 μ g/gm body weight of termite for C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. decidua* respectively). These treated termites were homogenized in 4.0 ml of 10% TCA with the help of glass-glass homogenizer. The obtained homogenate was centrifuged at 15,000 \times g for 15 minutes. Each experiment was performed three times. Standard curve was prepared by using 10 μ g, 20 μ g, 40 μ g, 80 μ g and 100 μ g of Bovine serum albumen. Data obtained was statistically analyzed by ANOVA method.

In vivo Determination of enzymatic parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided sub-lethal doses (40% and 80% of LD₅₀) of C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. decidua* with the cellulose paper as diet. Insects were sacrificed at the 4 h interval up to 16 h for measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass-glass homogenizer and centrifuged at 4 °C for 25 minutes at 15,000 \times g. Supernatant was isolated in a glass tube and used as enzyme source.

Determination of acid and alkaline phosphatase

Level of alkaline phosphatase level was determined according to the method of Bergmeyer [17]. For this purpose 500 mg of termites were homogenized in 1 ml of PBS buffer at 4 °C and centrifuged at 15,000 \times g for 15 min. A 0.2 ml of supernatant was taken in a test tube and 1.0 ml of acid buffer substrate solution was added. Contents were mixed thoroughly and incubated for 30 minutes at 37 °C. Now 4.0 ml of 0.10N NaOH solution was added to the incubation mixture. Similarly, for determination of ALP, 0.10 ml of supernatant was taken

in a test tube and 1.0 ml of alkaline buffer substrate was mixed with it. The mixture was mixed thoroughly and incubated for 30 minutes at 37 °. Now 5.0 ml of 0.02 N NaOH was added to the incubation mixture. The reaction was stopped by adding excess of NaOH. The p-nitrophenol formed as result of hydrolysis of p-nitrophenyl phosphate gave a yellow colour with NaOH. Optical density was measured at 420 nm. Standard curve was drawn with the help of different concentrations of p-nitrophenol. Enzyme activity was expressed as μ moles of p-nitrophenol formed /30min/mg protein.

Determination of lactic dehydrogenase

Activity of lactic dehydrogenase was measured according to the method of Annon [18]. For this purpose, 100 mg of insects were homogenized in 1.0 ml of 0.1 M phosphate buffer (pH 7.5) in ice bath and centrifuged at 10000 \times g for 30 minutes in cold centrifuge at 4 °. Supernatant was used as enzyme source. For determination of enzyme activity 0.05 ml of enzyme source was added to 0.50 ml of pyruvate substrate. Now the contents were incubated at 37 °C for 45 minutes. Now 0.50 ml of 2,4- dinitrophenyl hydrazine solution was added and the contents were mixture and kept at the room temperature. After 20 minutes, 5.0 ml of 0.4 N NaOH was mixed and left for 30 minutes at room temperature. The optical density was measured at 540 nm and it was converted to LDH unit by drawing a standard curve. Enzyme activity has been expressed as m moles of pyruvate reduced/45min/mg protein.

Determination of glutamate pyruvate transaminase and glutamic-oxaloacetic transaminase

GPT and GOT activity was measured according to the method of Reitman and Frankel [19]. A total of 500 mg termites were homogenized in 2 ml ice cold PBS buffer and centrifuged at 15,000 \times g for 15 min at 4 °C. For determining the activity of GPT, 0.10 ml of enzyme source was taken and 0.50 ml of GPT substrate. Similarly, for determination of GOT, 0.10 ml of enzyme source was taken and 0.50 ml of GOT substrate was added to it. Now 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and contents were left stand for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 20 minutes. The optical density was read at 505 nm after setting the blank. Standard curve was prepared by using oxaloacetic acid as working standard. The enzyme activity was expressed in units of glutamate pyruvate transaminase or glutamate oxaloacetate transaminase activity/ hr/mg protein.

Determination of acetylcholinesterase

Acetylcholinesterase activity was determined according to the method of Ellman et al. [20]. For this purpose 500mg treated termites were homogenized 50 mM phosphate buffer (pH 8) in ice bath and centrifuged at $1000 \times g$ for 30 minutes in cold centrifuge at 4 °C. To the supernatant 0.10 ml (5×10^{-4} M) of freshly prepared acetylcholinethiodide solution, 0.05 ml of DTNB reagent (chromogenic agent) and 1.45 ml of PBS (pH 6.9) were added. The changes in optical density were monitored at 412 nm regularly for three minutes at 25 °C. Enzyme activity has been expressed as m moles 'SH' hydrolysed per minute per mg protein.

Statistical analysis

The LD₅₀ for each extract was determined by using Probit analysis. Mean, standard deviation, standard error and Student t-test were applied by ANOVA program [21]

3 Results

Toxicity determination

In the present study both *Capparis decidua* and its combinatorial mixtures have shown very high termiticidal activity as LD₅₀ values obtained were very low i.e. 22.68, 13.00, 1.56, 19.20, 15.68 and 26 μ g/gm body weight of termites for C-ST, C-BT, C-CoT, PCU, C-CuT and aqueous extract of *C. decidua* respectively.

In vivo treatment of 40% and 80% after 16h LD₅₀ of C-ST mixture significantly ($p < 0.05$) cut down the level of glycogen, amino acid, lipid, DNA, RNA and protein i.e. 47.23% and 45.09%; 84.87% and 87.41%; 78.58% and 75.71%; 73.41% and 67.64%; 77.14% and 73.26%; 68.07% and 59.61% respectively (Table 1).

Similarly 40% and 80% of 16h LD₅₀ of C-BT mixture caused significant ($p < 0.05$) decrease in glycogen level i.e. 39.26% and 48.46% ($p < 0.05$). Similarly amino acid level was found to be decreased up to 83.94% and 77.94% at similar dose of C-BT mixture (Table 2).

In another experiment 40% and 80% of LD₅₀ of C-CoT mixture caused significant decrease in glycogen level i.e. 65.03% and 53.37% after 4 h of treatment. Same mixture also caused increase in amino acid level i.e. 105.89% and 96.30% at 4 h of treatment (Table 3). While a significant ($p < 0.05$)

decrease was noted in lipid level at 16 h of treatment i.e. 56.59% and 66.44% in comparison to control respectively. Besides this, DNA and RNA levels were also found to be decreased i.e. 64.35% and 77.17% and 76.93% and 63.87% after 16 h of treatment in comparison to control. Same mixture caused a significant ($p < 0.05$) decrease in protein level i.e. 52.69% and 62.50% at 4 h respectively (Table 3).

In the fourth set of experiment 40% of LD₅₀ of photoactivated cow urine (PCU) caused maximum decrease in glycogen level i.e. 42.33% in comparison to control. Similarly amino acid level was found to be decreased up to 83.49% after 16 h of treatment, while lipid level was found to be decreased i.e. 81.16% and 55.83% in comparison to control respectively. A similar dose of photoactivated cow urine caused decrease in DNA and RNA level i.e. 77.41% and 62.82% and 52.95% and 57.55% after 16 h treatment in comparison to control respectively. Similar total protein level was also found to be decreased up to 52.88% and 50.76% after 16 h of treatment respectively (Table 4).

Similarly C-CuT mixture caused significant decrease in glycogen level 45.09% and 29.57% (Table 5) and amino acid level i.e. 87.41% and 78.98% after 16 h treatment of termite with 40% and 80% LD₅₀ of C-CuT mixture. Same mixture also caused regular decrease in lipid levels up to 16 h i.e. 75.71% and 71.13% in comparison to control respectively. A similar dose significantly ($p < 0.05$) cut down the level of both DNA and RNA levels i.e. 67.64% and 67.17% and 73.26% and 52.55% respectively (Table 5). C-CuT mixture also caused significant ($p < 0.05$) decrease in protein level up to 16 h i.e. 59.61% and 46.73% in comparison to control respectively (Table 5).

40% of *C. deciduea* aqueous extract caused significant ($p > 0.05$) decreases in glycogen level in treated termites in comparison to control at 16 h of treatment. Maximum decrease in glycogen level i.e. 67.17% was observed after 16 h of treatment of 80% of LD₅₀ of *C. deciduea* aqueous extract. A similar dose of *C. deciduea* aqueous extract caused very slight decrease in lipid contents after 4 h of treatment. Later on it was found to be significantly ($p > 0.05$) decreased in other successive treatments. Similarly DNA level was also significantly decreased after 16 h of treatment i.e. 77.53% and 73.88% in comparison to control. Besides this, both RNA and protein levels were found to be decreased when termites were treated with 40% and 80% of LD₅₀ of *C. deciduea* aqueous extract i.e. 94.08% and 90.92% and 66.73% and 64.54% after 16 h treatment in comparison to control respectively (Table 6).

Table 2: Effect of 40% and 80% of LD₅₀ of C-BT on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Parameters	0 (Control)	Time (in h)											
		4		8		12		16		20		24	
		40%	80%	40%	80%	40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	3.26±0.0115 (100)	1.61*±0.045 (49.38)	2.05*±0.029 (62.88)	1.89*±0.038 (57.97)	2.27*±0.037 (69.63)	1.31*±0.04 (40.18)	1.78*±0.029 (54.60)	1.28*±0.07 (39.26)	1.58*±0.034 (48.46)				
Amino acid	0.866±0.003 (100)	0.876*±0.028 (107.15)	1.15*±0.011 (132.79)	0.769*±0.0038 (88.79)	0.763*±0.011 (88.10)	0.783*±0.003 (90.41)	0.763*±0.003 (88.10)	0.727*±0.0067 (83.94)	0.675*±0.006 (77.94)				
Lipid	1.046±0.001 (100)	0.983*±0.004 (93.97)	1.27*±0.027 (121.41)	0.953*±0.0045 (91.10)	0.854*±0.0065 (81.64)	1.07*±0.028 (99.63)	0.934*±0.0038 (89.29)	0.909*±0.0017 (86.90)	0.823*±0.002 (78.68)				
D.N.A.	0.85±0.028 (100)	0.722*±0.0038 (84.94)	0.649*±0.0026 (76.35)	0.714*±0.0028 (84.00)	0.639*±0.003 (75.17)	0.55*±0.0034 (64.70)	0.585*±0.0045 (68.82)	0.713*±0.0038 (83.88)	0.539*±0.009 (63.41)				
R.N.A.	0.98±0.023 (100)	0.879*±0.0065 (89.69)	0.921*±0.0032 (93.97)	0.848*±0.0045 (86.53)	0.909*±0.0035 (92.75)	0.732*±0.003 (74.69)	0.896*±0.0014 (91.42)	0.633*±0.005 (64.59)	0.884*±0.005 (90.20)				
Protein	5.2±0.046 (100)	2.92*±0.03 (56.15)	3.08*±0.04 (59.23)	2.93*±0.0045 (56.34)	2.56*±0.012 (49.23)	2.38*±0.034 (45.76)	2.08*±0.043 (40.00)	2.34*±0.067 (45.00)	2.54*±0.028 (48.84)				

Values are mean ±SE of three replicates

Values are parantheses indicate percent level with control taken as 100%

*Significant (P < 0.05, student t-test)

Table 3: Effect of 40% and 80% of LD₅₀ of *C-CoT* on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Parameters	0 (Control)	Time (in h)					
		4		8		12	
		40%	80%	40%	80%	40%	80%
Glycogen	3.26±0.0115 (100)	2.12*±0.014 (65.03)	1.74*±0.023 (53.37)	2.04*±0.018 (62.57)	1.86*±0.009 (57.05)	1.93*±0.005 (59.20)	1.62*±0.0042 (49.69)
Amino acid	0.866±0.003 (100)	0.917*±0.003 (105.89)	0.834*±0.0017 (96.30)	0.578*±0.012 (66.74)	0.792*±0.009 (91.45)	0.545*±0.002 (62.93)	0.766*±0.0012 (88.45)
Lipid	1.046±0.001 (100)	0.975*±0.009 (93.21)	0.925*±0.002 (88.43)	0.892*±0.0025 (85.27)	0.756*±0.0012 (72.27)	0.713*±0.003 (68.16)	0.822*±0.0012 (78.58)
D.N.A.	0.85±0.028 (100)	0.723*±0.009 (85.06)	0.742*±0.0012 (87.29)	0.69*±0.004 (81.17)	0.715*±0.0014 (84.12)	0.672*±0.001 (79.06)	0.673*±0.0057 (79.17)
R.N.A.	0.98±0.023 (100)	0.913*±0.0012 (93.16)	0.885*±0.0022 (90.30)	0.876*±0.0059 (89.38)	0.865*±0.0021 (88.26)	0.79*±0.0042 (80.61)	0.715*±0.0018 (72.95)
Protein	5.2±0.046 (100)	2.74*±0.017 (52.69)	3.25*±0.051 (62.50)	2.82*±0.009 (54.23)	3.15*±0.024 (60.57)	3.11*±0.018 (59.80)	3.45*±0.023 (66.34)
							0.626*±0.0017 (76.93)
							0.656*±0.0012 (77.17)
							0.626*±0.0025 (63.87)
							3.27*±0.012 (62.88)

Values are mean ±SE of three replicates

Values are parentheses indicate percent level with control taken as 100%

*Significant (P < 0.05, student t-test)

Table 5: Effect of 40% and 80% of LD₅₀ of C-CuT on glycogen, amino acid, lipid, DNA, RNA and protein levels in *Odontotermes obesus* (Rambur)

Para- meters	Time (in h)												
	0 (Control)	4			8			12			16		
		40%	80%	40%	80%	40%	80%	40%	80%	40%	80%	40%	80%
Glycogen	3.26±0.0115 (100)	1.65*±0.021 (50.61)	1.53*±0.0012 (46.93)	1.93*±0.028 (59.20)	1.27*±0.0037 (38.95)	1.66*±0.045 (50.92)	1.46*±0.009 (44.78)	1.47*±0.013 (45.09)	0.964*±0.0031 (29.57)				
Amino acid	0.866±0.003 (100)	0.835*±0.0015 (96.42)	0.768*±0.0029 (88.68)	0.783*±0.013 (90.41)	0.756*±0.009 (87.29)	0.793*±0.001 (91.57)	0.718*±0.002 (82.91)	0.757*±0.002 (87.41)	0.684±0.0012 (78.98)				
Lipid	1.046±0.001 (100)	0.97*±0.014 (92.73)	0.921*±0.002 (88.05)	0.95*±0.003 (90.82)	0.925*±0.0011 (88.43)	0.92*±0.0018 (87.95)	0.815*±0.0023 (77.91)	0.792*±0.0088 (75.71)	0.744*±0.0015 (71.13)				
D.N.A.	0.85±0.028 (100)	0.727*±0.002 (85.52)	0.67*±0.008 (78.82)	0.714*±0.0027 (84.00)	0.642*±0.01 (75.53)	0.642*±0.003 (75.52)	0.623*±0.0035 (73.29)	0.575*±0.004 (67.64)	0.571*±0.015 (67.17)				
R.N.A.	0.98±0.023 (100)	0.884*±0.0013 (90.20)	0.785*±0.0021 (80.10)	0.854*±0.007 (87.14)	0.646*±0.0022 (65.91)	0.845*±0.002 (86.22)	0.635*±0.0012 (64.79)	0.718*±0.0015 (73.26)	0.515*±0.0019 (52.55)				
Protein	5.2±0.046 (100)	3.52*±0.006 (67.69)	3.54*±0.017 (68.07)	3.56*±0.012 (68.46)	2.95*±0.017 (56.73)	3.38*±0.021 (65.00)	2.34*±0.017 (45.00)	3.10*±0.029 (59.61)	2.43*±0.026 (46.73)				

Values are mean ±SE of three replicates

Values are parantheses indicate percent level with control taken as 100%

*Significant (P < 0.05, student t-test)

In vivo Determination of enzymatic parameters

In a similar experiment when termites were treated with 40% and 80% of LD₅₀ of C-ST mixture, it caused a significant ($p > 0.05$) increase in acid phosphatase (107.49% and 107.10%), alkaline phosphatase (105.06% and 104.40%), lactic dehydrogenase (102.65% and 103.29%) and glutamate pyruvate transaminase (106.51% and 106.83%) levels after 4 hr treatment in comparison to control respectively (Table 7). While glutamate oxaloacetate transaminase and acetyl cholinesterase level was found to be decreased after 16 h treatment i.e. 80.03% and 79.73% and 61.12% and 61.09% in comparison to control respectively (Table 7).

Similarly 40% and 80% of LD₅₀ of C-BT mixture caused a significant ($p < 0.05$) decrease in alkaline phosphatase (90.08% and 86.21%), glutamate oxaloacetate transaminase (91.26% and 92.19%) and acetyl cholinesterase (78.85% and 79.62%) levels, while slight increase was observed in the acid phosphatase (103.29% and 101.61%), lactic dehydrogenase (101.89% and 101.64%) and glutamate pyruvate transaminase (102.46% and 106.01%) levels at 12 h of treatment in comparison to control respectively (Table 8).

However, 40% of LD₅₀ of C-CoT mixture caused a significant ($p < 0.05$) increase in the acid phosphatase (104.38%), alkaline phosphatase (113.54%), lactic dehydrogenase (101.53%), glutamate pyruvate transaminase (106.11%) and glutamate oxaloacetate transaminase levels (101.31%), while 80% of LD₅₀ of C-CoT mixture caused significant decrease in alkaline phosphatase (89.54%), lactic dehydrogenase (99.62%), glutamate pyruvate transaminase (99.41%), glutamate oxaloacetate transaminase (86.97%) and acetyl cholinesterase (74.02%) levels after 12 h of treatment of termites (Table 9).

Similarly 40% of LD₅₀ of photoactivated cow urine caused a significant ($p < 0.05$) decrease in the acid phosphatase (98.52%), lactic dehydrogenase (99.68%), glutamate pyruvate transaminase (70.44%) and glutamate oxaloacetate transaminase (99.74%) levels after 4 h of treatment in comparison to control respectively. While 80% of LD₅₀ of photoactivated cow urine caused a significant ($p < 0.05$) increase in acid phosphatase (107.10%), alkaline phosphatase (105.60%), lactic dehydrogenase (103.24%) and glutamate pyruvate transaminase levels (106.51%), while decrease in the glutamate oxaloacetate transaminase (98.78%) and acetyl cholinesterase (97.98%) level after 4 h of treatment in comparison to control respectively (Table 10).

Table 7: Effect of 40% and 80% of LD₅₀ of C-ST on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Parameters	Time (in h)											
	4			8			12			16		
	0 (Control)	40%	80%	40%	80%	40%	80%	40%	80%	40%	80%	80%
ACP	4.85±0.022 (100.00)	5.21*±0.0088 (107.49)	5.19*±0.0078 (107.10)	5.11*±0.015 (105.36)	5.06*±0.088 (104.46)	4.93*±0.0328 (101.71)	4.91*±0.0058 (101.23)	4.50*±0.0088 (92.71)	4.62*±0.013 (95.32)	4.50*±0.0088 (92.71)	4.62*±0.013 (95.32)	4.62*±0.013 (95.32)
ALP	1.25±0.0057 (100.00)	1.31*±0.015 (105.06)	1.31*±0.0058 (104.40)	1.29*±0.002 (103.22)	1.30*±0.00031 (103.80)	1.28*±0.0017 (102.48)	1.11*±0.0052 (89.54)	1.13*±0.016 (90.40)	1.06*±0.0088 (85.33)	1.13*±0.016 (90.40)	1.06*±0.0088 (85.33)	1.06*±0.0088 (85.33)
LDH	14.59±0.039 (100.00)	14.97*±0.02 (102.65)	15.07*±0.0058 (103.29)	14.89*±0.013 (102.10)	14.93*±0.0087 (102.29)	14.87*±0.0058 (101.91)	14.53*±0.022 (99.62)	14.57*±0.02 (99.90)	14.23*±0.012 (97.57)	14.57*±0.02 (99.90)	14.23*±0.012 (97.57)	14.23*±0.012 (97.57)
GPT	6.55±0.015 (100.00)	6.97*±0.0033 (106.51)	6.99*±0.0091 (106.83)	6.88*±0.012 (105.13)	6.72*±0.0039 (102.55)	6.73*±0.018 (102.79)	6.51*±0.0012 (99.41)	6.46*±0.0088 (98.67)	6.41*±0.0024 (97.91)	6.46*±0.0088 (98.67)	6.41*±0.0024 (97.91)	6.41*±0.0024 (97.91)
GOT	1.64±0.023 (100.00)	1.63*±0.0058 (99.39)	1.61*±0.0012 (97.90)	1.56*±0.012 (95.32)	1.57*±0.0062 (95.83)	1.42*±0.012 (86.99)	1.43*±0.0088 (86.97)	1.311*±0.002 (80.03)	1.31*±0.0015 (79.73)	1.311*±0.002 (80.03)	1.31*±0.0015 (79.73)	1.31*±0.0015 (79.73)
AChE	0.0116±0.009 (100.00)	0.014*±28.10 ⁻⁴ (123.56)	0.012*±47.10 ⁻⁴ (104.31)	0.00893*±12.10 ⁻⁴ (77.01)	0.0096*±2.10 ⁻⁴ (82.55)	0.0081*±3.4.10 ⁻⁴ (69.45)	0.0086*±12.10 ⁻⁴ (74.02)	0.0071*±4.5.10 ⁻⁴ (61.12)	0.0071*±4.4.10 ⁻⁴ (61.09)	0.0071*±4.5.10 ⁻⁴ (61.12)	0.0071*±4.4.10 ⁻⁴ (61.09)	0.0071*±4.4.10 ⁻⁴ (61.09)

Values are mean ±SE of three replicates

Values are parentheses indicate percent

*Significant at (P < 0.05, student t-test)

- acid phosphatase (ACP) and alkaline phosphatase (ALP): μ moles of p-nitrophenol formed /30 minute/mg protein.
- lactic dehydrogenase (LDH): μ moles of pyruvate reduced/ 30min/mg protein.
- glutamate-Pyruvate transaminase (GPT): Units of glutamate-pyruvate transaminase activity/hour/mg protein.
- glutamate oxalo acetate transaminase (GOT): Units of glutamate oxalo acetate transaminase activity/ hour/ mg protein.
- Acetylcholine esterase (AChE): μ moles 'SH' hydrolysed/min/mg/protein.

Table 8: Effect of 40% and 80% of LD₅₀ of C-BT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Parameters	Time (in h)											
	4			8			12			16		
0 (Control)	40%	80%		40%	80%		40%	80%		40%	80%	
ACP	4.85±0.022 (100.00)	5.36*±0.029 (110.65)	5.39*±0.061 (111.07)	5.23*±0.014 (107.97)	5.22*±0.0016 (107.53)	5.01*±0.023 (103.29)	4.93*±0.012 (101.61)	4.63*±0.0176 (95.60)	4.50*±0.036 (92.78)			
ALP	1.25±0.0057 (100.00)	1.41*±0.0039 (112.77)	1.41*±0.003 (112.51)	1.25*±0.0012 (100.45)	1.35*±0.0042 (107.81)	1.13*±0.018 (90.08)	1.08*±0.016 (86.21)	0.96*±0.0117 (76.98)	0.893*±0.0061 (71.49)			
LDH	14.59±0.039 (100.00)	15.24*±0.02 (104.47)	15.15*±0.021 (103.83)	15.11*±0.014 (103.58)	15.05*±0.02 (103.12)	14.86*±0.0088 (101.89)	14.83*±0.0058 (101.64)	14.54*±0.014 (99.68)	14.37*±0.0136 (98.48)			
GPT	6.55±0.015 (100.00)	7.14*±0.012 (109.06)	7.24*±0.023 (110.58)	6.82*±0.0088 (104.17)	7.05*±0.021 (107.67)	6.71*±0.010 (102.46)	6.94*±0.021 (106.01)	6.46*±0.0090 (98.66)	6.52*±0.0087 (99.60)			
GOT	1.64±0.023 (100.00)	1.62*±0.0024 (99.01)	1.61*±0.0058 (98.11)	1.59*±0.0041 (96.99)	1.57*±0.0038 (96.02)	1.50*±0.0088 (91.26)	1.51*±0.00115 (92.19)	1.41*±0.0145 (86.13)	1.40*±0.002 (85.65)			
AchE	0.0116±0.009 (100.00)	0.0116*±24·10 ⁻⁴ (100.57)	0.0115*±12·10 ⁻⁴ (99.71)	0.0096*±1.7·10 ⁻⁴ (82.84)	0.0098*±2.2·10 ⁻⁴ (84.79)	0.0091*±1.7·10 ⁻⁴ (78.85)	0.0092*±1.5·10 ⁻⁴ (79.62)	0.0082*±0.0032 (70.38)	0.0083*±1.2·10 ⁻⁴ (71.17)			

Table 9: Effect of 40% and 80% of LD₅₀ of C-CoT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Parameters	Time (in h)											
	4			8			12			16		
0 (Control)	40%	80%		40%	80%		40%	80%		40%	80%	
ACP	4.85±0.022 (100.00)	5.51*±0.0048 (113.55)	5.19*±0.0078 (107.10)	5.46*±0.0051 (112.69)	5.06*±0.0088 (104.46)		5.06*±0.026 (104.38)	4.91*±0.0058 (101.23)		4.94*±0.017 (101.75)	4.72±0.0058 (97.31)	
ALP	1.25±0.0057 (100.00)	1.56*±0.012 (124.64)	1.31*±0.0058 (104.40)	1.49*±0.0033 (119.46)	1.30*±0.00031 (103.80)		1.42*±0.0047 (113.54)	1.11*±0.0052 (89.54)		1.34*±0.0085 (106.96)	1.29*±0.0078 (103.57)	
LDH	14.59±0.039 (100.00)	15.21*±0.0044 (104.22)	15.07*±0.0058 (103.29)	15.03*±0.012 (103.04)	14.93*±0.0087 (102.29)		14.81*±0.028 (101.53)	14.53*±0.022 (99.62)		14.57*±0.009 (99.91)	14.96*±0.015 (102.53)	
GPT	6.55±0.015 (100.00)	7.37*±0.0088 (112.62)	6.99*±0.0091 (106.83)	7.17*±0.012 (109.51)	6.72*±0.0039 (102.55)		6.95*±0.01 (106.11)	6.51*±0.0012 (99.41)		6.84*±0.02 (104.52)	6.66*±0.015 (101.67)	
GOT	1.64±0.023 (100.00)	1.78*±0.0017 (108.59)	1.61*±0.0012 (97.90)	1.75*±0.0015 (106.89)	1.57*±0.0062 (95.83)		1.66*±0.012 (101.31)	1.43*±0.0088 (86.97)		1.61*±0.0018 (98.45)	1.48*±0.0026 (90.52)	
AChE	0.0116±0.009 (100.00)	0.0118*±3.10 ⁻⁴ (101.72)	0.012*±47.10 ⁻⁴ (104.31)	0.014*±15.10 ⁻⁴ (124.13)	0.0096*±2.10 ⁻⁴ (82.55)		0.0096*±1.7.10 ⁻⁴ (83.10)	0.0086*±12.10 ⁻⁴ (74.02)		0.0092*±0.6.10 ⁻⁴ (79.45)	0.0086*±1.5.10 ⁻⁴ (74.45)	

Table 10: Effect of 40% and 80% of LD₅₀ of photoactivated cow urine on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Parameters	Time (in h)											
	4			8			12			16		
	0 (Control)	40%	80%	40%	80%	80%	40%	80%	40%	80%	40%	80%
ACP	4.85±0.022 (100.00)	4.78*±0.0044 (98.52)	5.19*±0.0078 (107.1)	4.59*±0.0094 (94.66)	5.06*±0.0088 (104.46)	4.46*±0.0023 (92.03)	4.46*±0.0023 (92.03)	4.91*±0.0058 (101.23)	4.26*±0.007 (87.89)	4.62*±0.013 (95.32)	4.26*±0.007 (87.89)	4.62*±0.013 (95.32)
ALP	1.25±0.0057 (100.00)	1.26*±0.0012 (100.51)	1.32*±0.0058 (105.60)	1.246*±0.0024 (99.65)	1.314*±0.0026 (105.14)	1.23*±0.0059 (98.09)	1.23*±0.0059 (98.09)	1.17*±0.0115 (93.60)	1.21*±0.0015 (96.56)	1.08*±0.017 (86.40)	1.21*±0.0015 (96.56)	1.08*±0.017 (86.40)
LDH	14.59±0.039 (100.00)	14.54*±0.02 (99.68)	15.06*±0.022 (103.24)	14.52*±0.0038 (99.49)	14.96*±0.0058 (102.53)	14.55*±0.0088 (99.71)	14.55*±0.0088 (99.71)	14.56*±0.0115 (99.79)	14.43*±0.023 (98.93)	14.37*±0.012 (98.51)	14.43*±0.023 (98.93)	14.37*±0.012 (98.51)
GPT	6.55±0.015 (100.00)	1.93*±0.02 (70.44)	6.98*±0.02 (106.51)	6.53*±0.011 (99.65)	6.76*±0.012 (103.15)	6.44*±0.022 (98.27)	6.44*±0.022 (98.27)	6.66*±0.0012 (101.62)	6.34*±0.0071 (96.76)	6.45*±0.02 (98.42)	6.34*±0.0071 (96.76)	6.45*±0.02 (98.42)
GOT	1.64±0.023 (100.00)	1.64*±0.0027 (99.74)	1.62*±0.01 (98.78)	1.62*±0.0015 (98.96)	1.58*±0.0042 (96.21)	1.6*±0.002 (97.83)	1.6*±0.002 (97.83)	1.46*±0.0029 (80.73)	1.58*±0.003 (96.58)	1.33*±0.0065 (80.91)	1.58*±0.003 (96.58)	1.33*±0.0065 (80.91)
AChE	0.0116±0.009 (100.00)	0.0143*±28·10 ⁻⁴ (123.56)	0.0114±79·10 ⁻⁴ (97.98)	0.0089*±12·10 ⁻⁴ (77.01)	0.0097*±2·10 ⁻⁴ (83.64)	0.0081*±3.4·10 ⁻⁴ (69.45)	0.0081*±3.4·10 ⁻⁴ (69.45)	0.0082*±3.2·10 ⁻⁴ (70.26)	0.0071*±4.5·10 ⁻⁴ (61.12)	0.007*±5.5·10 ⁻⁴ (60.60)	0.0071*±4.5·10 ⁻⁴ (61.12)	0.007*±5.5·10 ⁻⁴ (60.60)

Table 11: Effect of 40% and 80% of LD₅₀ of C-CuT on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, Glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Para- meters	Time (in h)											
	4			8			12			16		
	0 (Control)	40%	80%	40%	80%	40%	40%	80%	40%	80%	40%	80%
ACP	4.85±0.022 (100.00)	4.42*±0.0026 (91.20)	4.39*±0.0023 (90.41)	4.28*±0.013 (88.15)	4.16*±0.00088 (85.72)	4.18*±0.0018 (86.24)	4.18*±0.0018 (86.24)	4.04*±0.025 (83.40)	3.95*±0.004 (81.38)	3.81*±0.022 (78.52)	3.95*±0.004 (81.38)	3.81*±0.022 (78.52)
ALP	1.25±0.0057 (100.00)	1.25*±0.00069 (99.83)	1.24*±0.00074 (98.99)	1.23*±0.0017 (98.35)	1.16*±0.0012 (98.99)	1.15*±0.0031 (91.76)	1.15*±0.0031 (91.76)	1.08*±0.0074 (86.24)	1.06*±0.0031 (84.48)	0.98*±0.00088 (78.59)	1.06*±0.0031 (84.48)	0.98*±0.00088 (78.59)
LDH	14.59±0.039 (100.00)	14.15*±0.018 (97.01)	13.98*±0.0022 (95.83)	14.15*±0.065 (96.98)	13.65*±0.0029 (93.58)	13.95*±0.0043 (95.60)	13.95*±0.0043 (95.60)	13.10*±0.0013 (89.76)	13.15*±0.0005 (90.12)	12.26*±0.023 (84.03)	13.15*±0.0005 (90.12)	12.26*±0.023 (84.03)
GPt	6.55±0.015 (100.00)	6.46*±0.025 (98.63)	6.36*±0.013 (97.12)	6.28*±0.0029 (95.95)	6.07*±0.017 (92.74)	6.08*±0.0033 (92.77)	6.08*±0.0033 (92.77)	5.57*±0.011 (85.11)	5.68*±0.0036 (86.68)	5.28*±0.0088 (80.56)	5.68*±0.0036 (86.68)	5.28*±0.0088 (80.56)
GOT	1.64±0.023 (100.00)	1.62*±0.0014 (98.50)	1.61*±0.0026 (98.08)	1.61*±0.0018 (97.85)	1.59*±0.011 (97.14)	1.56*±0.0017 (95.41)	1.56*±0.0017 (95.41)	1.29*±0.0015 (78.43)	1.33*±0.0025 (80.85)	1.25*±0.016 (76.44)	1.33*±0.0025 (80.85)	1.25*±0.016 (76.44)
AChE	0.0116±0.009 (100.00)	0.011*±58.10 ⁻⁴ (94.54)	0.00985*±1.2.10 ⁻⁴ (84.89)	0.0096*±1.7.10 ⁻⁴ (83.10)	0.0092*±0.1.10 ⁻⁴ (79.05)	0.00923*±1.5.10 ⁻⁴ (79.60)	0.00923*±1.5.10 ⁻⁴ (79.60)	0.0089*±5.5.10 ⁻⁴ (76.32)	0.008±2.6.10 ⁻⁴ (76.72)	0.0082*±43.10 ⁻⁴ (70.72)	0.0089*±5.5.10 ⁻⁴ (76.32)	0.0082*±43.10 ⁻⁴ (70.72)

Table 12: Effect of 40% and 80% of LD₅₀ of *Capparis decidua* aqueous extract on acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur)

Parameters	Time (in h)											
	0 (Control)			4			8			12		
	40%	80%		40%	80%		40%	80%		40%	80%	
ACP	4.85*±0.022 (100.00)	4.67*±0.0058 (96.28)	4.62*±0.0058 (95.26)	4.59*±0.0088 (94.63)	4.48*±0.0375 (92.37)	4.28*±0.0346 (88.25)	4.31*±0.0546 (88.87)	4.036*±0.0218 (83.22)	4.14*±0.0546 (85.36)			
ALP	1.25±0.0057 (100.00)	1.243*±0.0029 (99.44)	1.234*±0.0023 (98.72)	1.224*±0.0023 (97.92)	1.217*±0.0041 (97.36)	1.194*±0.0035 (95.52)	1.179*±0.0029 (94.32)	1.146*±0.0061 (91.68)	1.132*±0.0027 (90.56)			
LDH	14.59±0.039 (100.00)	14.55*±0.0176 (99.73)	14.21*±0.015 (97.40)	14.44*±0.0218 (98.97)	14.10*±0.042 (96.64)	14.63*±0.0185 (100.27)	14.10*±0.043 (95.89)	14.246*±0.012 (97.64)	13.91*±0.0208 (95.34)			
GPT	6.55±0.015 (100.00)	6.53*±0.053 (99.69)	6.37*±0.0176 (97.25)	6.42*±0.029 (98.02)	6.31*±0.02 (96.33)	6.64*±0.0317 (101.37)	6.1*±0.0176 (93.13)	6.19*±0.034 (94.50)	5.97*±0.021 (91.15)			
GOT	1.64±0.023 (100.00)	1.75*±0.0176 (106.70)	1.4*±0.0425 (85.37)	1.66*±0.012 (101.22)	1.16*±0.024 (70.73)	1.56*±0.0185 (95.12)	1.04*±0.02 (63.41)	1.464*±0.0208 (89.27)	0.99*±0.0033 (60.48)			

Similarly 40% and 80% of LD₅₀ of C-CuT mixture caused a significant ($p < 0.05$) decrease in acid phosphatase (81.38% and 78.52%), alkaline phosphatase (84.48% and 78.59%), lactic dehydrogenase (90.12% and 84.03%), glutamate pyruvate transaminase (86.68% and 80.56%), glutamate oxaloacetate transaminase (80.85% and 76.44%) and acetyl cholinesterase (76.72% and 70.72%) levels after 16 h of treatment in comparison to control respectively (Table 11).

40% and 80% of LD₅₀ of *C. decidua* aqueous extract caused significant ($p < 0.05$) decrease in acid phosphatase (83.22% and 85.36%) and alkaline phosphatase level (95.52% and 94.32%) after 16 h of treatment. Contrary to this Lactic dehydrogenase level was found to be increased after 12 h treatment of termites with *C. decidua* aqueous extract. Same extract has also shown slight variation in glutamate pyruvate transaminase level at different treatment in comparison to control (Table 12). Similarly 40% and 80% of LD₅₀ of Capparis decidua aqueous extract caused a significant decrease in glutamate oxaloacetate transaminase and acetyl cholinesterase level at all the test time interval and the level recorded 89.27% and 60.48% and 83.01% and 74.31% after 16 h treatment in comparison to control respectively (Table 12).

4 Discussion

Plant products are used as pesticides to kill insect pests. However, in the present study efforts have been made to explore insecticidal potential of *C. decidua* and its mixtures. In various bioassays *C. decidua* extract and its mixtures were found highly toxic which is proved by low LD₅₀ value obtained i.e. ranged from 1.56 to 26.0 $\mu\text{g/g}$ body weight after 16 h treatment. Similarly neem bark and cedar wood Douglas fir wood [22, 23] and black heartwood of *Cryptomeria japonica* abs red cedar, Tectona grandis have shown good termiticidal activity against *Coptotermes formosanus* Shiraki [10]. Besides this, limonoids such as obacunone (113 ppm), nomillin (4475 ppm) and azadirachtin (65,293 ppm) exhibited very high toxicity against *R. speratus* [24]. Similarly essential oils like vetiver grass, Cassia leaf, clove bud, cedar wood, Eucalyptus, lemon grass, geranium were also found highly effective against termites at a very low dose of 1% (W/V). Similarly, anti-termite activity is reported in *Curcuma longa* rhizome oil against *O. obesus* at 2,000ppm concentration [25].

Besides showing toxicity, various extracts have significantly altered the level of various macromolecules and enzymes in treated termites. However, 40% and 80% of LD₅₀ of each mixture i. e. C-ST, C-CoT, C-CuT caused significant ($p < 0.05$) reduction in glycogen, amino acid, DNA, RNA, protein and lipid

contents in termites after 16 h of treatment. C-ST mixture has decreased lipid contents up to 78.58% and 75.71% after 16 h treatment respectively (Table 1) while 40% and 80% of LD₅₀ of *C. decidua* mixed with borate and other natural components caused a significant ($p < 0.05$) decrease in glycogen, amino acid, DNA, RNA, protein and lipid level in termites (Table 2). Similarly *C. decidua* mixed with copper sulphate (C-CoT) mixture has shown significant ($p < 0.05$) decrease in the level of different macromolecules (Table 3). Similar results were obtained in photo-activated cow urine and its combinatorial mixtures with *C. decidua* (Table 4 and 5). It was mostly found that on an average *C. decidua* and its combinatorial mixtures have shown higher toxicity in comparison to other mixture. More specifically, termites treated with 40% and 80% of LD₅₀ of *C. decidua* aqueous extract showed reduction in glycogen, amino acid, DNA, RNA and protein levels at regular time intervals (Table 6). after response to stress caused by pesticide treatment

In insects, glycogen is a major energy reserve found in fat body and muscles. It is synthesized from glucose units but indirectly it is also synthesized from glucogenic amino acids which indicate utilization of amino acids. Normally free glycogen floats in the haemolymph/blood. Its breakdown or help to maintain glucose level in insect tissues [26]. With this, its utilization exceeds to cope up the insecticide-induced stress [27, 28]. Therefore, phosphorylation of energy molecules is increased which indicates major utilization of food reserves and release of high energy in insect tissues. Therefore, more and more utilization of energy reserves occurs to fight against pesticides generated stress.

Similarly cypermethrin affect the level glycogen level in *Pimpla turionella* wasp larvae, pupae and adult female after treatment [29]. It also increased the protein level in *Spodoptera litura* larvae [30]. Contrary to this, organophosphorus insecticides i. e. chlorpyrifos, thiamethoxam, fipronil, and malathion caused significant depletion of total protein in haemolymph and fat body of silk worm *Bombyx mori* [31]. Similarly, lipid depletion occurs in haemolymph, fat body and oocytes of *Tenebrio molitor* after malathion treatment [32]. It indicates excess utilization of lipids. Hence, to maintain the level of lipids large portions of absorbed carbohydrates are converted into lipids [33]. It may be due to breakdown of glycerides and diglycerides. As insects required lipids was essential dietary constituents if anyhow lipid metabolism is exceeded more then it indirectly cut down carbohydrate reserves. Usually in insects, fatty acids are accumulated in fat body as triglycerides, which serve as energy reserves. Hence, lipid reserves are built up during active feeding. On the other hand mobilization of more lipids may induce hydrolysis of triglycerides and diglycerides by an enzyme lipase. If lipid reserve increases, it means hydrolytic

enzymes are not functioning well and over deposition of lipid may cause oxidative stress in insects. Similarly, excessive utilization of protein and nucleic acids caused physiological stress that result into low availability of these nutrients. Moreover, protein catabolism results in an increase in amino acid. Further, *in vivo* exposure of 40% and 80% of LD₅₀ of C-ST, C-BT, C- CoT, PCU, C-CuT and aqueous extract of *C. decidua* caused significant ($p < 0.05$) reduction in the level of acid phosphatase, alkaline phosphatase, lactic dehydrogenase, glutamatepyruvate transaminase, glutamateoxaloacetate transaminase and acetyl cholinesterase levels (Table 7-12). Similarly solvent and aqueous extracts of *Gloriosa superba* [34], *Paronia emodi* [35], *Corydalis incise* [36], *Cassia obtusifolia* [37], *Artemisia annua* [38], *Teucrium royleanum* [39], *Andrache cardifolia* [40], *Angelica archangelica* and *Geranium sylvatica* caused significant inhibition in the level of acetyl cholinesterase, lipoxygenase, urease and alkaline phosphatase, amino transferase of insects [41]. Alkaloids isolated from amaryllidaceae plants significantly inhibited acetylcholinesterase level in insects [42, 43].

Similarly, *C. decidua* extract and its mixtures significantly inhibited the level of ACP and ALP. It may be caused due to induction of hydrolytic activities to fight against toxic effect of pesticide that lead to significant reduction in the level of acid and alkaline phosphatase. Besides this, alkaline phosphatase level might be increased due to very high lysosomal activity in cells, which leads to biochemical stress in insects [44]. In addition to it pesticide intoxication increase transamination activity that affected the level of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in treated termites [45]. However, fat body and haemolymph exhibit higher glutamate oxaloacetate transaminase activity than the glutamate pyruvate transaminase. Hence, the level of haemolymph aminotransferase get significantly decreased. Similarly an increase in glycogenesis causes a significant decrease in free amino acid level [46]. Therefore, a sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects. However, inhibition of phosphatase activity and increase in lactic dehydrogenase level shows tissue necrosis in insects [47]. However, this imbalance in enzyme level indicates inhibition of important metabolic pathways [48]. Similar effects on phosphatases activity were observed in *Pectinophora gossypiella* (Saund.) by Abdel-Hafez et al. [49] after insecticide treatment. Hence, all significant changes in the level of ALP, ACP, GPT, GOT, LDH and AchE indicate very high insecticidal activity of above mixtures to the *O. obesus* the Indian white termite. However, it can be concluded that *C. decidua* possess few active ingredients that might be highly effective against termites. It is proved by the

results that these ingredients cause high lethality in termites at a very low dose and caused significant inhibition or induction of metabolic enzymes. Therefore, it is recommended that *C. decidua* active ingredients could be used for preparation of formulation to control field termites.

Acknowledgements

Authors are highly grateful to University Grants Commission, New Delhi for funding the work through project grant no. 34-417/2008 (SR).

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