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Toxic Effects of *Ficus benghalensis* Latex Based Combinatorial Formulations on Various Enzymatic Parameters in Indian White Termite *Odontotermes obesus*

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Abstract

In the present investigation, various bioassays were conducted to evaluate the alteration in levels of various enzymes *i.e.* alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase. For this purpose worker termites were treated with sub-lethal doses of 40% and 80% of 24 hrs LD_{50} of latex-based combinatorial formulations and observations were taken at 4 hours intervals up to 24 hours. *Ficus benghalensis* crude latex and its combinatorial mixtures, like C-MLT-A, CU-MLT-A, AQ-MLT, P-MLT and EA-MLT significantly altered the level of enzymes in *Odontotermes obesus* and this effect found time and dose-dependent. Reduction or increase in enzymes was calculated by using the corresponding control. The maximum decrease in acid phosphatase level was observed at 16 h when termites were treated with 80% of LD_{50} of B-MLT-B and P-MLT *i.e.* 82.84% at 16 h of treatment. A similar dose caused a very slight decrease in glutamate pyruvate transaminase at 4 h of treatment but with the increase in time. Further decrease was noted in other successive treatments that were significant at $p > 0.05$. Similarly, 40% and 80% of LD_{50} of the C-MLT-B mixture caused significant ($p > 0.05$) decrease in alkaline phosphatase and acid phosphatase level at 16 h treatment *i.e.* 93.42%, 89.46% and 95.89%, 88.17% respectively. The level of acetylcholinesterase was also found to be decreased when termites were treated with 40% and 80% of LD_{50} of C-MLT-B mixture *i.e.* 92.72% and 97.27% respectively. All the above alterations noted in levels of various enzymes confirm the action of latex ingredients on worker termites that were anti-feedant or deterrent types. These ingredients can be used to control not only termites but also other phytophagous insects.

Keywords

Ficus benghalensis, Enzymes, Alteration, *Odontotermes obesus*, Latexes, Anti-Feedant, Termiticides

1. Introduction

Latex is a milky fluid secreted by approximately 10% of flowering plant families. Latex exudes from plant parts after having an injury. It is a secondary metabolite and a complex emulsion that coagulates on exposure to air, consisting of proteins, alkaloids, starches, sugars, oils, tannins, resins, and gums [1]. It protects plants from physical damage caused by chewing herbivores and insect pests [2]. Latex causes itching, inflammation, redness and swelling to appear after skin contact with latex. The most common signs of latex reaction include skin irritation, rash, hives, runny nose and difficulty breathing. It causes an allergic reaction upon inhalation of latex particles inhale (breathe in) latex particles or come into physical contact with latex.

Plant latex contains alkaloids mainly glycosides which heavily deter herbivorous insects and target insect pests effectively [3]. Alkaloid glycosides also display strong anti-termitic activity [4]. Termites infest at various stages of plant growth and cause severe losses in sugarcane, maize, wheat, fruits, etc [5]. In crop fields, termites cause 50% - 100% losses in yield [6]. Components of plant latex inhibit metabolism in termites and kill them due to anti-feedant, repellent and toxic action. These were found in active insects German cockroach *Blatella germanica* [7].

Termites are destructive polyphagous insects that cause serious damage to crops. Termites seriously infest garden trees, timber, building materials, stored products, paper and clothing and cause enormous economic damage to them [8]. Termites infest the plant at different stages of growth [9]. They attack crops such as sugarcane, corn, wheat, barley, rice and vegetable crops. They cause about 50% - 100% yield loss in these crops. They also act as decomposers and increase their activity [10]. Soil fertility, yield and people also use them for their own benefit all over the world. However, some termite species become a threat to agricultural producers because they directly and indirectly cause serious damage to the agricultural system [11]. Termites are the main soil macrofauna and control of termite populations is very important to prevent damage to crops [12]. There are various preventive methods to control termite infestation like reducing openings that offer termites' access to the structure, not piling or storing firewood or wood debris next to the house and inspecting periodically to help ensure that termite colonies do not become established. Some chemical methods are also used as liquid soil-applied termiticides, termite baits, building materials impregnated with termiticides and wood treatments to control the termite population. But these synthetic insecticides are very harmful to man and

his environment. Hence, bio-insecticides are preferred for controlling insects because these are safer to non-target organisms because they have no residual effect in the environment [13].

Termites form huge mounds for colonization and protection against environmental stress and shelter from predators [14]. They participate in many ecological processes, mainly the decomposition of plant waste and dead wood and transform it into soil [15]. The global economic impact of termites is estimated to be approximately $40 billion per year, with subterranean termites accounting for approximately 80% of the total impact [16]. Predatory ants attack termite populations in forests [17].

However, harmful synthetic chemical pesticides are widely used to control termite attacks [18]. Various synthetic insecticides such as cyclodiene [19] [20], cypermethrin [21], hydroquinone and indoxacarb [22] have been used to control termites in crops. Dursban spray has been found to be very effective against wood-destroying termites [23]. Thiamethoxam shows high mortality in workers of the Asian subterranean termite *Coptotermes gestroi* after 1 - 3 days of exposure [24]. These chemicals have serious adverse effects on non-target biotic and abiotic factors in the environment [25]. Although chemical insecticides are very effective against termites, they are dangerous to non-target organisms in the ecosystem. Its associated residues remain in the environment longer and through different trophic levels they enter the food chain [26].

Many toxic synthetic chemical pesticides, bio-insecticides and plant substances have been used to control termites, but the treatment of plant latex is very scary [27] [28]. Plant latexes and some plant substances *Calotropis procera*, *Ipomoea fistulosa*, *Maesa lanceolata*, *Croton macrostachyus*, *Targets minuta*, *Datura stramonium* and *Azadirachta indica* are used for termite control [29] [30] [31]. Latex is produced and stored in special cells called "laticifers". Laticifers form a tubular system consisting of a row of elongated cells that branch to form an internal network covering the entire plant [32]. Plants secrete latex sap in response to physical damage [33]. Fig latexes are more effective natural therapeutic antibacterial agents against pathogenic bacteria [34]. The antiviral properties of *C. majus* latex are due to both the alkaloids and the proteins it contains, which affect different stages of the virus reproduction cycle [35]. There are so many herbal botanicals that can fight against termites in fields and vegetation [36]. Indeed, about 250,000 plant species in the world have been reported to contain compounds with insecticidal properties [37].

Ficus benghalensis, the banyan tree, is popular medicinal plant species. This is a huge plant having height more than 30 m in height. It is found in tropical areas of Asia and used extensively in Ayurveda treatment as a hypoglycemic, diuretic, tonic, rheumatism, astringent, and inflammation [38]. The plant belongs to the family Moraceae, its laticiferous tissue produces milky latex upon wounding. *F. benghalensis* contains a high amount of good quality natural rubber. The natural rubber (*cis*-1,4-polyisoprene) content in the latex of *F. benghalensis* is approx-

imately 17% [39]. Its latex serum contains a small number of mainly around 26-31-kDa protein which works as catalytically-active rubber particles. Plants produce secondary metabolites which are known to play a crucial role in pest control because some are selective, biodegradable, non-toxic products, and have few harmful effects on non-target organisms and the environment [40] [41]. In this study, efforts have been made to evaluate the toxic effects of *Ficus benghalensis* latex on metabolic enzymes alkaline phosphatase, acid phosphatase; glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in *Odontotermes obesus* (Rambur) the Indian white termite.

2. Experimental

2.1. Latex Collection

Latex was collected from *Ficus benghalensis* Banyan or Bargad Tree (Family: Moraceae) located in Deen Dayal Upadhyaya Gorakhpur University Garden. This specimen was authenticated by an expert in botany and help was taken from the Taxonomy of Indian Angiosperms. The herbarium specimen is healthy and preserved in the Botanical garden of Gorakhpur University for Future Reference. This plant is extensively used for shade, nutritional, and therapeutic purposes by local people not only in India but also in Southeast Asia. Fresh latex was collected by making an incision or cut mark with a knife over the tree trunk. Collected plant latex samples were lyophilized and powdered in a vacuum in cold. Lyophilized latex was extracted with different solvents by changing the polarity. Active fractions from the latex were portioned between different solvents on the basis of their polarity. For better fractionation, solvent extraction was performed using polar and non-polar solvents. Latex was allowed to evaporate in a SpeedVac vacuum concentrator to get residue. It was dried and weighed and re-dissolved in a known volume of distilled water. Dissolved residues were stored in cold at 4˚C for experimental purposes. All chemicals used in this study were purchased from CDH-laboratory chemicals suppliers in India supplied by Eastern Scientific Company, Gorakhpur.

2.2. Preparation of Combinatorial Formulations

2.2.1. *In Vivo* Determination of Enzymatic Parameters

To observe the effect on enzymatic parameters 500 mg of adult termite workers were provided 40% and 80% of LD_{50} of *Ficus benghalensis* latex-based combinatorial mixtures with the diet. Insects were sacrificed at the 4 h interval up to 16 h for the measurement of various enzyme levels. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass homogenizer and centrifuged in cold for 25 minutes at 15,000 rpm. The supernatant was isolated in a glass tube and used for the estimation.

2.2.2. Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase level was determined according to the method of Andrech and Szeypiaske and modified by Bergmeyer [42]. For this purpose 500 mg

treated termites were homogenized in 1 ml ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 min. For measurement of alkaline phosphatase level, 0.10 ml of supernatant was added to 1.0 ml of alkaline buffer substrate and incubated for 30 minutes at 37˚C. Alkaline buffer substrate was prepared by addition of 375 mg glycine, 10 mg $MgCl_2 \cdot 6H_2O$ and 165 mg p-nitrophenyl phosphate sodium salt in 42 ml of 0.1 N NaOH. The reaction was stopped by adding an excess of alkali (5.0 ml of 0.02 N NaOH). The p-Nitrophenol formed after the hydrolysis of p-nitrophenyl phosphate gave a yellow color with NaOH. Optical density was measured at 420 nm. The standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as μmoles of p-nitrophenol formed/30 min/mg protein. Three replicates were set in each experiment and the data obtained was statistically analyzed by the ANOVA method.

2.2.3. Determination of Acid Phosphatase (ACP)

Acid phosphatase activity in termites was determined according to the method of Andrech and Szeypiaske and modified by Bergmeyer [42]. For the determination of acid phosphatase level, whole body extract of termites was prepared similarly as mentioned above. For this purpose, 0.1 ml of supernatant was added to 1.0 ml of acid buffer substrate solution (0.41 gm citric acid, 1.125 gm sodium citrate and 165 mg p-nitrophenyl phosphate sodium salt to 100 ml of double distilled water). Contents were mixed thoroughly and incubated for 30 minutes at 37˚C. To this tube, 4.0 ml of 0.10 N NaOH was added to stop the reaction. A yellow color was developed which was measured at 420 nm. The standard curve was prepared by using different concentrations of p-nitrophenol. Enzyme activity was expressed as the amount of p-nitrophenol formed/30 min/mg protein. Three replicates were set in each experiment and the data obtained was statistically analyzed by the ANOVA method.

2.2.4. Determination of Glutamic-Oxaloacetic Transaminase (GOT)

GOT activity was measured according to the method of Reitman and Frankel [43]. For this purpose 500 mg treated termites were homogenized in 2 ml ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 min. For estimation of GOT 0.10 ml of centrifuged supernatant was taken out and to it and 0.50 ml of GOT substrate was added. GOT substrate was prepared by adding 0.292 gm of α-ketoglutaric acid and 26.6 gm of DL-aspartic acid into a 1.0 liter volumetric flask. Contents were mixed thoroughly and 1 N NaOH was added slowly to the above solution. PH of the solution was adjusted to 7.4 by using PBS buffer. A total volume of the solution was maintained at 1000 ml by adding buffer (13.97 gm K_2HPO_4 and 2.69 gm KH_2PO_4 in 1000 ml water). To this tube, 0.50 ml of 2 - 4 dinitrophenyl hydrazine solution (Dissolved 0.198 gm of 2, 4-dinitrophenyl hydrazine in 1 N HCl to make 1000 ml.) was added and kept standing for 15 minutes at room temperature. Then 5.0 ml of 0.4 N NaOH (1.6 gm NaOH dissolved in 100 ml distilled water) was added and mixed well. Now contents were left for 20 minutes at room temperature. Optical density was recorded at 505 nm

by setting the blank with distilled water. The standard curve was prepared by using oxaloacetic acid as the standard. Enzyme activity was expressed in units of glutamate oxaloacetate transaminase/30 min/mg protein.

2.2.5. Determination of Glutamate-Pyruvate Transaminase (GPT)

GPT activity in whole body extract of termites was measured according to the method of Reitman and Frankel [43]. For this purpose 500 mg treated termites were homogenized in 2 ml ice-cold PBS buffer and centrifuged at 15,000 Xg for 15 min. 0.10 ml of centrifuged supernatant was added to 0.50 ml of GPT substrate. GPT substrate was prepared by dissolving 0.292 gm of α-ketoglutaric acid and 17.8 gm of DL alanine in a 1.0 lite volumetric flask. 1 N NaOH was slowly added to the above mixture. It was mixed well until all solids dissolved completely. The PH of the substrate was adjusted to 7.4 by adding a sufficient volume of buffer and the total volume was maintained at 1000 ml. The buffer was prepared by dissolving 13.97 gm K_2HPO_4 and 2.69 gm KH_2PO_4 in 1000 ml distilled water. In the above supernatant, 0.5 ml of GPT substrate and 0.50 ml of 2 - 4 dinitrophenyl hydrazine solution (Dissolved 0.198 gm of 2,4-dinitrophenyl hydrazine in 1 N HCl to make 1000 ml) were added and kept for 15 minutes at room temperature. Now 5.0 ml of 0.4 N NaOH (1.6 gm NaOH dissolved in 100 ml distilled water) was added, mixed well and allowed to stand at room temperature for 20 minutes. The optical density was noted at 505 nm and the blank was set with water to make the background absorbance zero. The standard curve was prepared by using oxaloacetic acid as the standard. The enzyme activity was expressed in units of glutamate-pyruvate transaminase activity/mg protein. Three replicates were set for each test and the control and data obtained were statistically analyzed by the ANOVA method.

2.2.6. Determination of Acetylcholinesterase (AchE)

Acetylcholinesterase activity was determined according to the method of Ellman [44]. For this purpose, 500 mg treated termites were homogenized in ice-cold PBS buffer for 5 minutes in a glass-glass homogenizer. It was centrifuged at 15,000 rpm in cold to get the supernatant. For estimation of AchE level 0.050ml of supernatant was mixed with (10 mm path length cuvette) 0.10 ml freshly prepared acetyl cholinethioiodide solution (5×10^{-4} M) and into it 0.05 ml DTNB (0.19818 gm/l) a chromogenic agent and 1.45 ml of PBS (pH 6.9) were added. The change in absorbance was recorded at 412 nm regularly for three minutes at 25˚C. Enzyme activity was expressed in μ moles "SH" hydrolyzed per minute per mg protein.

2.2.7. Statistical Analysis

The LD_{50} in termite workers was determined for each extract and combinatorial mixture by using Probit analysis. Mean, standard deviation, standard error, correlation and Student t-test were applied by the ANOVA program. The Chi-Square test was applied to establish the repellent activity [45].

3. Results

In this study, the toxic effects of *Ficus benghalensis* latex-based combinatorial formulations on certain metabolic enzymes such as alkaline phosphatase, acid phosphatase; glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase were evaluated. Levels of these enzymes were calculated in whole-body extracts of termites treated with 40% and 80% of LD_{50} of *Ficus benghalensis* latex-based combinatorial formulations separately for 4, 8, 12 and 16 h (Figures 1-20).

40% and 80% of LD_{50} of Combinatorial mixture S-MLT-A, S-MLT-B and S-MLT-C caused significant ($p > 0.05$) decreases in alkaline phosphatase *i.e.*

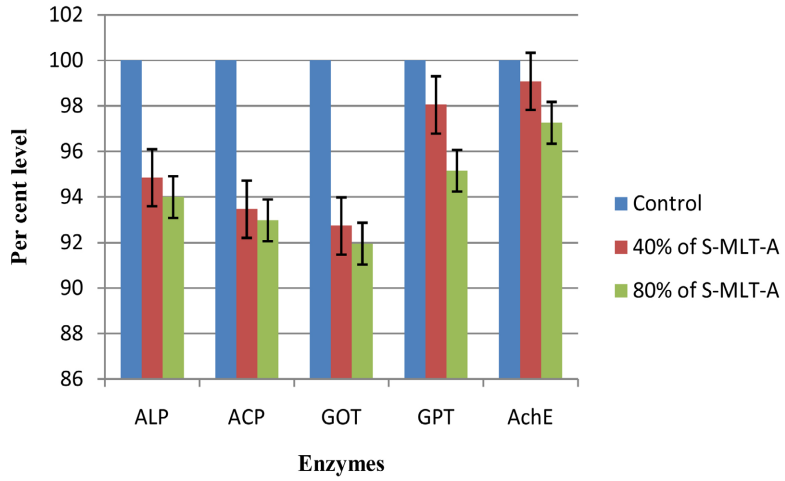

Figure 1. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-MLT-A mixture at 16 h.

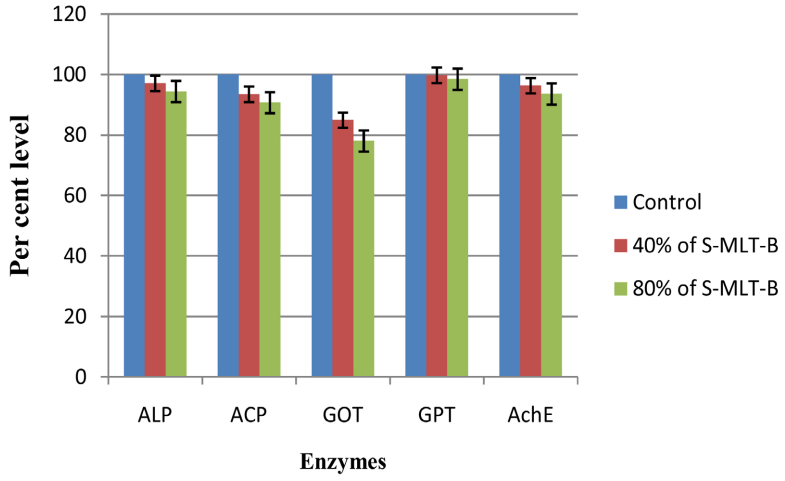

Figure 2. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-MLT-B mixture at 16 h.

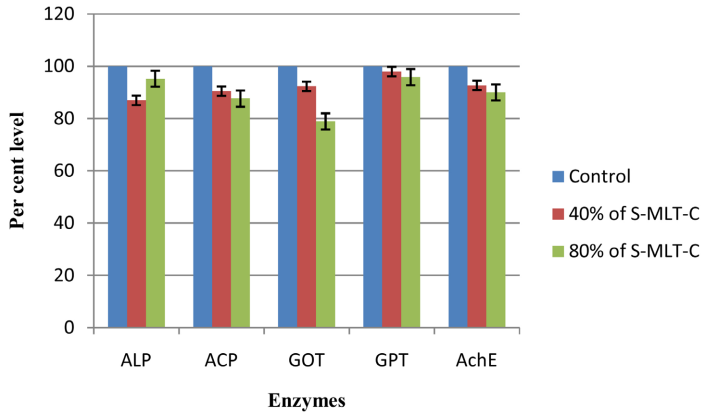

Figure 3. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-MLT-C mixture at 16 h.

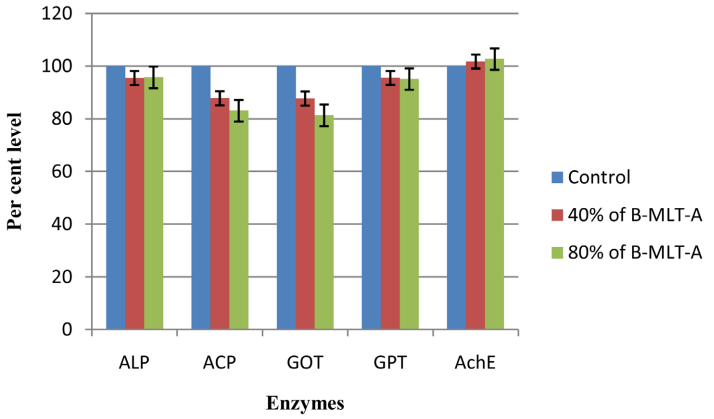

Figure 4. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-MLT-A mixture at 16 h.

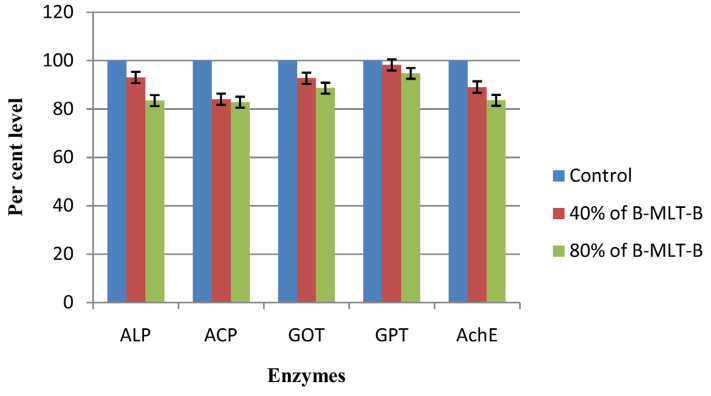

Figure 5. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-MLT-B mixture at 16 h.

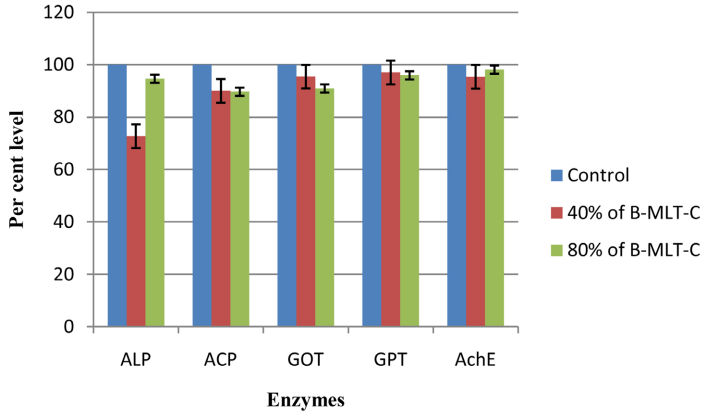

Figure 6. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-MLT-C mixture at 16 h.

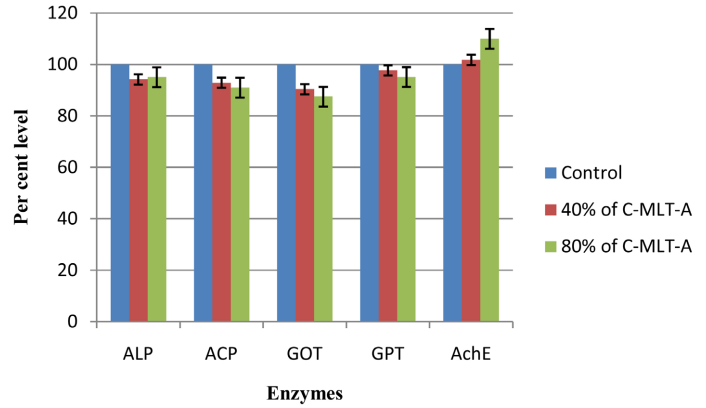

Figure 7. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-MLT-A mixture at 16 h.

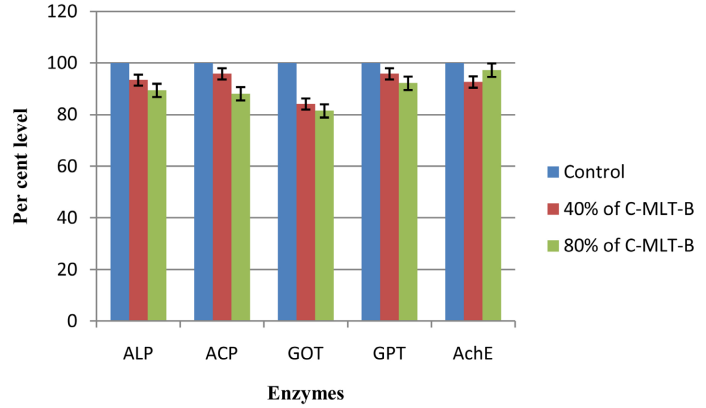

Figure 8. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-MLT-B mixture at 16 h.

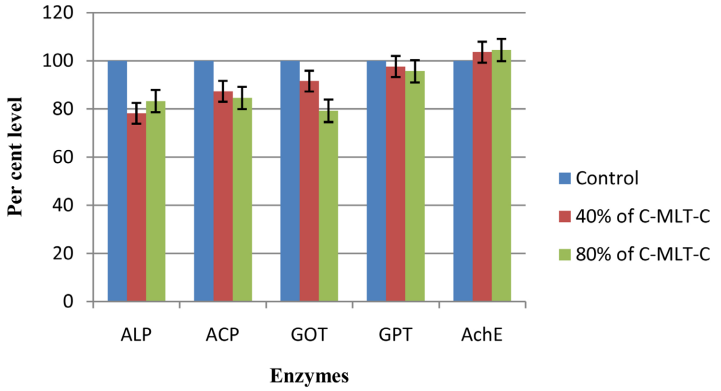

Figure 9. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-MLT-C mixture at 16 h.

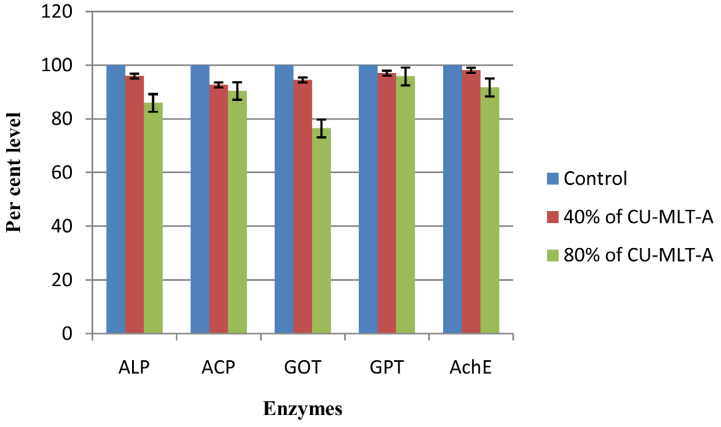

Figure 10. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-MLT-A mixture at 16 h.

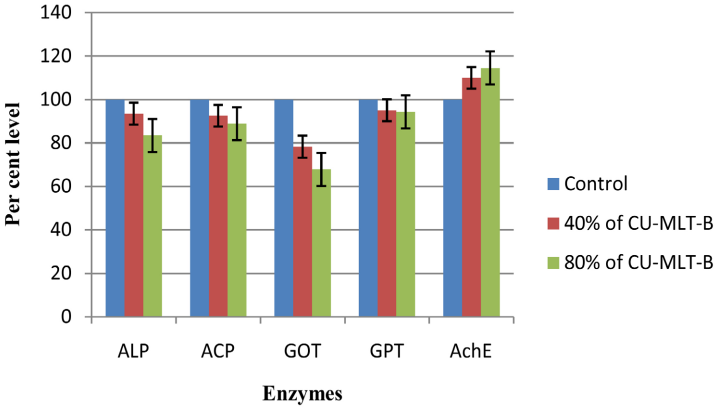

Figure 11. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-MLT-B mixture at 16 h.

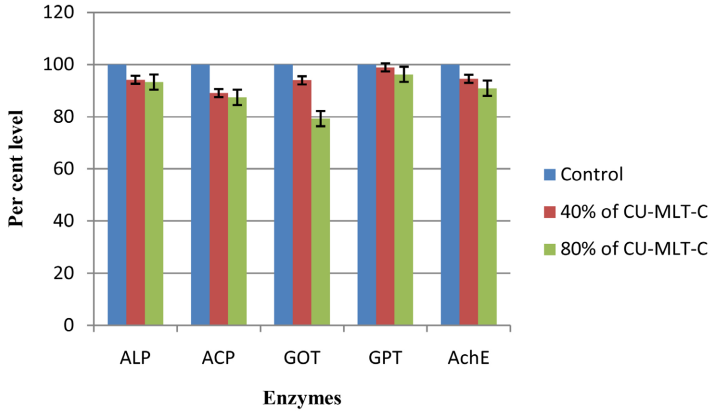

Figure 12. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-MLT-C mixture at 16 h.

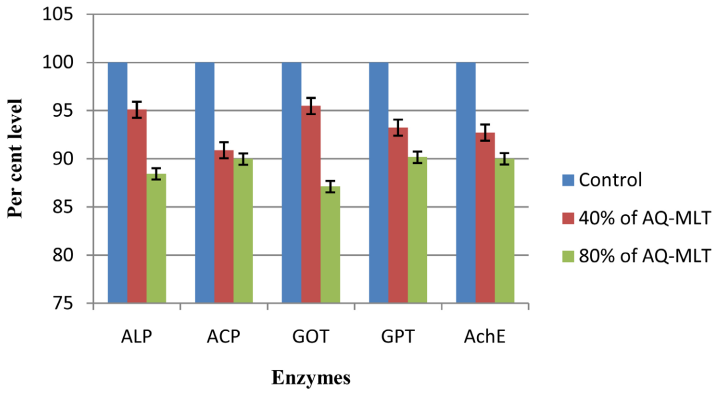

Figure 13. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of AQ-MLT mixture at 16 h.

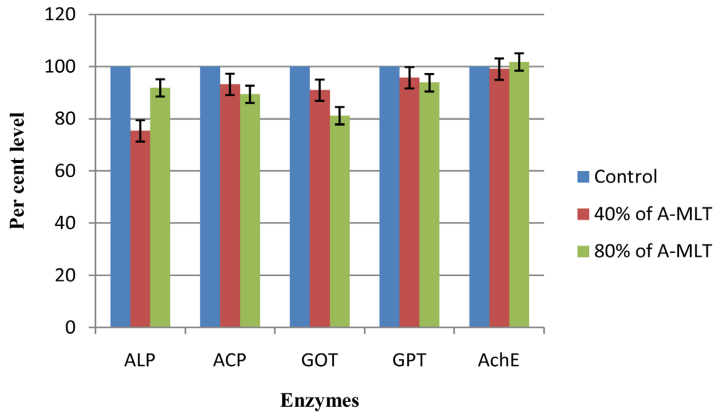

Figure 14. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of A-MLT mixture at 16 h.

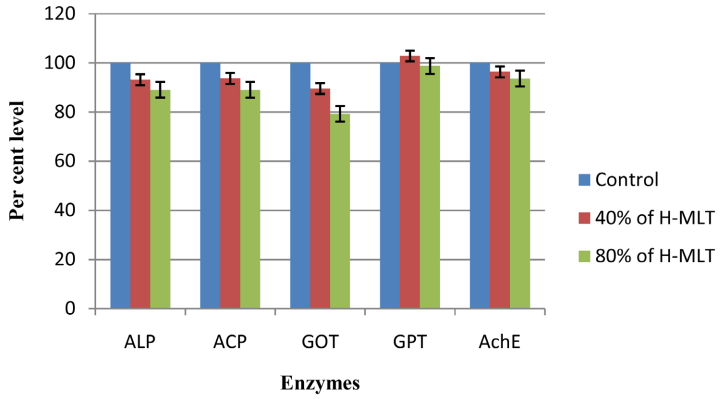

Figure 15. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of H-MLT mixture at 16 h.

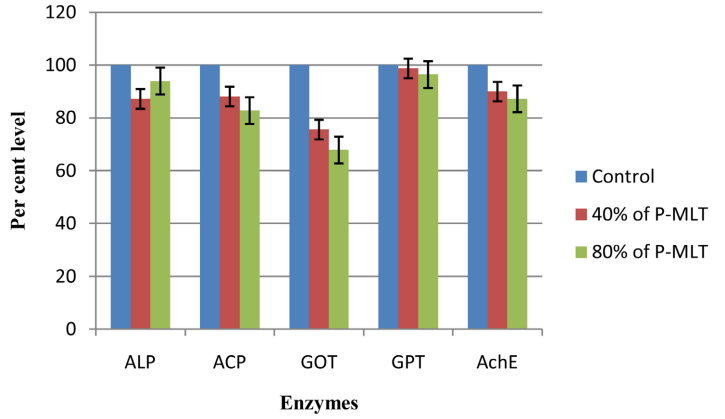

Figure 16. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of P-MLT mixture at 16 h.

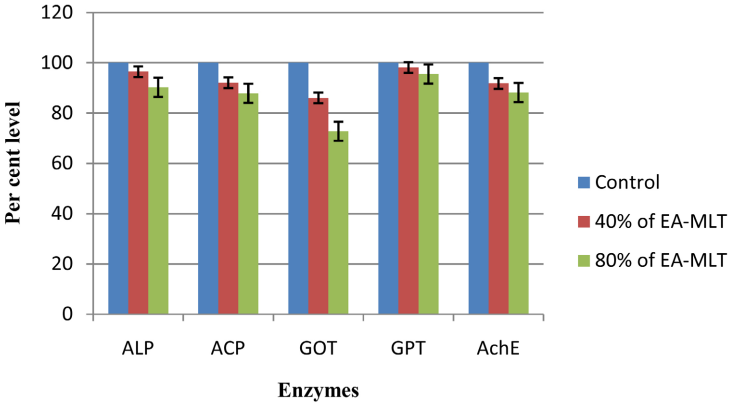

Figure 17. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of EA-MLT mixture at 16 h.

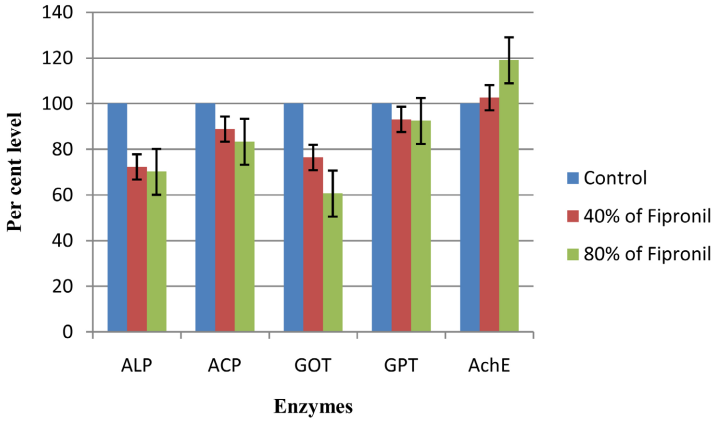

Figure 18. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of fipronil at 16 h.

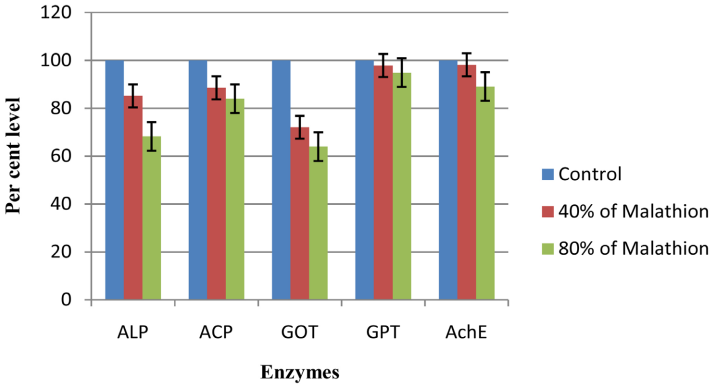

Figure 19. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of malathion at 16 h.

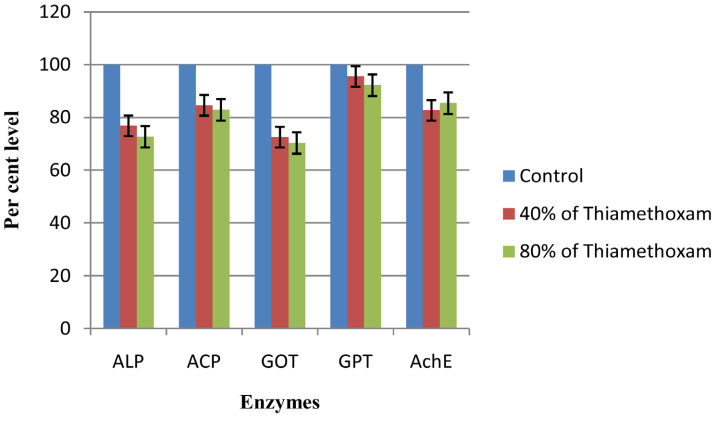

Figure 20. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of thiamethoxam at 16 h.

94.85%, 94.01% and 97.13%, 94.43%, 87.01% and 95.27% respectively at 16 h treatment (Figures 1-3). Similarly 40% and 80% of LD_{50} of Combinatorial mixture B-MLT-A, B-MLT-B and B-MLT-C caused a significant decrease in acid phosphatase level at 16 h treatment in comparison to control, the level recorded 87.85%, 83.13% and 84.05% & 82.84%, 90.07% and 89.72% respectively (Figures 4-6).

When termites were treated with 80% of LD_{50} of C-MLT-A, C-MLT-B and C-MLT-C these combinatorial mixtures caused significant ($p > 0.05$) alteration in acetyl cholinesterase level at 16 h of treatment *i.e.* 97.27% to 110.00% in comparison to control respectively (Figures 7-9), but in CU-MLT-A, CU-MLT-B and CU-MLT-C level was found to be significantly ($p > 0.05$) changed in comparison to control *i.e.* 91.81%, 114.54% and 90.90% respectively (Figures 10-12). Similarly, acetylcholinesterase level was found significantly ($p > 0.05$) decreased at 16 h of treatment in 80% of AQ-MLT, H-MLT, P-MLT and EA-MLT which were 90.00%, 93.63%, 87.27% and 88.18% respectively and slightly increased in A-MLT *i.e.* 101.81% in comparison to control (Figures 13-17). Glutamate oxaloacetate transaminase levels in 80% of synthetic pesticides fipronil, malathion and thiamethoxam were 60.71%, 63.98% and 70.39% respectively after 16 h treatment (Figures 18-20). Glutamate pyruvate transaminase enzyme level was slightly decreased in all tested combinatorial mixtures as well as synthetic pesticides except 40% of H-MLT (Figures 1-20).

4. Discussion

The latex of *Ficus benghalensis* is well known for its medicinal and therapeutic properties in the traditional medicinal system. In the present study, worker termites were treated with various combinatorial mixtures prepared from *Ficus benghalensis* and levels of various metabolic enzymes various enzymes such as alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase. For this purpose, termites have been treated with 40% and 80% of LD_{50} of *Ficus benghalensis* latex-based combinatorial mixtures and with some synthetic pesticides and inorganic insecticides like malathion, fipronil and thiamethoxam for comparison (Table 1 & Table 2; Figures 1-20). Effects on enzyme level alteration were measured in whole-body extracts of termites. In bioassays, combinatorial mixtures S-MLT-A, S-MLT-B, S-MLT-C, B-MLT-A, B-MLT-B and B-MLT-C mixtures have shown significant reduction in alkaline phosphatase and acid phosphatase, levels in a range of 72.76% to 95.78% and 82.84% to 93.49% respectively after 16 h treatment (Figures 1-6), while an alteration was measured in acetyl cholinesterase levels after 16 h treatment of 80% of C-MLT-A, C-MLT-B and C-MLT-C *i.e.* 110.00%, 97.27% and 104.54% respectively (Figures 7-9). More specifically, in another experiment, a similar dose of 80% of CU-MLT-A and CU-MLT-C mixture caused a slight decrease in acetyl cholinesterase levels after 16 h treatment in comparison to control termites *i.e.* 91.81% and 90.90% respec

Table 1. *Ficus benghalensis* and other ingredients used in preparation of combinatorial mixtures.

S. No.	Combinatorial Mixtures	Ingredients
1.	S-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Sulphur (3 gm) + Water (5 liter)
2.	S-MLT-B	*Ficus benghalensis* latexes (12 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Sulphur (3 gm) + Water (5 liter)
3.	S-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (50 ml) + Terpene oil (50 ml) + Glycerol (50 ml) + Sulphur (3 gm) + Water (5 liter)
4.	B-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
5.	B-MLT-B	*Ficus benghalensis* latexes (12 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
6.	B-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Borate (3 gm) + Water (5 liter)
7.	C-MLT-A	*Ficus benghalensis* latexes (9 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 gm) + Water (5 liter)
8.	C-MLT-B	*Ficus benghalensis* latexes peels (12 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 gm) + Water (5 liter)
9.	C-MLT-C	*Ficus benghalensis* latexes (18 gm) + Coconut oil (17 ml) + Terpene oil (17 ml) + Glycerol (17 ml) + Copper (3 gm) + Water (5 liter)
10.	CU-MLT-A	*Ficus benghalensis* latexes (9 gm) + Photoactivated Cow urine (10 g/L) + Water (5 liter)
11.	CU-MLT-B	*Ficus benghalensis* latexes (12 gm) + Photoactivated Cow urine (10 g/L) + Water (5 liter)
12.	CU-MLT-C	*Ficus benghalensis* latexes (18 gm) + Photoactivated Cow urine (10 g/L) + Water (5 liter)
13.	AQ-MLT	*Ficus benghalensis* latexes (40 gm) + Water (200 ml)
14.	A-MLT	*Ficus benghalensis* latexes (40 gm) + Acetone (200 ml)
15.	H-MLT	*Ficus benghalensis* latexes (40 gm) + Hexane (200 ml)
16.	P-MLT	*Ficus benghalensis* latexes (40 gm) + Petroleum Ether (200 ml)
17.	EA-MLT	*Ficus benghalensis* latexes (40 gm) + Ethyl Alcohol (200 ml)
18.	Malathion*	Malathion powder (7.5 gm/liter) + Water (5 liter)
19.	Fipronil*	Fipronil powder (7.5 gm/liter) + Water (5 liter)
20.	Thiamethoxam*	Thiamethoxam powder (7.5 gm/liter) + Water (5 liter)

*Synthetic pesticides.

tively (Figures 10-12).

In vivo exposure of 40% and 80% of LD_{50} of AQ-MLT and A-MLT mixtures caused a very significant ($p > 0.05$) reduction in all the test enzymes *i.e.* alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase expect acetyl cholinesterase level in 80% of A-MLT after 16 h all tested treatments in comparison to control (Figure 13 & Figure 14). Contrary to this, similar dose of H-MLT, P-MLT and EA-MLT caused

Table 2. Determination of LD_{50} value in various combinatorial mixtures.

S.N.	Name of combinatorial Mixture	LD_{50} µg/gm	LD_{40} µg/gm	LD_{20} µg/gm	0.95 confidence limit UCL-LCL	Chi-Square	Slope function	Degree of freedom	Heterogeneity
1.	S-MLT-A	368.529	147.41	73.70	550.006 - 276.516	4.0815	−0.118078	4	1.0204
2.	S-MLT-B	521.701	208.68	104.34	1000.726 - 342.461	10.476	−0.134144	4	2.6191
3.	S-MLT-C	716.570	286.62	143.31	914.105 - 587.428	3.996	−0.132971	4	0.999
4.	B-MLT-A	323.034	129.21	64.60	757.042 - 191.141	10.497	−0.108045	4	2.6244
5.	B-MLT-B	421.634	168.65	84.32	582.976 - 312.699	5.6838	−0.131763	4	1.4210
6	B-MLT-C	670.349	268.13	134.06	992.442 - 495.051	5.5602	−0.139478	4	1.3901
7	C-MLT-A	295.197	118.07	59.03	494.773 - 195.430	7.2238	−0.109047	4	1.8060
8	C-MLT-B	548.854	219.54	109.77	1049.929 - 368.438	7.9930	−0.126025	4	1.9983
9	C-MLT-C	717.439	286.97	143.48	927.577 - 538.238	3.231	−0.129877	4	0.808
10	CU-MLT-A	323.776	129.51	64.75	479.273 - 238.499	4.1271	−0.110143	4	1.0318
11	CU-MLT-B	555.295	222.11	111.05	1402.248 - 343.231	10.558	−0.124327	4	2.6396
12	CU-MLT-C	717.609	287.04	143.52	1353.574 - 481.076	8.4164	−0.133737	4	2.1041
13	AQ-MLT	27.719	11.08	5.54	48.771 - 19.287	6.7998	−0.643967	4	1.7000
14	A-MLT	19.078	7.63	3.81	24.405 - 15.488	1.693	−0.542454	4	0.423
15	H-MLT	11.887	4.75	2.37	37.163 - 6.857	11.181	−0.417258	4	2.7953
16	P-MLT	25.634	10.25	5.12	39.564 - 19.464	1.793	−0.535656	4	0.448
17.	EA-MLT	13.902	5.56	2.78	19.602 - 10.885	3.592	−0.430078	4	0.898
18.	Malathion*	67.026	26.81	13.40	95.511 - 52.909	2.083	−0.875498	4	0.521
19.	Fipronil*	27.891	11.15	5.57	58.871 - 18.100	11.839	−0.715511	4	2.9597
20.	Thiamethoxam*	50.255	20.10	10.05	63.329 - 41.833	2.844	−0.872107	4	0.711

*Synthetic pesticides.

significant reduction in all the test enzymes *i.e.* alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase (except 40% of H-MLT) including acetyl cholinesterase levels in comparison to control at 16 h treatment (**Figures 15-17**).

Aqueous extracts of *Gloriosa superba* [46], *Paronia emodi* [47], *Corydalis incise* [48], *Artemisia annua* [49], *Teucrium royleanum* [50], *Andrache cardifolia* [51], *Angelica archangelica* and *Geranium sylvatica* efficiently altered various enzymes level such as acetyl cholinesterase, alkaline phosphatase and amino transferase in insects [52]. The 40% of LD_{50} of synthetic pesticides fipronil, malathion and thiamethoxam caused a significant decrease in glutamate oxaloacetate transaminase levels *i.e.* 76.53%, 72.09% and 72.61% respectively at 16 h treatment, while 80% of LD_{50} of malathion and thiamethoxam caused decrease in all the enzymatic parameters at 16 h treatment in comparison to control (**Figures 18-20**). Similarly, certain alkaloids found in amaryllidaceae family

plants inhibit acetyl cholinesterase levels in various insect pests [53]. A large number of alkaloids are found abundantly in plants [54]. Phenolic compounds like phosphorus oxychloride showed acetylcholinesterase inhibition at a sub-lethal dose in termite *C. formosanus* [55]. Contrary to this malathion also potentially inhibits acetylcholinesterase activity more than malaxon and isomalathion [56]. Similarly, an increased acetylcholine esterase activity was noted in mice after administration of an aqueous extract of the seed kernel of *Thevetia peruviana* [57]. The increased levels of AChE show CNS poisoning followed by severe inflammation.

C. procera latex and leaf extract as well as abamectin markedly elevated the activities of serum CK-MB, AST and LDH at the two tested periods in a dose-dependent manner. Lipid peroxidation was significantly increased while GSH level and GPx, GST and SOD activities were significantly depleted in the heart and testis of all treated rats [58]. Contrary to this treatment with dried latex of *Calotropis procera* did not alter the serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, urea and urinary levels of glucose and protein as compared to the normal rats. It exhibited a modulatory role in maintaining the levels of blood glucose and serum insulin [59].

Commonly used organophosphorus insecticide shows genotoxicity both *in vivo* and *in vitro* treatments [60]. It is a well-known fact that in the presence of any toxicant or pesticide insects show stress [61] [62]. To fight against this stress, insects show significant induction in hydrolytic activities in the body tissues which elevate or reduced the acid and alkaline phosphatase levels. Acid and alkaline phosphatase is found in blood, liver, plasma, and intestine in human beings [63] [64]. Similarly in the presence of toxicant transamination of amino acids get an increase, hence the level of glutamate pyruvate transaminase, glutamate oxalo acetate transaminase enzymes get altered [65]. By using biochemical analyses, many ALPs have been found in *Culex tarsalis* [66]. In the temporal lobe and hippocampus, AChE activity is 67% lower than normal levels as AD progresses [67]. Transaminases are also affected by insecticides in different ways in fishes [68]. These display tissue damage in aquatic animals. Although ALP has functional relevance in vertebrate epithelia such as the gut, kidney, placenta, and mammary gland, cell-specific *in vivo* roles for ALPs are yet unclear [69]. Furthermore, the midgut of *Bombyx mori* larval expresses at least two ALP isoforms that are encoded by different genes [70].

Ficus benghalensis latex contains a highly stable serine protease Benghalensin. It possesses a molecular mass of 47 kDa. This is a glycoprotein having a molecular mass of 47 kDa, it shows enzymatic activity that is inhibited by PMSF and chymostatin [71]. Latexes from *Euphorbia bupleuroides* [72], *Calotropis procera* and *Jatropha curcas* contain diterpenoids [73]. *Ficus benghalensis* contains terpinoids Bengalensursenyl diglycoside and Ficusbengursenyl diglycoside [74]. These were found highly effective against *Odontotermes obesus* Rambur [72] [75]. The latex of four plants viz. *Euphorbia royleana*, *Jatropha gossypifolia* (Euphor-

biaceae), *Nerium indicum* and *Thevetia peruviana* (Apocynaceae) caused a significant reduction in acid/alkaline phosphatase activity and anti-acetylcholinesterase activity in nervous tissue of freshwater air-breathing fish *Channa marulius*. The reduction in the activity of both phosphatases and AChE was time as well as dose-dependent [76]. Synthetic pesticides change the biological parameters of the surrounding environment and have negative short- and long-term consequences on human and animal health. These persist in the residual form in the medium, enter into the food chain and show biomagnifications. Synthetic pesticides can cause burning eyes, blisters, rashes, blindness, nausea, diarrhea, and eventually death of target and non-target organisms [77].

In this investigation alteration in the level of certain enzymes in whole body extract of termites may be due to physiological alterations which are induced by *Ficus benghalensis* and its combinatorial mixtures. However, *Ficus benghalensis* constituents significantly altered the phosphatases, transaminase and esterase levels, which indicate very high toxic effects on the body tissues of termites. More often, elevation or reduction in enzyme level is associated with metabolic disturbances in insects. These obstructions in their chemical pathways led to the formation of abnormalities in the insect metabolism and make insects unable to survive. These have shown more significant termiticidal efficacy than synthetic pesticides.

5. Conclusion

In the present investigation alteration in the level of certain enzymes in whole body extract of termites may be due presence of different bio-organic constituents in *Ficus benghalensis* latex. This altered level of various enzymes indicates toxic effects on the body tissues of termites and obstruction in the physiology of termite workers. More specifically, it may lead intoxication of termites and make them unable to survive. No doubt these *Ficus benghalensis* latex-based combinatorial mixtures could be used for termite control in a sustainable and eco-friendly manner and become a safe alternative to synthetic pesticides.

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Authors' Contributions

Ravi Kant Upadhyay and Abhishek Kumar Tripathi were responsible for conception, experiments, writing and revising the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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