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Effect of *Ficus benghalensis* Latex Based Combinatorial Formulations on Various Bio-Molecules in Indian White Termite *Odontotermes obesus*

Abhishek Kumar Tripathi, Ravi Kant Upadhyay

Department of Zoology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, India
Email: rkupadhya@yahoo.com

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Abstract

In present investigation, various bioassays were conducted to evaluate the alteration in levels of certain biomolecules, such as glycogen, protein, amino acid, DNA, RNA and lipids. For this purpose, worker termites were treated with sub-lethal dose 40% and 80% of 24 hrs LD50 values and observations were taken at 4 hrs interval up to 24 hrs. Crude latex and its combinatorial mixtures, like S-MLT-A, B-MLT-A, C-MLT-A, CU-MLT-A, AQ-MLT significantly altered level of bio-molecules in *Odontotermes obesus*. This effect was found time and dose dependent. Reduction or increase in biomolecules was calculated by using corresponding control. Maximum decrease in glycogen level was observed at 16 h when termites were treated with 80% of LD_{50} of *Ficus benghalensis* aqueous extract *i.e.* 56.88% at 16 h of treatment. A similar dose caused very slight decrease in lipid contents at 4 h of treatment but it was found further significantly ($p > 0.05$) decreased in other successive treatments. 40% and 80% of LD_{50} of C-MLT-B mixture caused significant ($p > 0.05$) decrease in DNA and RNA level at 16 h treatment. The level of DNA and RNA level was recorded 83.90%, 90.18% and 85.42% and 74.05% respectively. Similarly, total proteins were also found to be decreased with 40% and 80% of LD_{50} of C-MLT-B mixture *i.e.* 71.47% and 66.45% respectively. All these alterations found in levels of various bio-molecules confirm the action of latex ingredients on worker termites that was antifeedant or deterrent types. These ingredients can be used to control not only termites but also other phytophagous insects in a sustainable and eco-friendly way.

Keywords

Ficus benghalensis, *Odontotermes obesus*, Latexes, Antifeedant or Deterrent

Termiticides

1. Introduction

Termites are highly destructive polyphagous pests that cause severe damage to crops [1]. Termites can seriously infect garden trees, timber, building materials, supplies, paper and clothing, causing enormous economic losses. Termites infest at various stages of plant growth. These attack crop plants such as sugarcane, maize, wheat, barley, rice and vegetable crops [2] [3]. These cause approximately 50% - 100% yield losses in these crops [4] [5]. They also act as decomposers, improving soil fertility, yields, and are also used by people around the world to their benefit. It poses a threat to farming communities as it indirectly causes large losses [6]. Termites are major soil macrofauna and control of termites population is very essential to avoid crop damage [7].

Termites build huge mounds to protect from colonization and environmental stress, and to hide from predators [8]. They are mainly involved in many ecological processes, such as breaking down plant debris and dead wood and converting them into soil [9]. The impact of termites on the global economy is estimated at about $40 billion annually, with subterranean termites accounting for about 80% of the total impact [10]. Forest termite populations are under attack by predatory ants [11].

However, for controlling termite attack, harmful synthetic chemical pesticides are extensively applied [12]. Various synthetic pesticides such as cyclodiene [13] [14], cypermethrin [15], hydroquinone and indoxcarb [16] have been used to control termites in crops. Dursban spray has been shown to be highly effective in controlling wood-destroying termites [17]. Thiamethoxam shows high mortality in Asian subterranean termite *Coptotermes gestroi* workers after 1 - 3 days exposure time [18]. These chemicals have severe adverse effects on untargeted biotic and abiotic environmental factors [19]. Though chemical insecticides are highly effective against termite, they are hazardous to non-target organisms in the ecosystem [20]. Its bound residues persist for longer duration in the environment, and through various trophic levels they entered into the food chain.

For control of termites, many toxic synthetic chemical insecticides, bio-insecticides and botanicals were used but work on plant latex is very scare [21] [22]. Plant latexes and some botanicals from Plant species *Calotropis procera*, *Ipomoea fistulosa*, *Maesa lanceolata*, *Croton macrostachyus*, *Targets minuta*, *Datura stramonium* and *Azadirachta indica* are used for the management of termites [23] [24] [25]. Latex is produced and stored in specialized cells named "laticifers". Laticifers form a tubing system composed of rows of elongated cells that branch and create an internal network encompassing the entire plant [26]. Plants exude latex sap in response to physical damage [27]. Fig latex is more effective natural therapeutic antibacterial agents against the pathogenic bacteria [28].

Antiviral properties of *C. majus* latex arise both from alkaloids and proteins contained therein, acting on different stages of the viral replication cycle [29]. There are so many plant-based botanicals that could act against termites in crop fields and vegetations [30].

2. Materials and Methods

2.1. Combinatorial Formulations

Ficus benghalensis and other ingredients were used in preparation of combinatorial mixtures. The details of combinatorial mixtures are given in Table 1 and Table 2.

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.

Table 2. Different latex based combinatorial mixture data analysis of LD50 values.

S.N.	Name of Latex/ Combinatorial Mixture	LD50 µg/gm	0.95 confidence limit UCL-LCL	Chi-Square	Slope function	Degree of freedom	LD40 µg/gm	LD 20 µg/gm	Heterogeneity
1.	S-MLT-A	368.529	550.006 - 276.516	4.0815	−0.118078	4	147.41	73.70	1.0204
2.	S-MLT-B	521.701	1000.726 - 342.461	10.476	−0.134144	4	208.68	104.34	2.6191
3.	S-MLT-C	716.570	914.105 - 587.428	3.996	−0.132971	4	286.62	143.31	0.999
4.	B-MLT-A	323.034	757.042 - 191.141	10.497	−0.108045	4	129.21	64.60	2.6244
5.	B-MLT-B	421.634	582.976 - 312.699	5.6838	−0.131763	4	168.65	84.32	1.4210
6	B-MLT-C	670.349	992.442 - 495.051	5.5602	−0.139478	4	268.13	134.06	1.3901
7	C-MLT-A	295.197	494.773 - 195.430	7.2238	−0.109047	4	118.07	59.03	1.8060
8	C-MLT-B	548.854	1049.929 - 368.438	7.9930	−0.126025	4	219.54	109.77	1.9983
9	C-MLT-C	717.439	927.577 - 538.238	3.231	−0.129877	4	286.97	143.48	0.808
10	CU-MLT-A	323.776	479.273 - 238.499	4.1271	−0.110143	4	129.51	64.75	1.0318
11	CU-MLT-B	555.295	1402.248 - 343.231	10.558	−0.124327	4	222.11	111.05	2.6396
12	CU-MLT-C	717.609	1353.574 - 481.076	8.4164	−0.133737	4	287.04	143.52	2.1041
13	AQ-MLT	27.719	48.771 - 19.287	6.7998	−0.643967	4	11.08	5.54	1.7000
14	A-MLT	19.078	24.405 - 15.488	1.693	−0.542454	4	7.63	3.81	0.423
15	H-MLT	11.887	37.163 - 6.857	11.181	−0.417258	4	4.75	2.37	2.7953
16	P-MLT	25.634	39.564 - 19.464	1.793	−0.535656	4	10.25	5.12	0.448
17.	EA-MLT	13.902	19.602 - 10.885	3.592	−0.430078	4	5.56	2.78	0.898
18.	Malathion*	67.026	95.511 - 52.909	2.083	−0.875498	4	26.81	13.40	0.521
19.	Fipronil*	27.891	58.871 - 18.100	11.839	−0.715511	4	11.15	5.57	2.9597
20.	Thiamethoxam*	50.255	63.329 - 41.833	2.844	−0.872107	4	20.10	10.05	0.711

*Synthetic pesticides.

2.2. Determination of Bio-Molecular Parameters

Termite workers were treated with 40% and 80% of LD_{50} of *Ficus benghalensis* latex based combinatorial mixtures separately. Whole body extracts was prepared by homogenization of termites in PBS buffer and centrifuged. Changes in the levels of different biomolecules were measured after different time intervals mainly at 4, 8, 12 and 16 hours. Several important biomolecules such as glycogen, total free amino acids, total lipids, nucleic acids (DNA and RNA) and total protein were determined in whole body extract of termites.

2.3. Determination of Glycogen

Glycogen content was determined by the Dubois's method [31]. For this purpose, 500 mg of termites were homogenized in 2 ml of 5% TCA using a glass homogenizer and centrifuged. After centrifugation, the supernatant was transferred to a 100 ml graduated cylinder and 3 volumes of 95% ethyl alcohol was added. The mixture was stirred until the precipitate started to flocculate, then a

small amount of NaCl was added to enhance flocculation. The graduated cylinder was then heated by placing it in hot boiling water until a precipitate formed. After centrifuging the contents again, the precipitate was dissolved in 5 ml of distilled water. To 0.5 ml of the unknown deproteinized glycogen supernatant was then added 6 ml of concentrated H_2SO_4 and the contents were kept in boiling water for 5 minutes, then the mixture was cooled to room temperature. The optical density of the content was observed at 530 nm. Glycogen levels in unknowns (supernatants) were calculated using a standard curve plotted with known amounts of glucose. A blank was prepared using 0.50 ml of 5% TCA and 6 ml of concentrated H_2SO_4. The amount of glycogen is expressed in gm/100gm of termite body weight. Three replicates were set up to maintain accuracy and precision. The data obtained were statistically analyzed using the ANOVA method.

2.4. Determination of Total Free Amino Acid

Free amino acid content was determined by using Spies's method [32]. For this purpose, 500 mg of treated termites were homogenized in 2 ml of 95% ethyl alcohol. The homogenate was centrifuged at 15,000× g for 20 minutes and the supernatant separated. For estimation, 0.1 ml of supernatant was taken and mixed with 0.1 ml of distilled water and 2.0 ml of ninhydrin reagent. Ninhydrin reagent was prepared by mixing 1.0 g of ninhydrin in 25 ml of absolute ethanol and 0.04 g of stannous chloride in 25 ml of citrate buffer (pH 5.0). The citrate buffer was prepared by mixing 20 ml of reagent A (1.05 g citric acid in 50 ml distilled water) and 29.5 ml reagent B (2.94 g sodium citrate in 100 ml distilled water). The reaction mixture was kept in boiling water for 15 minutes. Now 2 ml of 5.0% ethyl alcohol was added to the above boiled mixture. A purple color developed in the reaction mixture measured at 575 nm. For calculations, a standard curve was constructed using known amounts of glycine and expressed in gm/100gm termite body weight. Three replicates are used and the data are analyzed statistically by the ANOVA method.

2.5. Determination of Total Lipid

Total lipid content in termite whole body extracts was determined by the Folch's method [33]. For this purpose, 500 mg of termite workers were homogenized in 5 ml of a mixture of chloroform and methanol (2:1 v/v). The homogenized material was suspended and held at room temperature for 30 minutes. After that, it was filtered through Whatmann paper no. 1. The residue was resuspended in the same volume of mixture for 1 hour and the supernatant filtered again. Both filtrates were mixed with an equal volume of 0.6% NaCl (w/v). The separatory funnel containing the above mixture was kept in the dark at room temperature for 12 hours. The upper solvent layer (chloroform:methanol) was collected and the unsaponified portion was kept unused. The contents of the lower layer were allowed to evaporate by placing in an oven at 60˚C. Finally, total lipid levels were weighed and expressed as gm/100gm of termite body weight. Three replicates

were set and data were statistically analyzed by his ANOVA method.

2.6. Determination of Nucleic Acids

The content of nucleic acids in termite whole body extracts was determined by the Schneider's method [34]. For this purpose, 500 mg of termite workers he separately fed with 40% and 80% LD50 of aqueous extracts and their combinatorial mixtures. Termites were sacrificed and homogenized in 5% TCA with glass homogenizer and centrifuged at 15,000× g for 25 minutes.

2.7. DNA Estimation

For DNA determination, 0.2 ml of supernatant was taken and diluted by adding 3.8 ml of distilled water. Then 4.0 ml of diphenylamine reagent (1 g diphenylamine, 100 g glacial acetic acid, 2.5 ml concentrated H_2SO_4) was added. The contents were kept in the boiling water bath for 10 minutes. Blue color developed in solution measured at 600 nm.

2.8. RNA Estimation

For RNA measurement, 0.2 ml of supernatant was taken and diluted by adding 4.8 ml of distilled water. Then 2 ml of orcinol reagent (1 g orcinol, 100 ml concentrated HCl and 0.5 g ferric acid) was added. A green color developed when the solution was kept in a boiling water bath for 10 minutes, measured at 600 nm. In both cases, three replicates were set up and the resulting data were statically analyzed by her ANOVA method.

2.9. Determination of Total Protein

Termite total protein was estimated by using the Lowry's method [35]. For this purpose, 500 mg of termite workers were treated separately with mixtures of aqueous extracts of *Ficus benghalensis* latex based combinatorial mixtures. These treated termites were homogenized in 4.0 ml of 10% TCA using a glass homogenizer. The resulting homogenate was centrifuged at 15,000× g for 15 minutes. The supernatant was discarded, the precipitate was dissolved in 5% TCA, centrifuged again at the same speed for 10 minutes, and the supernatant was discarded. The precipitate was then dissolved in 1 ml of 1*N* NaOH and centrifuged again at the same speed. The supernatant was used for protein determination. For this purpose, 0.5 ml of alkaline copper solution (reagent C) was added to 0.5 ml of supernatant. Reagent C was prepared by addition of 50.0 ml reagent A (2% sodium carbonate in 0.1N NaOH) and 1 ml of reagent B (1.0% sodium potassium tartarate, 0.5% copper sulphate mixed in 1:1 ratio at the time of experiment). The reaction mixture was kept for 10 minutes at room temperature and then 0.5 ml Folin and Ciocalteu's phenol reagent (diluted 1:2 ratio with distilled water at the time of experiment) was added. Then 1.5 ml of 0.2N NaOH and 3.5 ml of distilled water were added and mixed thoroughly. After 10 minutes, the reaction developed a blue color measured at 620 nm. Three replicates were set

up for each experiment. A standard curve was generated using various concentrations of bovine serum albumin. The data obtained were statistically analyzed by using the ANOVA method.

2.10. Statistical Analysis

The LD_{50} in termite workers were 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. A chi-square test was used to assess repellency [36].

3. Results

The investigation of biochemical estimation of various biomolecules glycogen, amino acid, lipid, DNA, RNA and protein were done in treated and control termites. For this purpose adult worker termites were provided with 40% and 80% LD_{50} of *Ficus benghalensis* latex and its other combinatorial mixtures with the diet. After 4 h of treatment each biomolecule level was determined up to 16 h. Whole body extract of both treated and control termites were prepared. For this purpose termites were scarified, homogenized and centrifuged and level of various biomolecules was determined. Reduction or increase in biomolecules was calculated by using corresponding control (**Figures 1-20**).

40% of *Ficus benghalensis* aqueous extract caused significant ($p > 0.05$) decrease in glycogen level in treated termites in comparison to control at 16 h of treatment. Maximum decrease in glycogen level was observed at 16 h when termites were treated with 80% of LD_{50} of *Ficus benghalensis* aqueous extract *i.e.* 56.88% at 16 h of treatment. A similar dose caused very slight decrease in lipid contents at 4 h of treatment but it was found further significantly ($p > 0.05$) decreased in other successive treatments. Similarly 80% of LD_{50} of *Ficus benghalensis* aqueous extract caused significant ($p > 0.05$) decrease in DNA level at 16 h of treatment *i.e.* 77.32% 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 LD_{50} of *Ficus benghalensis* aqueous extract *i.e.* 82.38% and 68.47% & 55.06% and 61.28% at 16 h treatment in comparison to control respectively (**Figure 13**).

Treatment of 40% and 80% of LD_{50} of C-MLT-B mixture caused a significant decrease in glycogen levels up to 63.44% and 64.14% at 16 h treatment in comparison to control respectively. In another experiment when termites were treated with 40% of LD_{50} of C-ST3 mixture show decrease in amino acid level at 4 h of treatment *i.e.* 61.93% but later it increased up to 78.58% at 16 h of treatment respectively (**Figure 8**). In the same experiment 40% and 80% of LD_{50} of C-MLT-B mixture caused significant ($p > 0.05$) decrease in DNA and RNA level at 16 h treatment. The level of DNA and RNA level was recorded 83.90%, 90.18% and 85.42% and 74.05% respectively. Similarly total proteins were also found to be decreased with 40% and 80% of LD_{50} of C-MLT-B mixture *i.e.* 71.47% and 66.45% respectively (**Figure 8**).

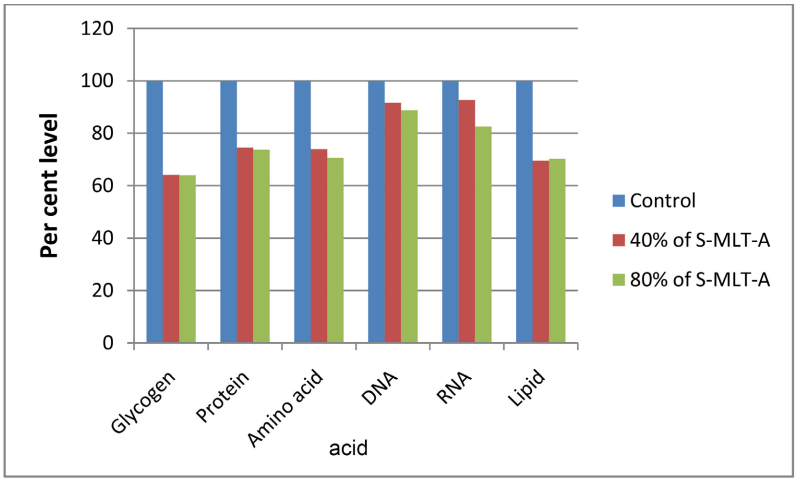

Figure 1. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-MLT-A mixture at 16 hour.

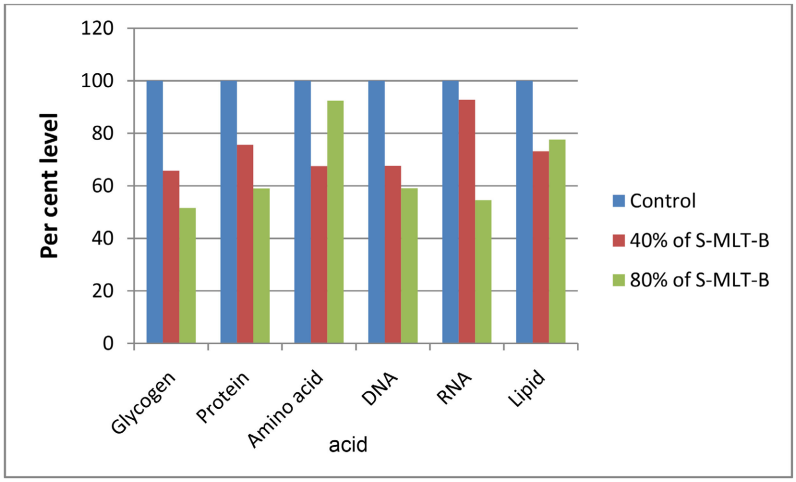

Figure 2. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-MLT-B mixture at 16 hour.

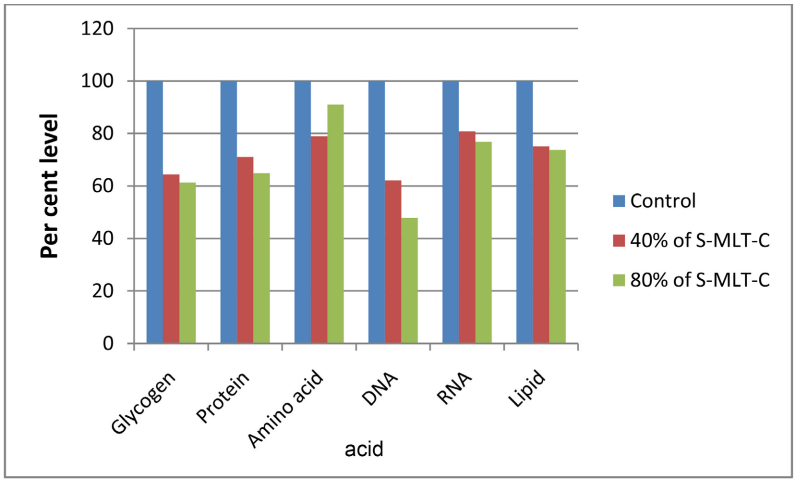

Figure 3. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-MLT-C mixture at 16 hour.

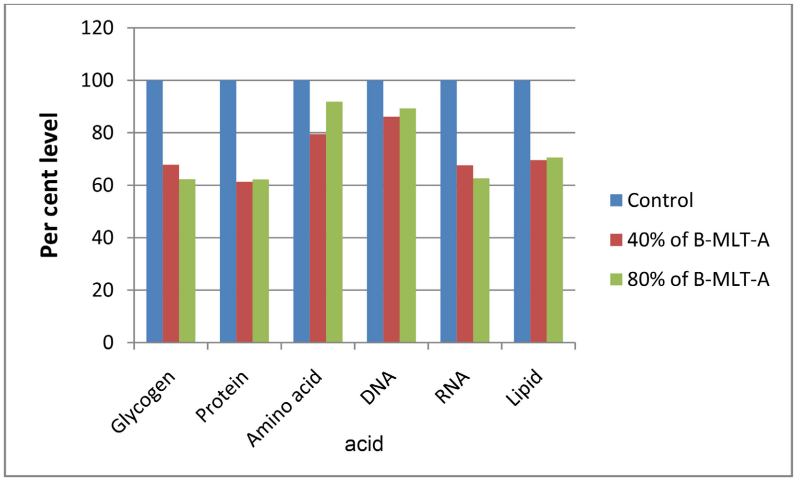

Figure 4. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-MLT-A mixture at 16 hour.

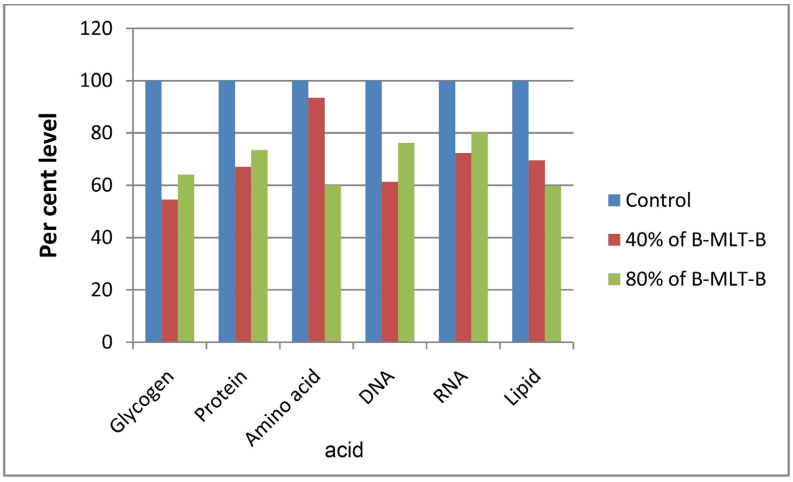

Figure 5. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-MLT-B mixture at 16 hour.

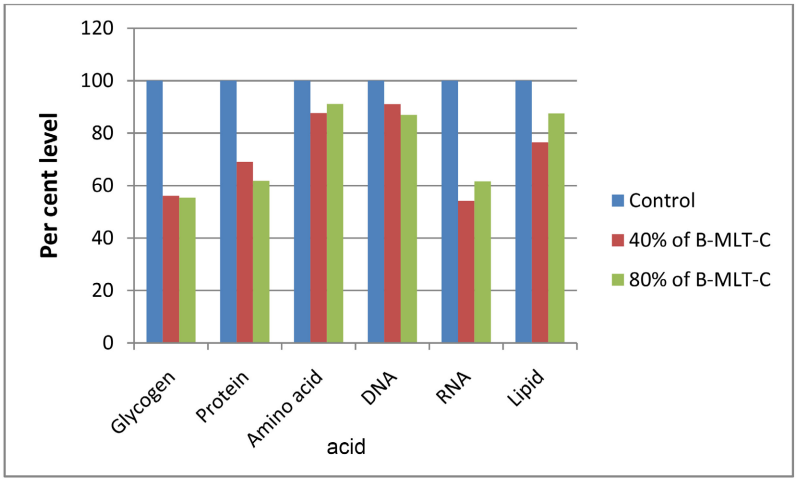

Figure 6. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-MLT-C mixture at 16 hour.

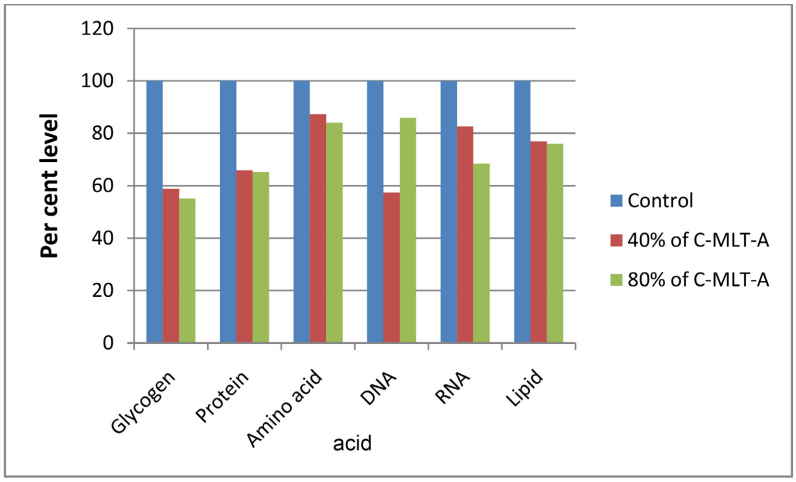

Figure 7. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-MLT-A mixture at 16 hour.

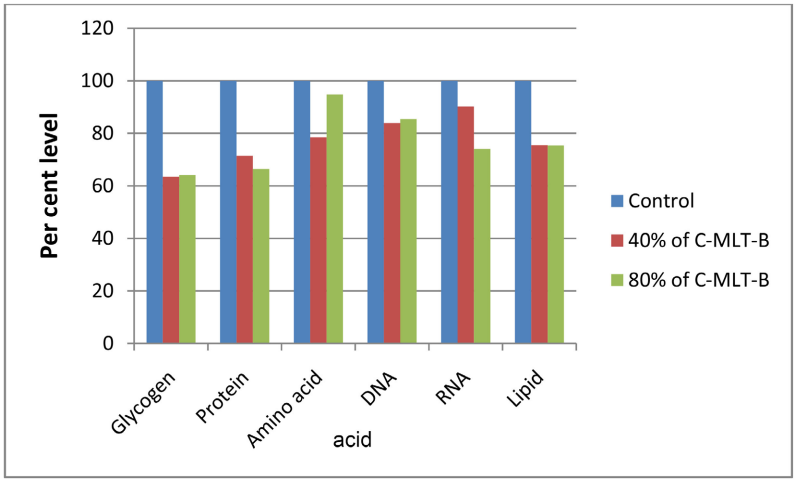

Figure 8. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-MLT-B mixture at 16 hour.

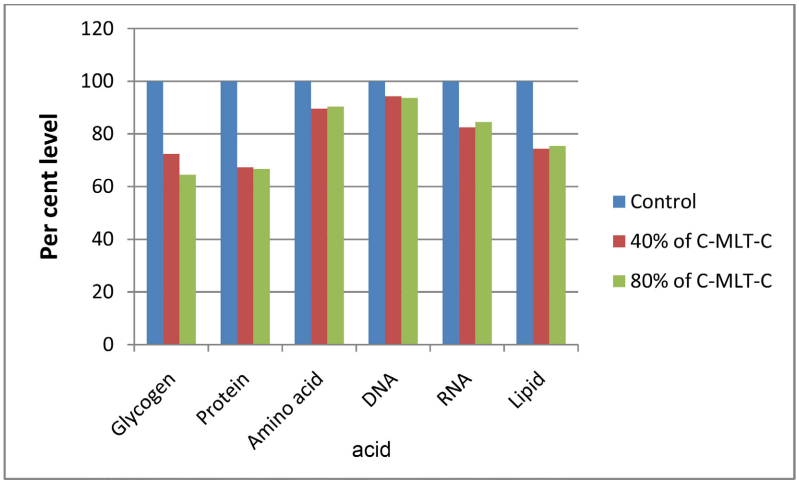

Figure 9. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-MLT-C mixture at 16 hour.

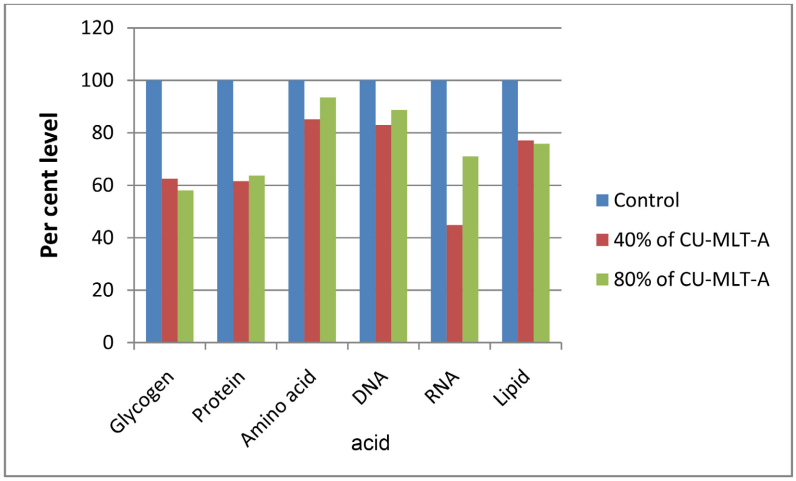

Figure 10. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-MLT-A mixture at 16 hour.

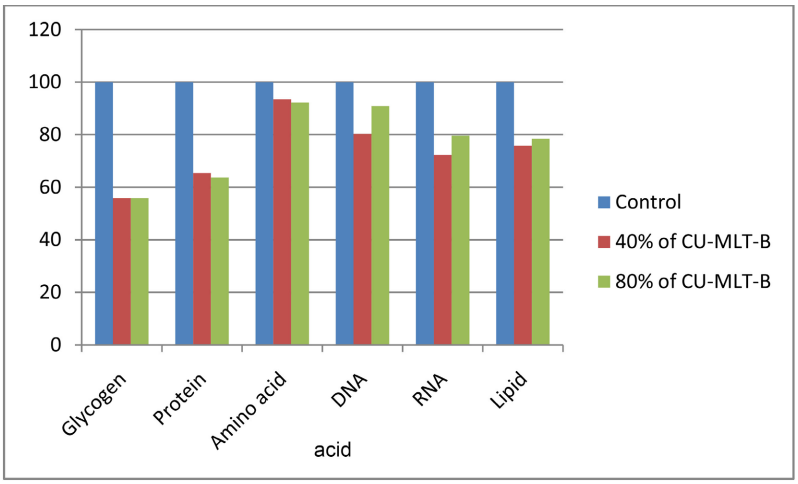

Figure 11. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-MLT-B mixture at 16 hour.

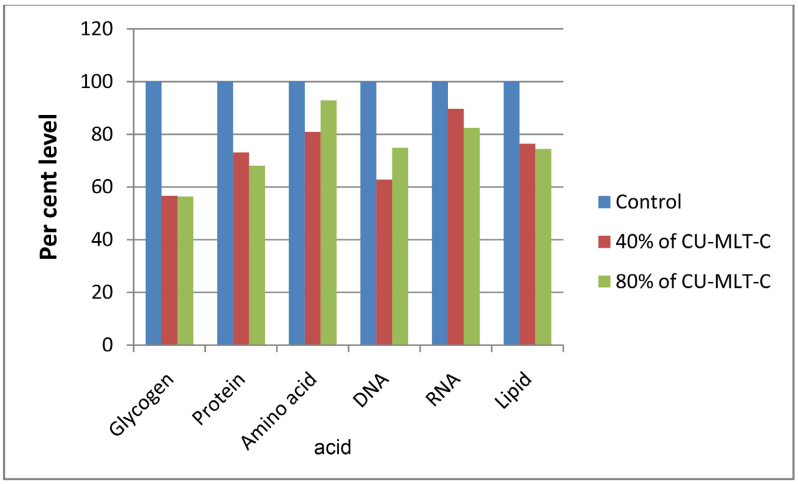

Figure 12. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-MLT-C mixture at 16 hour.

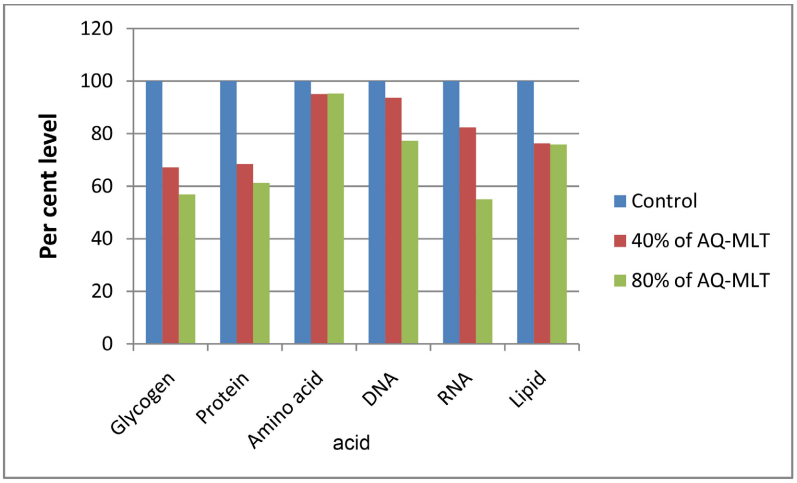

Figure 13. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of AQ-MLT mixture at 16 hour.

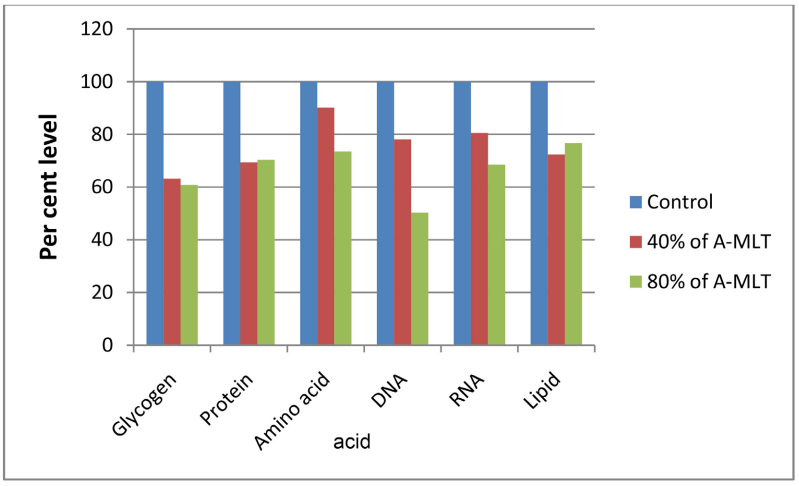

Figure 14. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of A-MLT mixture at 16 hour.

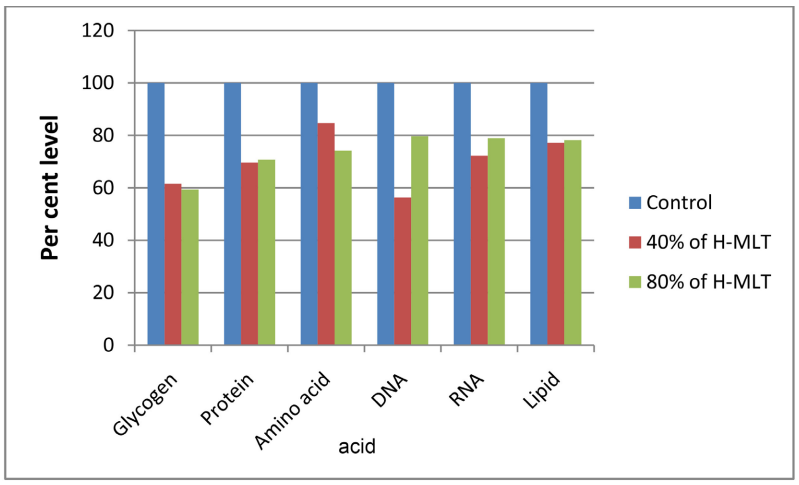

Figure 15. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of A-MLT mixture at 16 hour.

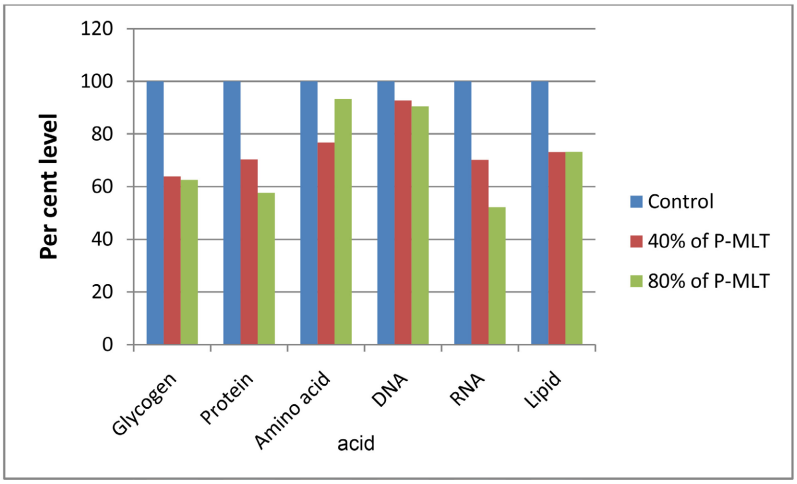

Figure 16. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of P-MLT mixture at 16 hour.

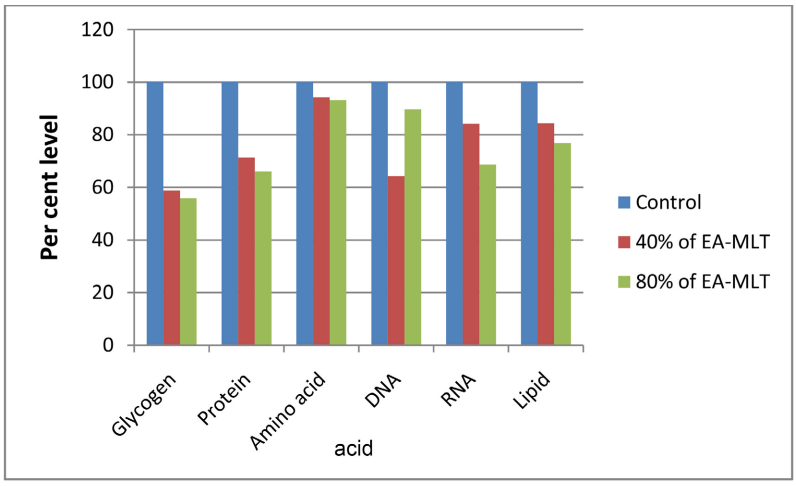

Figure 17. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of EA-MLT mixture at 16 hour.

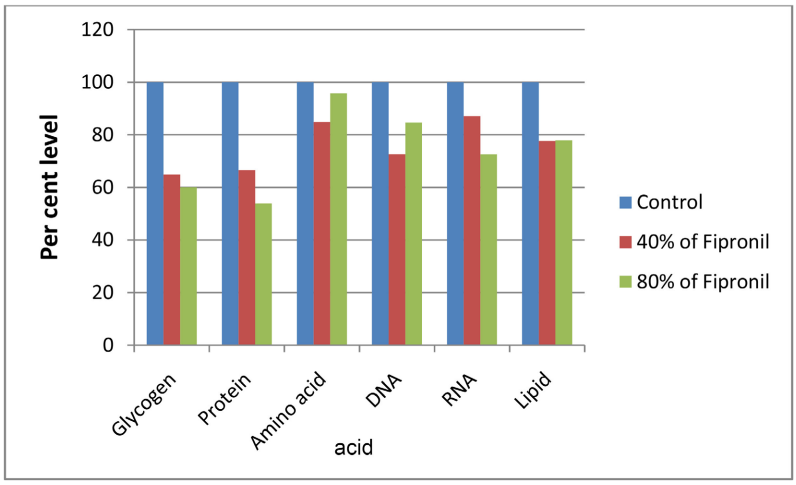

Figure 18. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of fipronil mixture at 16 hour.

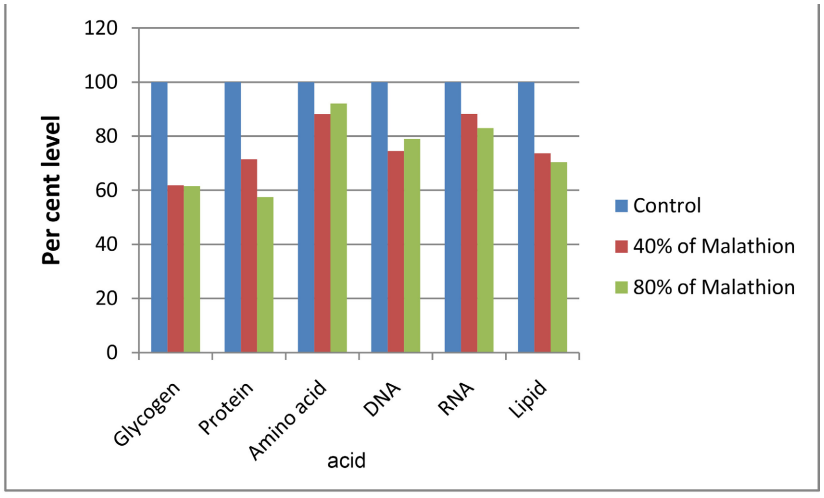

Figure 19. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of malathion mixture at 16 hour.

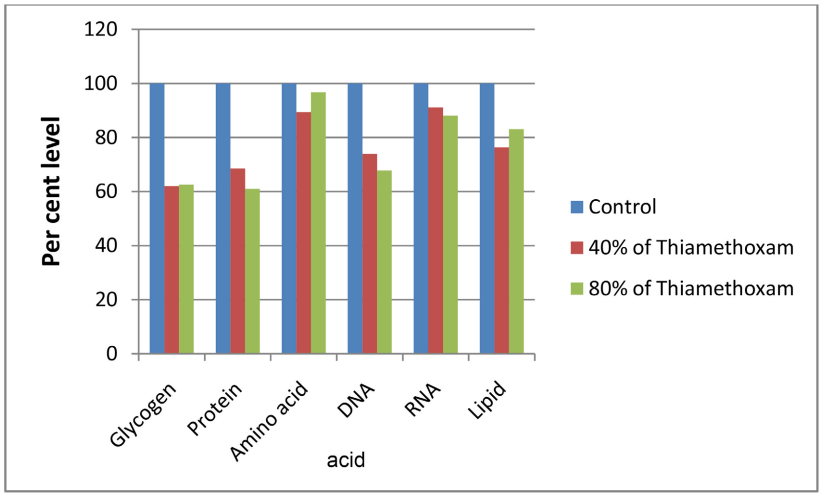

Figure 20. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of thiamethoxam mixture at 16 hour.

In another experiment when termites were treated with 40% and 80% of LD_{50} of B-MLT-C mixture, it has shown continuous decrease in glycogen level *i.e.* 56.05% and 55.44% at 16 h of treatment in comparison to control respectively. While at the same dose amino acid level was found to be decreased at 4 h treatment *i.e.* 86.08% and 73.80% but later on start increasing simultaneously. Similarly the lipid level was also found to be decreased at 40% and 80% of LD_{50} of B-MLT-C mixture up to both at 4 h and 8 h *i.e.* 75.29%, 75.69% and 78.39% and 72.67% respectively in comparison to control. At a similar dose DNA level was also found to be decreased at 16 h treatment *i.e.* 91.06% and 86.95% respectively. Similarly RNA level was also found to be decreased up to 54.21% and 61.60% at 40% and 80% of LD_{50} of B-MLT-C mixture respectively. Maximum inhibition was observed in protein level *i.e.* 69.06% and 61.75% at 40% and 80% of LD_{50} of 16 h of treatment respectively (**Figure 6**).

4. Discussion

For the determination of toxicity of *Ficus benghalensis* latex and its combinatorial mixture its effect was determined in biomolecules. For this purpose termites were treated with 40% and 80% of LD_{50} of *Ficus benghalensis* aqueous extract and its combinatorial mixtures. Effects were observed on glycogen, amino acid, DNA, RNA and protein levels at regular time intervals (**Figures 1-20**). A significant ($p > 0.05$) decrease was observed in all the above biomolecules when termites were treated with S-MLT-A combinatorial mixture. A similar decrease was obtained in glycogen, amino acid, DNA, RNA and protein levels when termites were treated with 40% and 80% of LD_{50} of B-MLT-A mixture, while lipid contents were found to be decreased two fold (**Figure 4**). Similarly 40% and 80% of LD_{50} of S-MLT-C mixture also caused significant ($p < 0.05$) decline in glycogen, amino acid, DNA, RNA and protein contents after 16 h of treatment. Lipid content was found to be decreased up to 75.05% and 73.78% at 16 h treatment respectively. Plant natural product based combinatorial formulations have shown very high lethality in *Odontotermes obesus*. This is proved by very low LD 50 values obtained in each case. These types of formulations showed strong anti-termite potential as natural pesticides [37] [38]. These gave an overall sustainable way to termite control in crop field mainly maize and millet crops [39] [40].

In another experiment termite were treated with 40% and 80% of LD_{50} of *Ficus benghalensis* mixed with borate and other natural components, it caused a significant ($p > 0.05$) decrease in glycogen, amino acid, DNA, RNA, protein and lipid level in termites (**Figures 4-6**). Similar *Ficus benghalensis* mixed with copper sulphate mixture has shown significant ($p > 0.05$) decrease in the level of all macromolecules in comparison to untreated termites (**Figures 7-9**). Besides this, photo-activated cow urine and its combinatorial mixtures with *Ficus benghalensis i.e.* CU-MLT-A, B and C have also shown significant ($p > 0.05$) decrease in levels of different macromolecules (**Figures 13-15**). It was mostly found that on an average *Ficus benghalensis* and its combinatorial mixtures have shown better toxicity than synthetic pesticides (**Figures 1-20**). It is well known that stored lipids are the most important and convenient stores of metabolic energy, meeting the long-term energy needs of insects under stress. Physiologically, lipids play an important role in insect development, reproduction, and flight. Inhibition of lipid metabolism interferes with most of the normal physiological activities of termites, resulting in indirect control of termites. In insects, carbohydrate metabolism also plays an important role in flight mechanisms and reproduction. In insects trehlose a disaccharide is an important energy molecule that upon hydrolysis give rise three molecules of glucose in the presence of enzyme trehlase, which synthesizes in midgut epithelium of insects. Besides trehlose, glycogen is a major energy reserve found in fat body and muscles. Glycogen is synthesized from glucose units but indirectly it is also synthesized from glucogenic amino acids which indicate utilization of amino acids. Therefore, more phosphorylation indicates major utilization of food reserves and release of high energy in in-

sect tissues. Another reason of decrease in levels of glycogen and protein is toxic stress imposed by extract. In this study glycogen amount was reduced after treatment of *Ficus benghalensis* aqueous fraction and its combinatorial mixtures in termites. Similarly amount of total lipids was also increased. It may be due to breakdown of glycerides and diglycerides. As insects obtained lipids are essential dietary constituents. If any how lipid metabolism is induced 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, diglycerides by an enzyme lipase. If lipid reserve increases it means hydrolytic enzymes are not working and over deposition of lipid may cause oxidative stress in insects. Similarly increased protein level is also related to an increase in RNA concentration while at the same time DNA amount may be lesser. Subsequently the ratio of RNA and DNA has wide concern with protein metabolism. At time of active protein synthesis DNA ratio falls but later on increases. Reduction in protein synthesis may lead to decrease in protein concentration and RNA, at the same time DNA level may increase. More specifically, all macromolecules serve as initial substrate for oxidation and energy production. Plant latex and other exudates are sap that exude from plant injury sites caused by mechanical or insect injury. Latex is secreted from 10% of plant families, has evolved independently many times, and is the most effective defense of milkweeds against its chewing herbivores [41] [42]. Latex contains two specialized metabolites such as terpenoids, cardenolides, alkaloids, and phenolics, which are show broad spectrum such as antibacterial, antifungal, anthelmintic, cytotoxic, and insect-repellent activities. Latex is a type of sticky endogenous fluids derived from diverse plants. It contains unique compounds mainly defense molecules which protect plants against microbes and herbivores [43] [44]. Latex secreted from *E. fischeriana* show anti-feedant activity against *H. armigera* [45] while *Plumeria rubra* plant latex shows activity against *Aedes aegypti* and *Anopheles stephensi* [46]. Latex of *Synadenium grantii* shows activity nematicidal activity on *Meloidogyne incognita* and *Panagrellus redivivus* [47].

Plant latex contains defense metabolites which assist them to protect against insect herbivores and pathogens [48]. Latex was used traditinonal folk medicine to treat papillae, warts, condylomas, human papilloma virus (HPV) infections [49] [50]. Latex of *P. pudica* show inflammatory ulcerative colitis [51]. The synthesized AgNps synthesized from *P. rubra* latex were formed highly toxic than crude latex extract termite species. Latex of *Thevetia peruviana* show antifungal activity against *P. amapa* [52], latex shows activity against change *C. megacephala* post embryonic development [53]. Plant exudates mainly latex, sap, gums, resins etc. are known to possess diverse biological activities including, antimicrobial, anti-inflammatory, antioxidant, and wound healing and anti-termite [54]. *Calotropis procera* is known to produce contact dermatitis and the latex of this plant produces intense inflammation. It also found quite effective against

termites [55].

To prove antitermitic activity of *Ficus benghalensis* latex and its various combinatorial mixtures, level of various biomolecules were determined in termites. *Ficus benghalensis* aqueous extract and its combinatorial mixtures have shown significant alterations in biomolecules level. This led to the formation of abnormal state in the insects and make insects unable to survive.

5. Summary

In present investigation, *Ficus benghalensis* latex and its combinatorial mixture, and its effect were determined in biomolecules. For this purpose, termites were treated with 40% and 80% of LD_{50} of *Ficus benghalensis* aqueous extract and its combinatorial mixtures. Effects were observed on glycogen, amino acid, DNA, RNA and protein levels at regular time interval. A significant ($p > 0.05$) decrease was observed in all the above biomolecules when termites were treated with S-MLT-A combinatorial mixture. A similar decrease was obtained in glycogen, amino acid, DNA, RNA and protein levels when termites were treated with 40% and 80% of LD_{50} of B-MLT-A mixture, while lipid contents were found to be decreased two fold. Similarly 40% and 80% of LD_{50} of S-MLT-C mixture also caused significant ($p < 0.05$) decline in glycogen, amino acid, DNA, RNA and protein contents after 16 h of treatment. Lipid content was found to be decreased up to 75.05% and 73.78% at 16 h treatment respectively. Plant natural product based combinatorial formulations have shown very high lethality in *Odontotermes obesus*. This is proved by very low LD 50 values obtained in each case. Toxicity in aqueous extract was much higher than solvent extracts. This is the main merit in these formulations as they have strong anti-termite potential as natural pesticides. Plant latex protects plants from insect predation and damage. It contains strong antifeedants and toxic components. These gave an overall sustainable way to termite control in crop field, mainly maize and millet crops. This led to the formation of abnormal state in the insects and made insects unable to survive.

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Conflicts of Interest

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

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