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Termiticidal and Biochemical Activity of Combinatorial Essential Oil Ingredients of *Tagetes erecta* on Indian White Termite *Odontotermes obesus*

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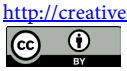

Abstract

In present investigation, various bio-molecular parameters *i.e.* glycogen, protein, amino acid, DNA, RNA and lipid, were determined for evaluation of anti-termite efficacy of *Tagetes erecta* essential oil based combinatorial formulations against Indian white termite *Odontotermes obesus*. For this purpose, 40% and 80% of 24 hrs of LD_{50} values of various formulations were provided for treatment of termite workers. Observations were taken at 4 hrs time interval up to 16 hrs to know inhibitory activity of these combinatorial mixtures on termite species *Odontotermes obesus*. Significant ($p > 0.05$) alterations were observed in the level above mentioned bio-molecules when termites were treated with different combinatorial essential oils ingredients of *Citrus maxima*. Combinatorial mixtures of *Tagetes erecta* essential oils have shown synergistic activity against termites. This study will provide an overall sustainable way to termite control in crop field, gardens and houses. It also suggests use of essential oils as better alternative of synthetic termiticides as these are safer for environmental and human health.

Keywords

Tagetes erecta, *Odontotermes obesus*, Plant Essential Oils, Termiticides, Bio-Molecules, Inhibition, Sustainable

1. Introduction

Termites are most destructive pests on the planet. These small size insects feed primarily on trees, crop plants and building materials, cereal grains, wood filaments, cloths and papers [1]. These too harm green foliages, seedlings, wood,

strands, and other family cellulose based materials. These also feed on dead plant matter such as woodchips. These intensely overrun post-harvest put away items, cereal grains, wood strands, cloths and papers. Dry land forest and woodland termites make burrows, termitarium, and tunnels to hide during the day and feed on green biomass, vegetation, or crops of adjoining areas during night time. Termites live in the wet, tropical regions of the world. They digest plant debris, conifers, and other cellulosic fabric materials. Termite species attack plants, essentially resulting in reduced trimming losses and biomass production.

Termites feed and burrow into the wood by eating it, compromising the structural integrity of the structure. This forces the wood structure to bend, swell, sag, or sag. Termites also leave external traces of their presence, including mud hoses, abandoned wings, and wood-colored debris called feces. Subterranean termites connect their subterranean soil colonies to aboveground food sources via mud pipes (sometimes called galleries or tunnels). They are destroying forest and agricultural biomass in the environment and contributing to various environmental forms. Termites feed on mostly green forests, forming huge mounds. These attacks on standing dead trees in tropical forests damage the wood and degrade its quality [2]. Around 2800 termite species have been identified worldwide, belonging to 282 genera. Termites decompose deadwood and prevent floating and standing deadwood from consolidating in the soil [3], with the exception of *R. virginicus*, *Reticulitermes virginicus* and *Reticulitermes flavipes* (Collana). Humidity, temperature and light influence of termite feeding [4].

Few common plant products, such as flavonoids [5], sesquiterpenes [6] phenylpyrazoles, pyrethroids, chloronicotinyls, and pyrroles and thiophenes [7] have been used for the control of Formosan subterranean termite, *Coptotermes formosanus* Shiraki (*Isoptera*: *Rhinotermitidae*). Fipronil is termiticide that belongs to phenyl-pyrazole class of chemical compounds. Although it effectively kills termites and other household insects vary efficiently [8]. It remains inside soil in the form of bound residues for longer period [9]. It is toxic to mammals and seeps into ground water [10]. Of the past 100 pesticides that have been established over the years, such as chlordane [11] and cypermethrin, few of them act at the cellular and physiological level [12]. Novi flumuronis used as a damage bait to kill molting populations of the Taiwan subterranean termite, *Coptotermes formosanus* Shiraki-Innards [13]. Polyacrylamide/attapulgite composites lings are also bonded in agricultural soils. They attract termite colonies to begging [14]. These gels may contain systemic damage or insecticides to kill termites. Carbon dioxide emissions attract termites and enhance the suitability of sprays to kill termites in the home [15]. Termite-specific populations can be controlled or suffocated using damage traps and slaughtering at pre-adult stages [16].

Plant-derived compounds are used as one of the pesticides made to control termites. Herbal insecticides are environmentally friendly and control termites without harming non-pest species. For termite control, few bait formulations have been thoroughly tested against underground termite colonies. *Chrysan-*

themum roseum capital and *C. cinerriefolium* are dried, powdered and used as an insecticide [20]. Combinatorial mixtures of *Tagetes erecta* significantly repelled termites in two choice bioassays. These have also shown powerful anti-nutrition effects in termite workers [21].

Plants are rich in natural chemical compounds, including flavonoids, alkaloids, polyphenols, polysaccharides, quinonoid, saponins, etc. These natural plant products such as essential oils, can be used in poison baits, and act as deterrents, repellents, fumigants against white termite [22]. The present study signifies insecticidal potential and target specificity of *Tagetes erecta* L plant essential oil against Indian white termite. Plant belongs to family (Asteraceae) and contains active ingredients as polyphenols, phenolic acids, flavonoids, acetylenes and triterpenes [23]. Its activity was tested by exposing termites with sub-lethal dose (40% and 80% of 24 hrs LD50) of various combinatorial fractions and alterations in various level of various bio-molecules in whole body extract of termites was determined at various time intervals.

2. Materials and Methods

2.1. Preparation of Combinatorial Mixtures

Tagetes erecta Extract *and* other ingredients were used in the preparation of combinatorial mixtures. The details of all combinatorial mixtures are mention in following **Table 1**.

2.2. Determination of Biomolecular Parameters

The aqueous *Tagetes erecta* extract of 40% and 80% of LD50 and its various combinatorial mixes were administered to termite workers individually. To prepare the whole body extracts of the termites for bio-molecular analysis, they were slaughtered, homogenised, and centrifuged. After different time periods, such as 4, 8, 12 and 16 hours, changes in the level of various bio-molecules were measured. A few significant bio-molecules were identified, including glycogen, total free amino acids, total lipid, nucleic acids (DNA and RNA), and total protein.

2.3. Determination of Glycogen

Glycogen substance was measured according to method of Dubois [24]. For this purpose 500 mg of termites were homogenized in 2 ml of 5% TCA with the assistance of glass-glass homogenizer and centrifuged. After centrifugation supernatant was exchanged into a 100 ml graduated cylinder and after that included three times volume of 95% ethyl liquor. Blend was blended until accelerate begun flocculating at that point a squeeze of NaCl was included to strongly the flocculation. Presently the graduated barrel was warmed by keeping it in hot bubbling water until the precipitate is shaped. The substances were once more centrifuged and after that accelerate was broken up in 5 ml refined water. Then 0.5 ml of obscure de-proteinized glycogen supernatant was included with 6 ml of

concentrate H_2SO_4 and after that substance were kept in bubbling water for 5 minutes, at that point blend was cooled to room temperature. Optical thickness of the reactant was perused at 530 nm. Glycogen substance in obscure (supernatant) was calculated by utilizing standard bend drawn with known sum of glucose. The clear was set by taking 0.50 ml of 5% TCA and 6 ml of concentrate H_2SO_4. The sum of glycogen was communicated in gm/100gm of body weight of termites. Three replicates were set to get precision. Information gotten was factually dissected by utilizing ANOVA method.

Table 1. *Tagetes erecta* and other ingredients used in preparation of combinatorial mixtures.

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

*Synthetic pesticides.

2.4. Determination of Total Free Amino Acid

Level of free amino acids was decided by utilizing Spies method [25]. For this reason 500 mg treated termites were homogenized in 2 ml of 95% ethyl liquor. Homogenate was centrifuged at 15,000× g for 20 minutes and supernatant was isolated. For estimation 0.1 ml of supernatant was taken and to it 0.1 ml of refined water and 2.0 ml Ninhydrin reagent were blended. The Ninhydrin reagent was arranged by blending 1.0 gm Ninhydrin in 25 ml of absolute ethanol and 0.04 gm stannous chloride in 25 ml citrate buffer (pH 5.0). Citrate buffer was arranged by blending 20 ml of reagent A (1.05 gm of citric corrosive in 50 ml of refined water) and 29.5 ml of reagent B (2.94 gm sodium citrate in 100 of refined water). The response blend was kept in bubbling water for 15 minutes. Presently 2 ml of 5.0% ethyl liquor was included to the over boiled mixture. A violet colour was created within the response mixture which was measured at 575 nm. For calculation standard bend was arranged by utilizing known sum of glycine and was communicated in gm/100gm body weight of termites. Three reproduces were utilized and information is measurably analyzed by ANOVA method.

2.5. Determination of Total Lipid

The total lipid levels in the termite whole-body extracts were determined according to Floch [26]. For this purpose, 500 mg of worker termites were homogenized in 5 ml of a mixture of chloroform and methanol (2:1 v/v). The homogenized material was suspended and kept at room temperature for 30 minutes. Homogenate was filtered through Whatmann No. 1. The residue was re-suspended in the same volume of the mixture for 1 hour and the supernatant filtered again. Both filtrates were mixed with an equal volume of 0.6% (w/v) NaCl. The separatory funnel containing the above mixture was kept in the dark at room temperature for 12 hours. The top solvent layer (chloroform:methanol) was collected and the non-saponified portion was left unused. The contents of the lower layer were allowed to evaporate by keeping it in an oven at 60˚C. The total lipid content was finally weighed and expressed as g/100g termite body weight. Triplicate experiments were set up and the data were analyzed statistically by ANOVA.

2.6. Determination of Nucleic Acids

The nucleic acid levels in the termite whole body extracts were determined using the method of Scheidner [27]. For this purpose, 500 mg of termite mounds were fed separately with an aqueous extract with 40% and 80% LD50 and their combinatorial mixtures. Insects were killed and homogenized in 5% TCA using a glass-glass homogenizer at 15,000× g for 25 minutes.

2.7. DNA Estimation

For DNA evaluation, 0.2 ml of the supernatant was removed and diluted by adding 3.8 ml of distilled water. Now 4.0 ml diphenylamine reagent (1 g diphe-

nylamine, 100 g glacial acetic acid and 2.5 ml concentrate H_2SO_4 was added). The contents were kept in a boiling water bath for 10 minutes. A blue color developed in the solution measured at 600 nm (OD).

2.8. RNA Estimation

For RNA determination, 0.2 ml of the supernatant was removed and diluted by adding 4.8 ml of distilled water. Now 2 ml orcinol reagent (1 g orcinal, 100 ml conc. 5 g ferric acid was added). The solution was kept in a boiling water bath for 10 minutes, a green color appeared which was measured at 600 nm. In both cases, three replicates were assumed and the data obtained were statistically analyzed by ANOVA.

2.9. Determination of Total Protein

Total termite proteins were estimated using the method of Lowry [28]. For this purpose, 500 mg of worker termites were treated separately with aqueous extracts of *Tagetes erecta* and various combinations thereof. These treated termites were homogenized in 4.0 ml 10% TCA using a glass-glass homogenizer. The resulting homogenate was centrifuged at 15,000× g for 15 minutes. The supernatant was discarded and the pellet dissolved in 5% TCA and centrifuged again at the same speed for 10 min, after which the supernatant was discarded. The pellet was then dissolved in 1 ml of 1 N NaOH and centrifuged again at the same speed. The supernatant was used for protein assessment. For this purpose, 0.5 ml of the supernatant was added to 0.5 ml. 5 ml copper lye (reagent C). Reagent C was prepared by adding 50.0 mL of Reagent A (2% sodium carbonate in 0.1N NaOH) and 1 mL of Reagent B (1.0% sodium potassium tartrate, 0 NaOH, 1N). 5% copper sulphate mixed 1:1 in the experiment). The reaction mixture was kept at room temperature for 10 minutes, and then 0.5 ml of Folin-Phenol-Ciacalteu reagent (diluted 1:2 with distilled water during the experiment) was added. Then 1.5 ml 0.2N NaOH and 3.5 mL distilled water were added and mixed well. After ten minutes, the reagents turned blue as measured at 620 nm. Three replicates were set up for each experiment. A standard curve was constructed using various concentrations of bovine whey proteins. The data obtained were statistically analyzed by ANOVA.

2.10. Statistical Analysis

The LD50 in termite mounds was determined for each extract and combinatorial mixture by probit analysis (**Table 2**). Mean, standard deviation, standard error, correlation and Student's t-test were used in the ANOVA program. The chi-square test was used to determine the deterrent effect (Sokal and Rohlf) [29].

3. Results

The investigation of biochemical estimation of various bio-molecules glycogen, amino acid, lipid, DNA, RNA and protein were done in treated and control

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

S.N.	Name of Extract/ Combinatorial Mixture	LD50 µg/gm	0.95 confidence limit UCL-LCL	Chi-Square	Slope function	Degree of freedom	Heterogeneity	LD40 µg/gm	LD20 µg/gm
1.	S-AST-A	337.839	521.443 - 243.460	7.2207	−0.131622	4	1.8052	135.1	67.56
2.	S-AST-B	370.722	485.216 - 280.403	5.6770	−0.136581	4	1.4192	148.28	74.14
3.	S-AST-C	507.666	691.192 - 371.138	6.7542	−0.141483	4	1.6886	203.06	101.53
4.	B-AST-A	261.930	352.270 - 194.957	6.2448	−0.129716	4	1.5612	104.77	52.38
5.	B-AST-B	364.898	434.773 - 305.138	3.584	−0.123795	4	0.896	145.9	72.97
6	B-AST-C	494.352	683.442 - 352.598	7.1354	−0.140405	4	1.7839	197.74	98.87
7	Cu-AST-A	307.838	430.957 - 230.441	10.717	−0.133137	4	2.6792	123.1	61.5
8	Cu-AST-B	358.599	475.526 - 266.342	5.6228	−0.129255	4	1.4057	143.43	71.7
9	Cu-AST-C	531.550	715.288 - 397.738	10.489	−0.144219	4	2.6222	212.62	106.31
10	Cow-AST-A	236.106	328.254 - 164.877	9.0054	−0.130733	4	2.2513	94.44	47.22
11	Cow-AST-B	377.816	478.518 - 297.051	4.0440	−0.133482	4	1.0110	151.1	75.56
12	Cow-AST-C	577.159	734.248 - 459.912	4.1740	−0.154133	4	1.0435	230.86	115.43
13	AQ-AST	30.147	36.071 - 24.929	1.077	−0.708375	4	0.269	12.05	6.02
14	A-AST	30.212	36.543 - 25.045	2.400	−0.684713E	4	0.600	12.08	6.04
15	H-AST	50.601	90.739 - 34.517	8.6803	−0.822924	4	2.1701	20.24	10.12
16	P-AST	-	-	67.670	−0.458164	4	16.917	-	-

termites. For this purpose adult worker termites were provided with 40% and 80% LD_{50} of *Tagetes erecta* aqueous extract and its other combinatorial mixtures with the diet. After 4 h of treatment each bio-molecule level was determined up to 16 h. Whole body extract of both treated and control termites was prepared. For this purpose termites were scarified, homogenized and centrifuged and level of various bio-molecules was determined. Reduction or increase in bio-molecules was calculated by using corresponding control. 80% of B-AST-C combinatorial mixture caused significant ($p > 0.05$) 50.65% 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 S-AST-C combinatorial mixture *i.e.* 50.25% 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 40% and 80% of LD_{50} of S-AST-C combinatorial mixture caused significant ($p > 0.05$) decrease in DNA level at 16 h of treatment *i.e.* 58.78% and 97.07% 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 S-AST-C combinatorial mixture *i.e.* 55.42% and 61.24% & 56.48% and 57.28% at 16 h treatment in comparison to control respectively (**Figures 1-3**). All treatments done by using 40% and 80% of LD_{50} of various mixtures and its impact on various bio molecules have been displayed in (**Figures 4-10**).

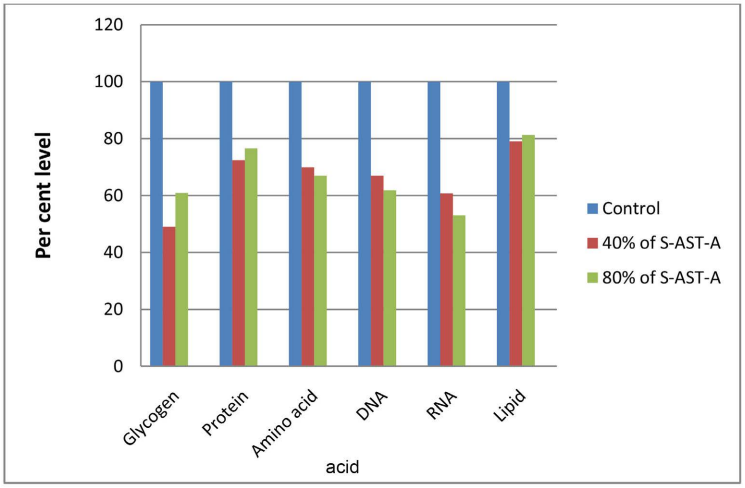

Figure 1. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-AST-A mixture at 16 hour.

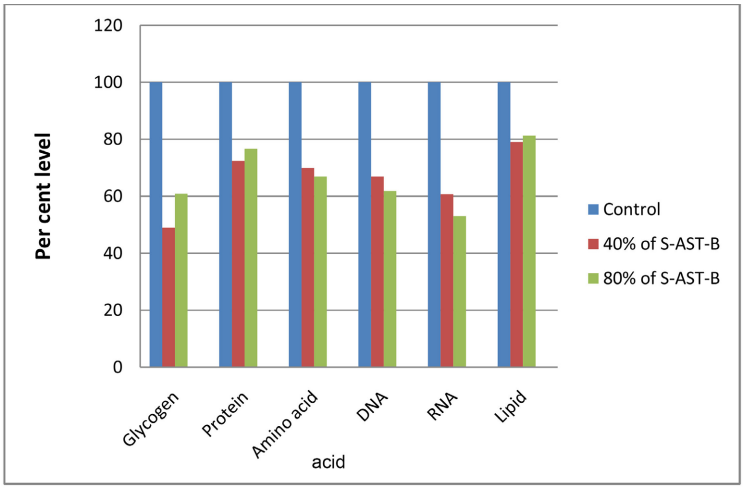

Figure 2. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-AST-B mixture at 16 hour.

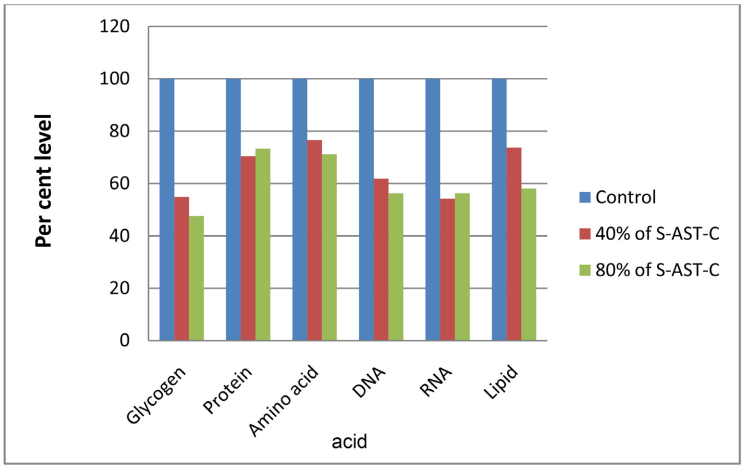

Figure 3. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of S-AST-C mixture at 16 hour.

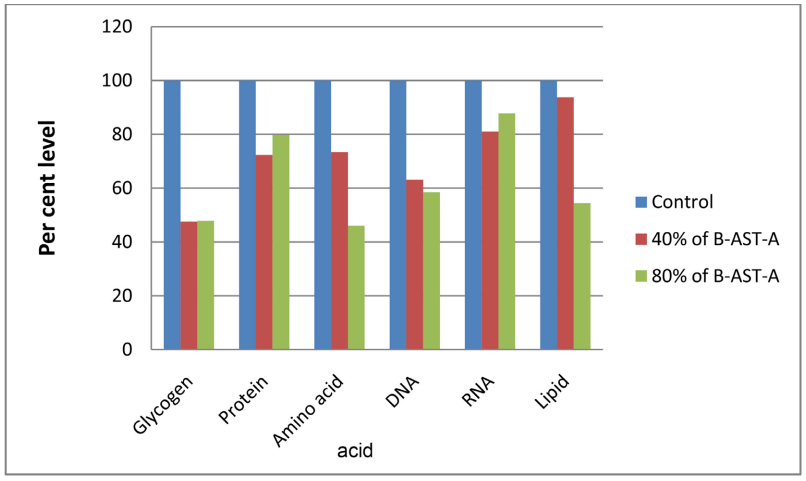

Figure 4. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-AST-A mixture at 16 hour.

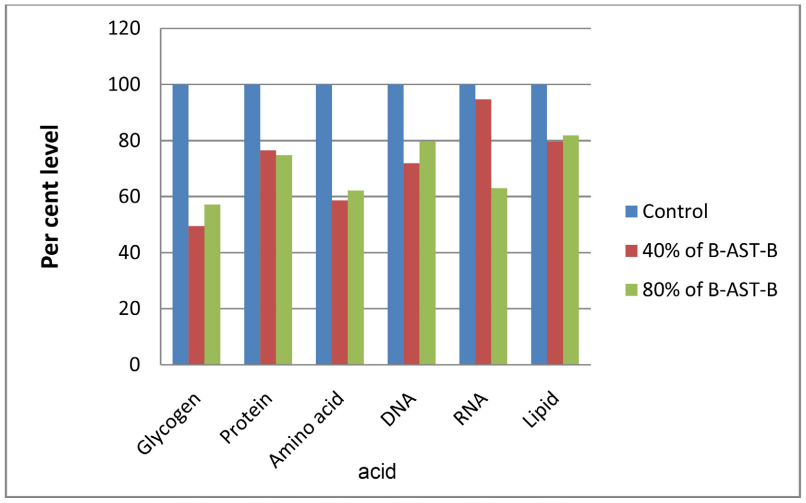

Figure 5. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-AST-B mixture at 16 hour.

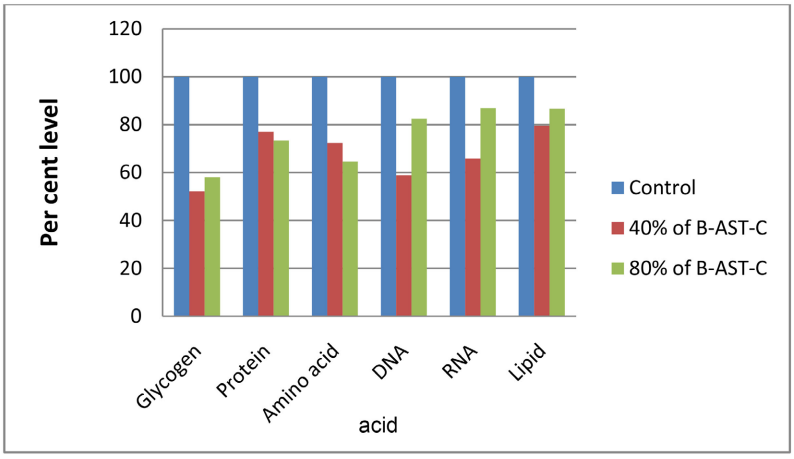

Figure 6. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of B-AST-C mixture at 16 hour.

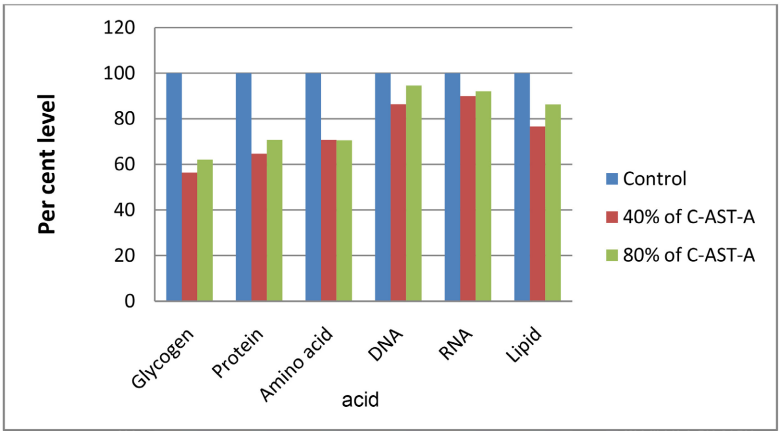

Figure 7. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-AST-A mixture at 16 hour.

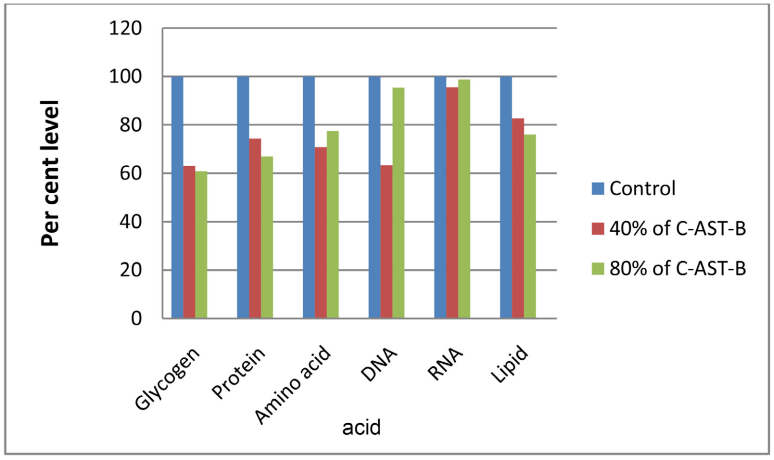

Figure 8. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-AST-B mixture at 16 hour.

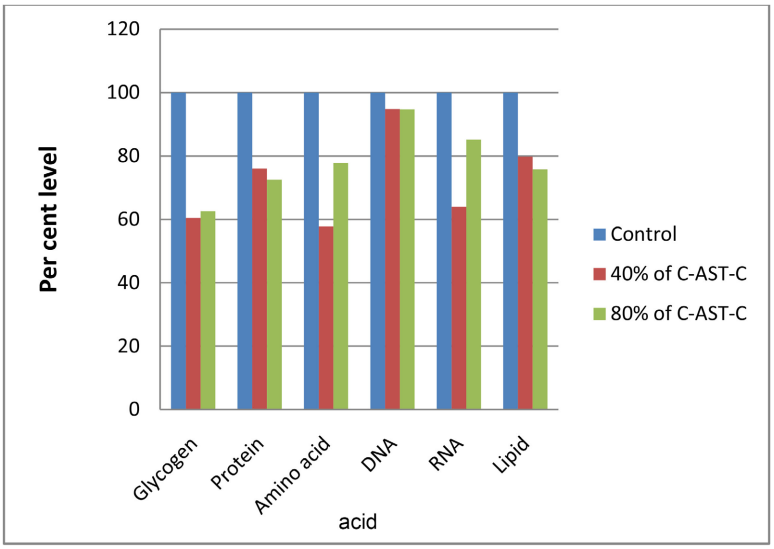

Figure 9. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of C-AST-C mixture at 16 hour.

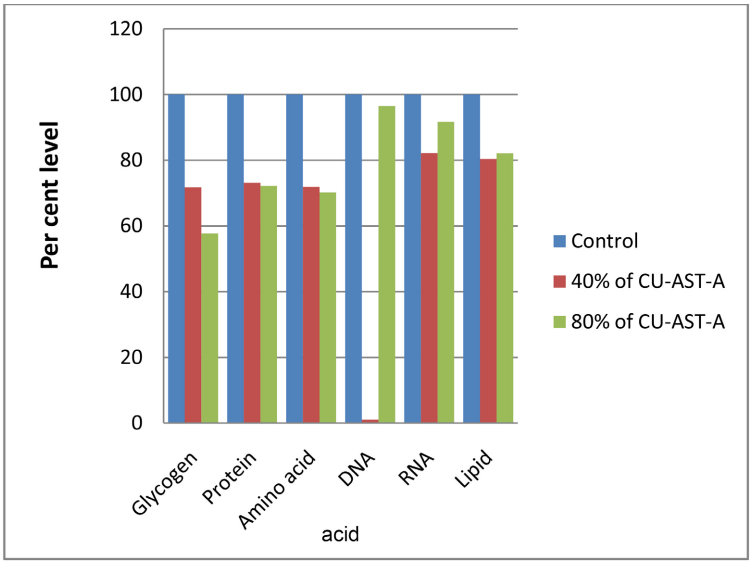

Figure 10. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-AST-A mixture at 16 hour.

Treatment of 40% and 80% of LD_{50} of CU-AST-B mixture caused a significant decrease in glycogen levels up to 52.47% and 54.38% at 16 h treatment in comparison to control respectively. In another experiment when termites were treated with 80% of LD_{50} of CU-AST-B mixture show slightly decrease in amino acid level at 4 h of treatment *i.e.* 94.78% but later it increased up to 97.46% at 16 h of treatment (Figure 11). In the same experiment, 40% of LD_{50} of CU-AST-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 97.43%, 92.44% while in 80% of LD_{50} of CU-AST-B mixture caused slightly increased in DNA level *i.e.* 100.7% and slightly decreased in RNA level *i.e.* 91.70%. Similarly total proteins were also found to be decreased with 40% and 80% of LD_{50} of CU-AST-B mixture *i.e.* 80.55% and 70.80% respectively (Figure 12).

In another experiment when termites were treated with 40% and 80% of LD_{50} of AQ-AST mixture, it has shown continuous decrease in glycogen level *i.e.* 56.87% and 58.62% at 16 h of treatment in comparison to control respectively. While at the same dose amino acid level was found to be slightly decreased at 4 h treatment *i.e.* 91.26% and 96.47%. Similarly the lipid level was also found to be decreased at 40% and 80% of LD_{50} of AQ-AST mixture up to both at 4 h and 8 h *i.e.* 92.83%, 96.71% and 81.88% and 81.80% respectively (Figure 12). Similarly RNA level was also found to be decreased up to 90.74% and 53.08% at 40% and 80% of LD_{50} of AQ-AST mixture respectively (Figure 13). Maximum inhibition was observed in protein level *i.e.* 67.20% and 62.88% at 40% and 80% of LD_{50} of 16 h of treatment respectively (Figures 14-19).

4. Discussion

For control of insect pests different synthetic pesticides were used. These have

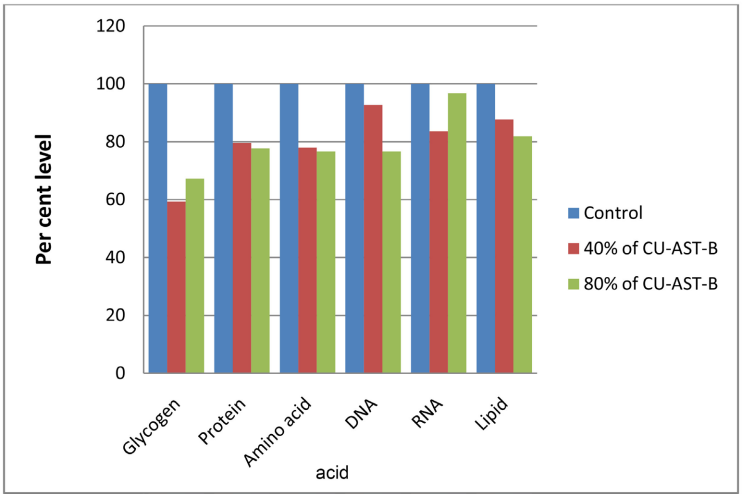

Figure 11. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-AST-B mixture at 16 hour.

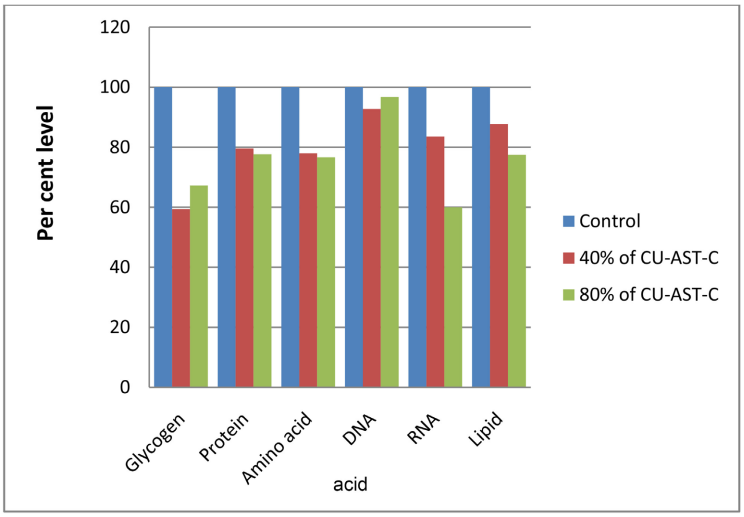

Figure 12. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of CU-AST-C mixture at 16 hour.

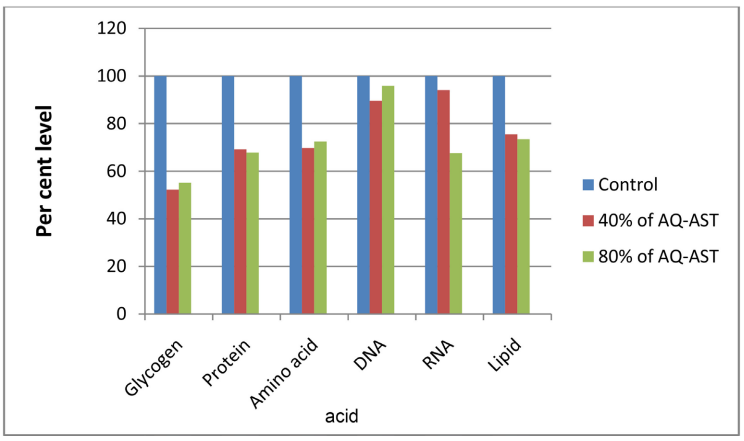

Figure 13. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of AQ-AST mixture at 16 hour.

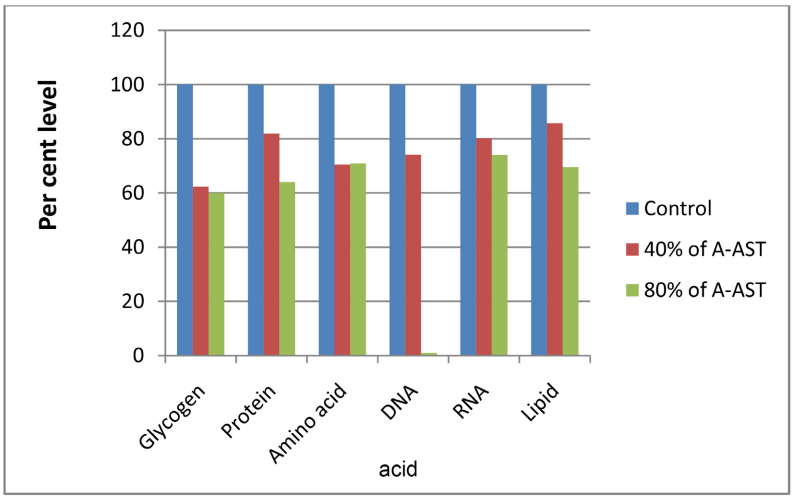

Figure 14. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of A-AST mixture at 16 hour.

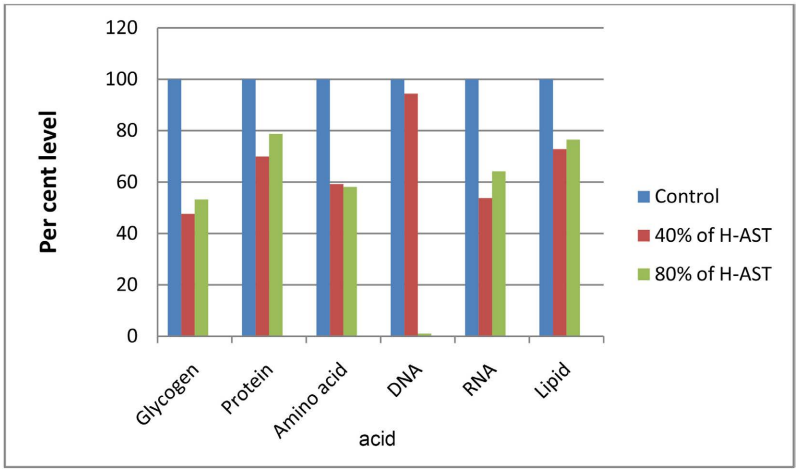

Figure 15. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of H-AST-mixture at 16 hour.

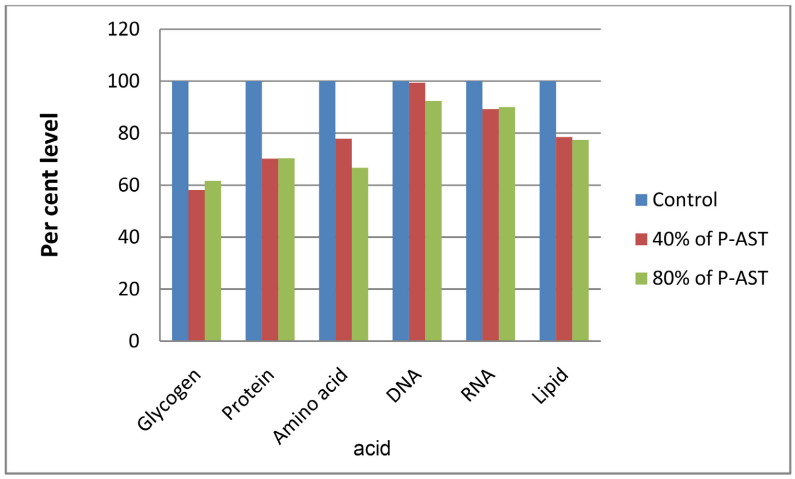

Figure 16. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of P-AST mixture at 16 hour.

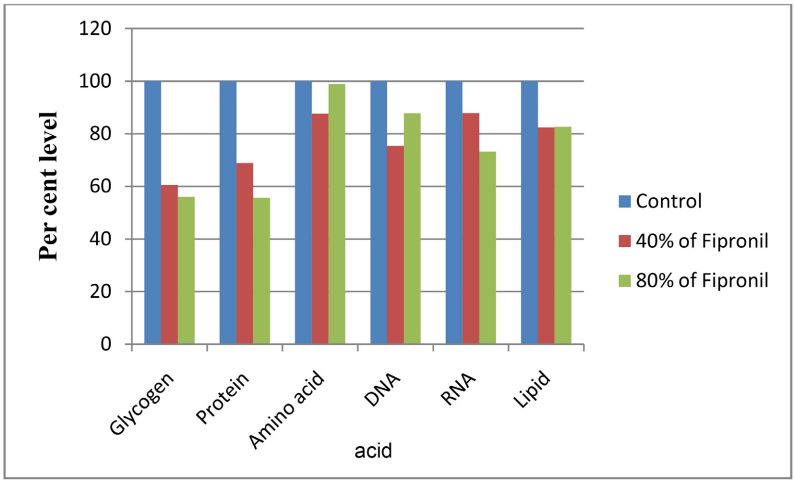

Figure 17. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of fipronil mixture at 16 hour.

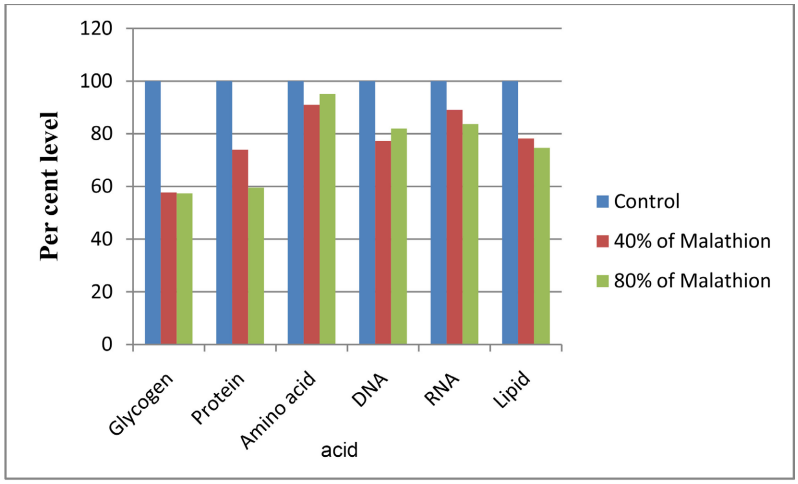

Figure 18. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of malathion mixture at 16 hour.

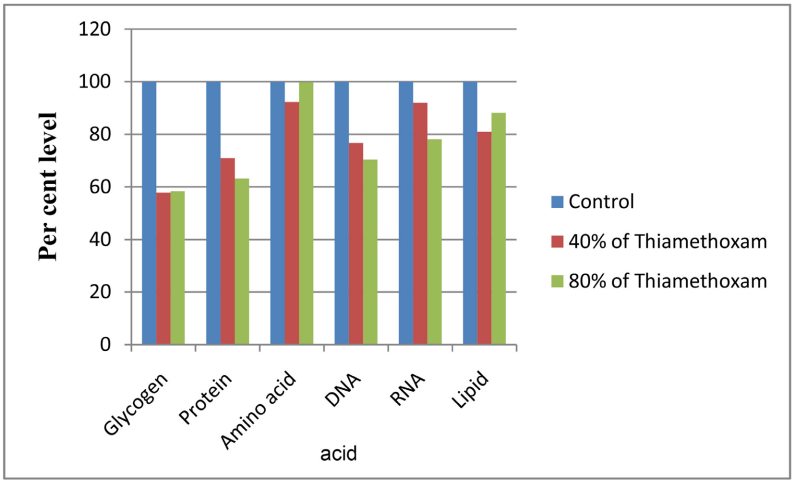

Figure 19. Comparison of glycogen, amino acid, lipid, DNA, RNA and protein in termites treated with 40% and 80% of thiamethoxam mixture at 16 hour.

shown adverse effects on non target organisms, human health and environment. Hence, new alternatives of synthetic pesticides were searched in form of natural pesticides or plant origin pesticides. For routine control of field termites, various synthetic pesticides such as chlordane, cypermethrin and thiomathxam were used [30]. These chemicals enter into the food chain and kill non-target organisms. These have been banned and its new alternatives are discovered in the form of natural pesticides. *Tagetes erecta* are rich in essential oils and exhibit toxicity towards various insect species. The essential oils are also an eco-friendly option for insect pest management. *Tagetes erecta* essential oil a waste product, characterized it, and evaluated its potential for insect pest management.

In present investigation toxic effects of *Tagetes erecta* extract and its combinatorial mixture were determined in various bio-molecules. For this purpose termites were treated with 40% and 80% of LD_{50} of *Tagetes erecta* aqueous extract, its combinatorial mixtures and synthetic pesticides. Effects were observed on glycogen, amino acid, DNA, RNA and protein levels at regular time intervals (**Figures 1-19**). A significant ($p > 0.05$) decrease was observed in all the above bio-molecules when termites were treated with S-AST-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-AST-A mixture, while lipid contents were found to be more decreased in B-AST-A in comparison to S-AST-A (**Figure 1** & **Figure 4**). Similarly 40% and 80% of LD_{50} of *C-AST-C* mixture also caused significant ($p < 0.05$) decline in glycogen, amino acid, DNA, RNA and protein contents after 16 h of treatment (**Figures 5-8**). Lipid content was found to be decreased up to 80.45% and 80.62% at 16 h treatment respectively (**Figure 9**).

Plant natural products are used in eco-friendly control of termites and its management. *Capparis decidua* and its combinatorial mixtures are used to control the Indian white termite *Odontotermes obesus* [31] and stored grain insects [32] showed strong termiticidal effects. Citral exhibited the highest fumigant toxicity against *C. maculatus* and *S. zeamais* with LC_{50} value 0.19 and 2.02 µL/L air at 24 h respectively. Acetylcholinesterase and Na^+/K^+-ATPase activities were significantly inhibited by *C. sinensis* oil and its constituents in both *C. maculatus* and *S. zeamais* as compared to control [33]. Due to their target specificity botanical pesticides are considered the most promising alternative to synthetic pesticides, considering their less negative impacts on the environment and human health. Similarly, Lemongrass *Cymbopogon citratus* essential oil (EO) was found effective against *Reticulitermes flaviceps*, as it slow down walking and gripping abilities in termite workers. Lemongrass essential oil and its major components dissolute cuticle and chitin in termites. Both lemongrass and citral were found safe to control *R. flaviceps* [34]. Similar anti-termite activity was reported in subterranean termites [35].

A combinatorial mixture of *Tagetes erecta* significantly repelled termites in a two-choice bioassay. These have shown potent anti-feeding effects against termite workers and act as deterrents, repellents, fumigants to control house and

filed termites [36].

Few plant species such as *Carum carvi*, *Salvia umbratica*, *Ilicium difengpi*, *Periploca sepium*, *Cephalotaxus sinensis*, *Murraya exotica*, *Rhododendron anthopogonoides*, *Ruta graveolens*, *Eucalyptus viminalis*, *Ocotea odorifera*, *Eucalyptus globulus*, *Eucalyptus dunnii*, *Anethum graveolens*, *Ilicium verum*, *Cryptocarya alba*, *Azadirachta indica*, *Chenopodium ambrosioides*, *Cupressus semperivens*, *Schinus molle*, *Piper hispidinervum*, *Mentha longifolia*, and *Croton pulegiodorus* extracts showed very low LC_{50} or LD_{50} values than synthetic toxic chemicals or pesticides, it signifies its good insecticidal properties. Various investigations showed that EOs have great potential to be used as bio-insecticides against *S. zeamais* [37]. Chemical composition indicated that the EOs were rich in essential phyto-chemicals including hydrocarbons, monoterpenes and sesquiterpenes. Conclusively, the tested essential oils could be used as eco-friendly alternatives in mosquitoes control programme [38].

The major terpenoids present in *Tagetes erecta* are Limonene and α-Pinene. Overall, the *Tagetes erecta* is therefore found to be a bioactive essential oil extracted from the wastes of pomelo (*C. maxima*) [39]. Plant essential oil based combinatorial formulations have shown very high lethality in *Odontotermes obesus*. Essential oils have very low LD50 value in each case. Essential oil based formulations showed strong anti-termite potential as natural pesticides [40]. *Tagetes erecta* combinatorial mixtures displayed significant protection to garden saplings and resisted against wood invasion in field experiments. *C. maxima* extracts have shown significant reduction (89%) in termite infestation in garden saplings. These gave an overall sustainable way to termite control in crop field mainly maize and millet crops [41]. *C. tinctorius* has recently been shown to have antioxidant, analgesic, anti-inflammatory and anti-diabetic activities, safflor yellow *A. safflamin C* and *luteolin* are the main constituents which are reported from this plant. Caryophyllene, p-allyltoluene, 1-acetoxytetralin and heneicosane were identified as the major components for *C. tinctorius* flowers essential oil [42]. *Lactuca sativa* L, identified constituents methyl esters of linoleic, oleic, palmitic, stearic, arachidic and myristic acids [43]. The oil extracted from marigold (*T. erecta*) to be a great repellent against *O. obesus* [44].

A. galanga fundamental oil was considered to be the ideal concentration that gave most extreme antifeedant impact [45]. Basic oils and ethanolic extricate from *C. konishii*, and their viable constituents, served as potential, eco-friendly termite-control agent [46]. Essential oils from Clove (*Eugenia caryophyllata*), Cubeb Pepper (*Piper cubeba L*), and Lemon Grass (*Cymbopogon winterianus Jowitt*) shows highest repellency performance against *Cryptotermes cynocephalus* (Kalotermitidae) [47]. Plant essential oils from *Corymbia citriodora*, *Croton sonderianus*, *Cymbopogon martini*, *Lippia alba*, *L. Gracilis*, *L. Sidoides* and *Pogostemon cablin* shows repellent activity on *N. Corniger* [48]. Wintergreen oil also kills termites, but it doesn't hurt people or their pets. Plant derived natural product, vulgarone B (isolated from *Artemisia douglasiana Besser*), apiol (iso-

lated from *Ligusticum hultenii* and cnicin (isolated from *Centaurea maculosa Lam.*) shows higher mortalities rates against *Coptotermes formosanus* Shiraki [49]. The essential oil from Indian *Tagetes patula*, Asteraceae, showed antifungal properties [50]. Cannabis and cannabinoids isolated from *Cannabis sativa*, or hemp shows several medicinal properties [51]. Its major components are piperitone, piperitenone, terpinolene, dihydro tagetone, cis-tagetone, limonene, and allo-ocimene. The oil exerted a good antifungal activity against two phytopathogenic fungi, *Botrytis cinerea* and *Penicillium digitatum*, providing complete growth inhibition at 10 microl/ml and 1.25 microl/ml, respectively [52].

In another experiment termite were treated with 40% and 80% of LD_{50} of *Tagetes erecta* 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 *Tagetes erecta* 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 *Tagetes erecta i.e.* CU-AST-A, CU-AST-B and CU-AST-C have also shown significant ($p > 0.05$) decrease in levels of different macromolecules (**Figures 10-12**). It was mostly found that on an average *Tagetes erecta* and its combinatorial mixtures have shown better toxicity than synthetic pesticides (**Figures 1-19**). It is well known that stored lipids are most important and convenient reservoirs of metabolic energy, which fulfil prolonged energy demand in insects in stress. Physiologically lipids play an important role in insect development, reproduction and flight. Once lipid metabolism is inhibited most of the normal physiological activities of termites get obstructed that results in to an indirect control of termites. In insects, carbohydrate metabolism also plays an important role in flight mechanism 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 insect tissues. In the present investigation glycogen amount was reduced after treatment of *Tagetes erecta* 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 di glycerides. 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.

To prove anti-termitic activity of *Tagetes erecta* and its various combinatorial mixtures level of various enzymes were also determined in termites. In the present investigation levels of various enzymes were found to be reduced that indicate obstruction in their chemical pathways. More specifically, *Tagetes erecta* aqueous extract and its combinatorial mixtures have shown significant alterations in enzyme activity. This led to the formation of abnormal state in the insects and made insects unable to survive.

5. Summary

In present investigation, toxic effects of *Tagetes erecta* extract and its combinatorial mixture were determined in various bio-molecules *i.e.* glycogen, amino acid, DNA, RNA and, lipid, protein. For this purpose, worker termites were treated with 40% and 80% of LD_{50} of *Tagetes erecta* aqueous extract, its combinatorial mixtures and synthetic pesticides.

Effects were observed on at regular time intervals. A significant ($p > 0.05$) decrease was observed in all the above bio-molecules when termites were treated with S-AST-A combinatorial mixture.

Maximum decrease in glycogen level was observed at 16 h when termites were treated with 80% of LD_{50} of S-AST-C combinatorial mixture *i.e.* 50.25% at 16 h of treatment.

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-AST-A mixture, while lipid contents were found to be more decreased in B-AST-A in comparison to S-AST-A.

AST-C combinatorial mixture caused significant ($p > 0.05$) decrease in DNA level at 16 h of treatment *i.e.* 58.78% and 97.07% 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 S-AST-C combinatorial mixture *i.e.* 55.42% and 61.24% & 56.48% and 57.28% at 16 h treatment in comparison to control respectively.

Similarly 40% and 80% of LD_{50} of *C-AST-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 80.45% and 80.62% at 16 h treatment respectively.

Tagetes erecta was selected as this plant possess polyphenols, phenolic acids,

flavonoids, acetylenes and triterpenes which have been used as insecticide against *Chrysanthemum roseum capital* and *C. cinerriefolium* in powdered. Plant natural products are used in eco-friendly to the non-target organisms and put no adverse effect on environment. Similarly, plant derived natural product, *vulgarone B* (isolated from *Artemisia douglasiana Besser*), apiol (isolated from *Ligusticum hultenii*) and cnicin (isolated from *Centaurea maculosa Lam.*) shows higher mortalities rates against *Coptotermes formosanus* Shiraki.

Combinatorial mixtures of *Tagetes erecta* essential oils have shown synergistic activity against termites. This study will provide an overall sustainable way to termite control in crop field, gardens and houses. To prove anti-termitic activity of *Tagetes erecta* and its various combinatorial mixtures level of various enzymes was also determined in termites. In the present investigation, levels of various enzymes were found to be reduced that indicate obstruction in their chemical pathways. More specifically, *Tagetes erecta* aqueous extract and its combinatorial mixtures have shown significant alterations in enzyme activity. 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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