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Toxicity of *Tagetes erecta* Essential Oil Based Combinatorial Formulations on Various Metabolic Enzymes in Indian White Termite *Odontotermes obesus*

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Abstract

Plant essential oils and their constituents have proven to be very effective against insects, especially termites. They are the best alternative to synthetic pesticides that are harmless to the environment and human health. In the present study, different enzymes, namely alkaline phosphatase, acid phosphatase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase and acetylcholinesterase, were evaluated in Indian termite *Odontotermes obesus* in a combination preparation based on *Tagetes erecta* essential oil. For the study of anti-termite effects against worker termites were treated topically with 40% and 80% of the 24-hour LD_{50} values of various combination formulations. Subsequent bioassays at 40% and 80% of LD_{50} of combinatorial preparations S-AST-A, S-AST-B, and S-AST-C resulted in a significant ($p > 0.05$) decrease in glutamate-oxaloacetate transaminase. After 16 hours of treatment, they were 87.92%, 80.17%, 89.30%, 79.17%, 81.92% and 73.17% respectively. *In vivo* exposures of 40% and 80% of the LD_{50} of the combination formulation caused a highly significant ($p > 0.05$) reduction ($p > 0.05$) of all test enzymes tested compared to controls. The effects of different oil formulations exhibited time- and dose-dependent responses, resulting in physiological changes in the concentrations of various enzymes. The combined mixture of *Tagetes erecta* essential oils has significantly better anti-termite ability compared to inorganic insecticides. Findings from this study will help support termite control in fields, gardens and homes in a sustainable way, without the downsides of insecticide resistance and pollution. These could potentially be used to produce commercial formulations for use against pests.

Keywords

Tagetes erecta, Essential Oils, *Odontotermes obesus*, Enzymes, Inhibition,

Termiticidal Action

1. Introduction

With over 1600 genera and over 2500 species, the Asteraceae family is one of the largest flowering plant families in the world. Some of the best-known taxa include lettuce, chicory, artichoke, daisy and dandelion. Asteraceae plant products have been used extensively for nutritional and medicinal purposes in many parts of the world for centuries. Plants are rich in natural compounds such as flavonoids, alkaloids, polyphenols, polysaccharides, quinonoids, saponins, phenolic acids, acetylenes and triterpenes [1] which show multiple therapeutic effects [2]. These natural plant products derived from essential oils are used to make baits, deterrents, repellents, fumigants, and insecticides for termite control [3]. Plant-based termite control agents are environmentally friendly and control termites without harming non-pest species [4]. The cores of *Chrysanthemum roseum* and *C. cinerriefolium* are dried, powdered and used as insecticides [5]. The combined mixture of *Tagetes erecta* significantly rejected termites in two selection bioassays. They have also shown potent anti-feedant effects on hardwood termites [6]. They are an environmentally friendly alternative to synthetic pesticides used to control termites and other pests.

Tagetes erecta essential oil contains terpenoids, limonene and *α*-pinene as main components and is toxic to various insect species [7]. They act at the cellular and physiological levels and have predominantly protective effects [8]. They also demonstrated biological benefits such as antioxidant, anti-inflammatory, anticancer, antibacterial, antifungal, and antiviral effects [9]. These phytochemicals also act as repellents [10] and inhibit feeding in termite *C. formosanus* [11]. The essential oil is obtained by steam distillation from the heads of *Tagetes erecta*, a member of the Asteraceae family, and tested for detection of phytochemicals [12] and evidence of their insecticidal and medicinal effects [13].

Termites are detritivorous social insects that live in colonies. They are classified as insects and belong to the family *Termitidae*. Termites are classified into 1231 different families. 112 species have been documented in India. Termites are a type of invasive and eusocial insects that often eat wood, leaf litter, and soil humus, among other elements from decaying plants. Short, beaded antennae and soft, non-pigmented shells are characteristics of termites. The phrase "white ants" also refers to their worker termites. They are not, however, closely related to ants. The destructive polyphagous pest known as the termite, *Odontotermes obesus* (Rambur) (Isoptera: Odontotermitidae) is present in eastern Uttar Pradesh.

Many people are killed by it, which also infests home objects, food crops, orchard crops, and timber. The dry wood termite *Cryptotermes brevis*, which is prevalent in tropical and subtropical regions, is the most destructive species of

termite. It is among the most significant wood structure pests in the world [14]. It is commonly found in woodlands, mainly on dead trees, under and above ground, termites rot dead wood and plant matter [15]. Given that some societies employ enormous termite mounds to cut bodies for burial, these have spiritual and social significance as well [16]. The use of baits made from essential oil components acts as a synergist to take advantage of termite feeding, tunneling, and reproductive behavior [17] [18].

Termites are vital for the global carbon cycle, decomposition processes, and nutrient-rich cellulose mineralization [19]. They are significant decomposers and have tremendous ecological impacts on agricultural and non-agricultural habitats. Crop output, soil fertility, nutrient availability, and water infiltration are all enhanced by termite tunneling activity [20]. Despite their beneficial qualities, termites seriously injure people, especially in environments that contain cellulose, such as libraries, buildings, standing trees, forests, grain storage items, books, and wooden structures [21]. Over $40 billion in economic damage is caused each year on a global scale [22]. They harm horticultural and agricultural crops by feeding on bark and underlying tissue [23]. Farmers are at risk from termites because they directly or indirectly destroy all main crops, including sugarcane, cotton, tobacco, grains, vegetables, fruits, legumes, oilseeds, and decorative plants [24]. Cassava, coffee, cotton, fruit trees, maize, peanuts, soybeans, and vegetables are among the other plants that are attacked by termites [25]. The lower termites (*Mastotermitidae*, *Kalotermitidae*, *Hodotermitidae*, *Termopsidae*, *Rhinotermitidae*, and *Serritermitidae*) and higher termites (*Termitidae*) are distinguished phylogenetically within the isopteran. Chinese researchers discovered *Chroniodiplogaster formosiana* sp. (Rhabditida: *Diplogastridae*) [26].

Crude essential oil extracts of *Tagetes erecta* were used to prepare compound formulations and to measure their effects on specific metabolic enzymes *i.e.* alkaline phosphatase, acid phosphatase, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase and acetylcholinesterase. It solves the problem of increasing drug resistance in insects, kills workers in an environmentally friendly way and replaces the production of bio-organic termiticides, protecting the environment and human health. It's an attempt.

2. Experimental

2.1. Preparation of Combinatorial Mixtures

Flowers from the Asteracae family, *Tagetes erecta* (Genda), were taken from the Deen Dayal Upadhyaya Gorakhpur University, garden (India). It is a native of Mexico and Central America and is a natural, attractive floral plant. A specialist in botany verified this specimen, with assistance from Taxonomy of Indian Angiosperms. The herbarium specimen is in good condition and has been stored for future use in Gorakhpur University's botanical garden. In Southeast Asia as well as India, this plant is widely used by locals for therapeutic and ornamental purposes.

Tagetes erecta is a herbaceous annual or perennial plant that grows to a height of 30 - 110 cm. The root is rotating and cylindrical, with a fibrous and shallow branching system. The stem is striated, occasionally ridged, and smooth or slightly with villi, cylindrical, oval, herbaceous to slightly woody, with fragrant resin channels in the bark when squeezed. The most characteristic of the flowers is that they are assembled in little heads or in singular inflorescences, on peduncles up to 15 cm long; they are liquids of yellow colors to ruddy. Within the flowers of the disc: 150 to 250 within the straightforward heads, within the copies it appears distinctive degrees of change in ligules, yellow to orange corollas, of 8 to 10 mm in length. The natural products and seeds are straight achenes 7 to 10 mm long, smooth or somewhat secured with hardened hairs at the corners. It includes a long blossoming period expanding all through the summer and drop, it reproduces effectively by seeds.

Fresh flower extract was utilised to prepare the combinatorial mixture w/v. The fresh flower was weighed, and the extract was made in a power mixer and grinder with distilled water. The resulting extract was chilled for later use after being dried in a rotating evaporator. All of the chemicals used in this investigation were bought from Gorakhpur-based Eastern Scientific Company, CDH-laboratory chemicals suppliers in India. The essential oils and bioactive components from *Tagetes erecta* extract were extracted using a Clevenger device.

Tagetes erecta extract and other ingredients have been used in the preparation of combination blends. Details of all combination mixtures are shown in the table below.

2.2. *In Vivo* Determination of Enzymatic Parameters

500 mg of adult termite workers were given 40% and 80% of LD_{50} of *Tagetes erecta* essential oil and its combinatorial mixtures with the food to investigate the effect on enzymatic parameters. To evaluate different enzyme levels, insects were sacrificed every 4 to 16 hours. Insects were homogenized in phosphate saline buffer (pH 6.9) in a glass homogenizer, and then centrifuged at 15,000 rpm in the cold for 25 minutes. In a glass tube, the supernatant was separated and used for the estimate.

2.3. Determination of Alkaline Phosphatase (ALP)

Alkaline phosphatase levels were assessed using a modified version of the Andrech and Szeypiaske (1947) method developed by [27]. This was accomplished by homogenising 500 mg of treated termites in 1 ml of ice-cold PBS buffer and centrifuging the mixture for 15 minutes at 15,000 rpm. The amount of alkaline phosphatase was determined by adding 0.10 ml of supernatant to 1.0 ml of alkaline buffer substrate and incubating at 37˚C for 30 minutes. Alkaline buffer substrate was made by mixing 42 ml of 0.1 N NaOH with 375 mg of glycine, 10 mg of $MgCl_2 \cdot 6H_2O$, and 165 mg of the sodium salt of p-nitrophenyl phosphate. By introducing an excessive amount of alkali (5.0 ml of 0.02 N NaOH), the process

was stopped. With NaOH, the p-nitrophenol that resulted from the hydrolysis of p-nitrophenyl phosphate took on a yellow hue. At 420 nm, optical density was observed. P-nitrophenol was used in various amounts to create the standard curve. The amount of p-nitrophenol produced every 30 minutes per mg of protein was used to express enzyme activity. Each experiment had three replicates, and the ANOVA method was used to statistically examine the results.

2.4. Determination of Acid Phosphatise (ACP)

Termite acid phosphatase activity was determined using the method of Andrech and Szeypiaske (1947), modified by [27]. To measure acid phosphatase levels, termite whole-body extracts were prepared in a manner similar to that described above. For this purpose, 0.1 ml of supernatant was mixed with 1.0 ml of acidic buffered substrate solution (0.41 g of citric acid, 1.125 g of sodium citrate and 165 mg of p-nitro phenyl phosphate sodium salt to 100 ml of double-distilled water). The contents were mixed well and incubated at 37˚C for 30 minutes. The reaction was quenched by adding 4.0 mL of 0.10N NaOH to the tube. A yellow colour developed and was measured at 420 nm. A standard curve was generated using various concentrations of p-nitrophenol. Enzyme activity was expressed as the amount of p-nitrophenol formed per mg of protein per 30 minutes. Three replicates were performed for each experiment and the resulting data were statistically analyzed using the ANOVA method.

2.5. Determination of Glutamic-Oxaolacetic Transaminase (GOT)

For this purpose, 500 mg of treated termites were homogenized in 2 ml of ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 minutes. To measure GOT, 0.10 ml of centrifuged supernatant was removed and 0.50 ml of GOT substrate was added. GOT substrate was prepared by adding 0.292 g α-ketoglutarate and 26.6 g DL-aspartate to a 1.0 liter volumetric flask. The contents were mixed well and 1N NaOH was slowly added to the above solution. The pH of the solution was adjusted to 7.4 using PBS buffer. The total volume of the solution was maintained at 1000 ml by adding buffer (13.97 g K_2HPO_4 and 2.69 g KH_2PO_4 in 1000 ml water). 0.50 mL of 2,4-dinitrophenylhydrazine solution (0.198 g of 2,4-dinitrophenylhydrazine dissolved in 1N HCl to make 1000 mL) was added to the tube and allowed to stand at room temperature for 15 minutes. Then 5.0 mL of 0.4 N NaOH (1.6 g of NaOH dissolved in 100 mL of distilled water) was added and mixed well. The contents were then left at room temperature for 20 minutes. Optical densities were recorded at 505 nm by treating blanks with distilled water. A standard curve was generated using oxaloacetate as standard. Enzyme activity was expressed in units of glutamate-oxaloacetate transaminase/30 min/mg protein.

2.6. Determination of Glutamic-Pyruvate Transaminase (GPT)

GPT activity in termite whole body extract was measured using the method of [28]. For this purpose, 500 mg of treated termites were homogenized in 2 ml of

ice-cold PBS buffer and centrifuged at 15,000 rpm for 15 minutes. 0.10 ml of the centrifuged supernatant was added to 0.50 ml of GPT substrate. GPT substrate was prepared by dissolving 0.292 g α-ketoglutarate and 17.8 g D-alanine in a 1.0 liter volumetric flask. 1N NaOH was slowly added to the above mixture. Mix well until all solids are completely dissolved. The substrate pH was adjusted to 7.4 by adding a sufficient buffer to keep the total volume at 1000 ml. A buffer was prepared by dissolving 13.97 g of K_2HPO_4 and 2.69 g of KH_2PO_4 in 1000 ml of distilled water. nitrophenylhydrazine in 1N HCl to make 1000 mL. was dissolved and kept at room temperature for 1 hour. 15 minutes. Then 5.0 ml of 0.4 N NaOH (1.6 g of NaOH dissolved in 100 ml of distilled water) was added mixed well and left at room temperature for 20 minutes. Optical densities were measured at 505 nm and blanks were adjusted to zero background absorbance with water. A standard curve was generated using oxaloacetate as standard. Enzyme activity was expressed in units of glutamate-pyruvate transaminase activity/mg protein. Three replicates were established for each test and control and the resulting data were statistically analyzed using the ANOVA method.

2.7. Determination of Acetylcholinesterase (AchE)

Acetylcholinesterase activity was measured according to the method of [29]. For this purpose, 500 mg of treated termites were homogenized in ice-cold PBS buffer for 5 min with a glass-to-glass homogenizer. It was refrigerated and centrifuged at 15,000 rpm to obtain the supernatant. To estimate the level of AchE, 0.050 mL of supernatant was added to 0.10 mL of freshly prepared acetylcholine thioiodide solution (5×10^{-4} M) (10 mm light path cuvette) and 0.05 mL chromogen DTNB (0.19818 g/L) mixed to 1.45 ml of PBS (pH 6.9). Changes in absorbance were recorded periodically at 412 nm for 3 min at 250˚C. Enzyme activity was expressed in μ moles of "SH" hydrolyzed and mg of protein hydrolyzed per minute.

2.8. Statistical Analysis

Worker termite LD_{50} were determined for each extract and combination mixture using probit analysis. Mean, standard deviation, standard error, correlation and Student's t-test were applied by the ANOVA program. A chi-square test was used to determine the repellent effect [30].

3. Results

In this investigation, toxic effects of *Tagetes erecta* essential oil and its combinatorial mixtures 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 determined in whole body extracts of termites treated with 40% and 80% of LD_{50} of *Tagetes erecta* essential oil and its combinatorial mixtures separately for 4, 8, 12 and 16 h (**Figures 1-19**).

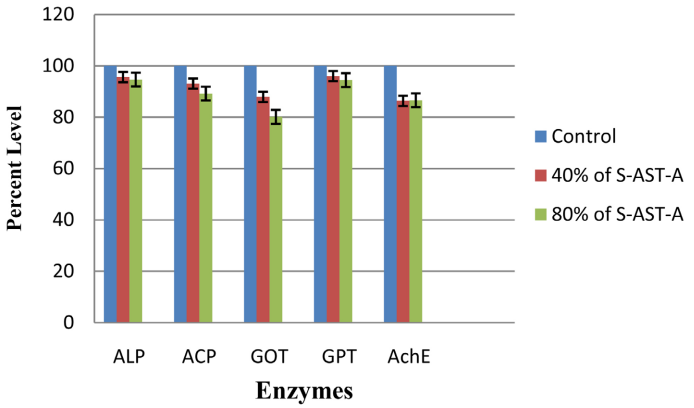

Figure 1. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-AST-A mixture at 16 h.

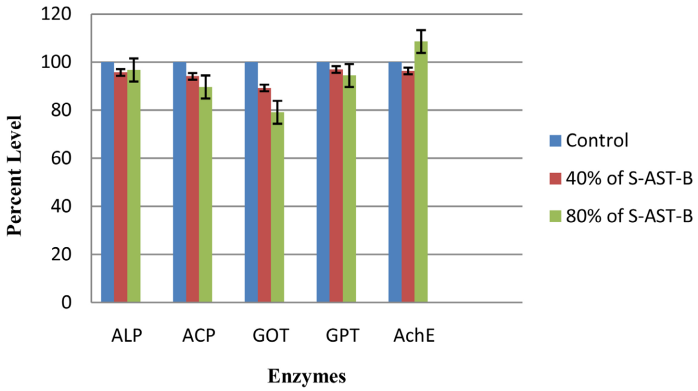

Figure 2. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-AST-B mixture at 16 h.

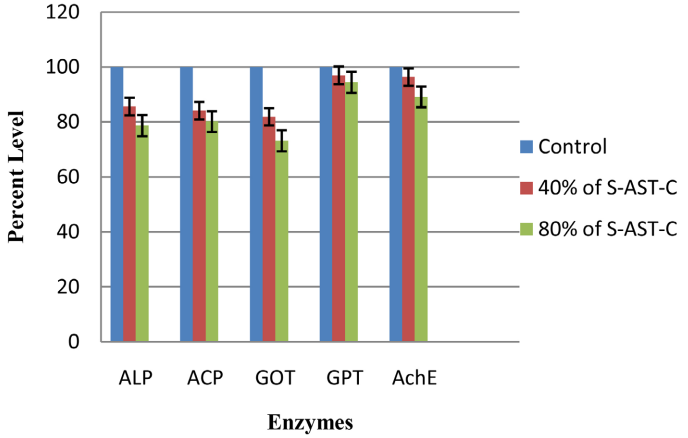

Figure 3. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of S-AST-C mixture at 16 h.

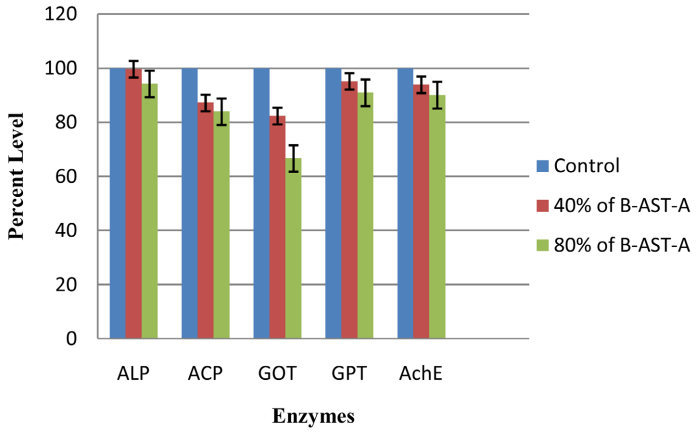

Figure 4. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-AST-A mixture at 16 h.

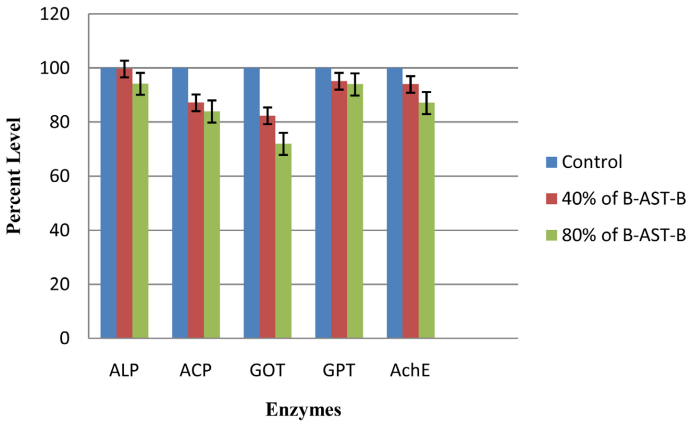

Figure 5. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-AST-B mixture at 16 h.

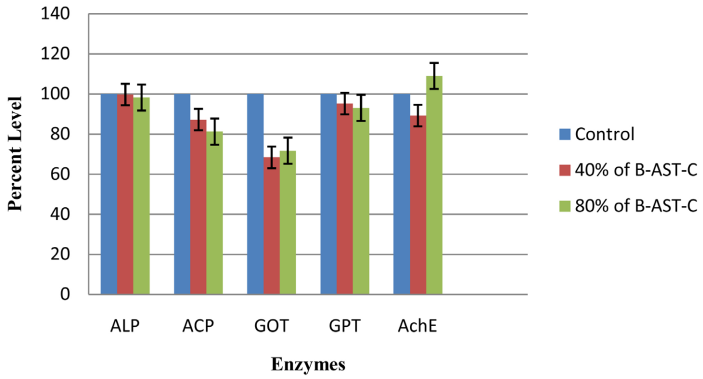

Figure 6. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of B-AST-C mixture at 16 h.

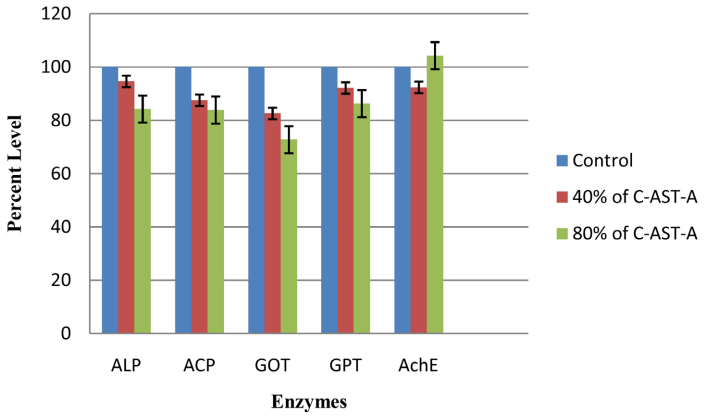

Figure 7. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-AST-A mixture at 16 h.

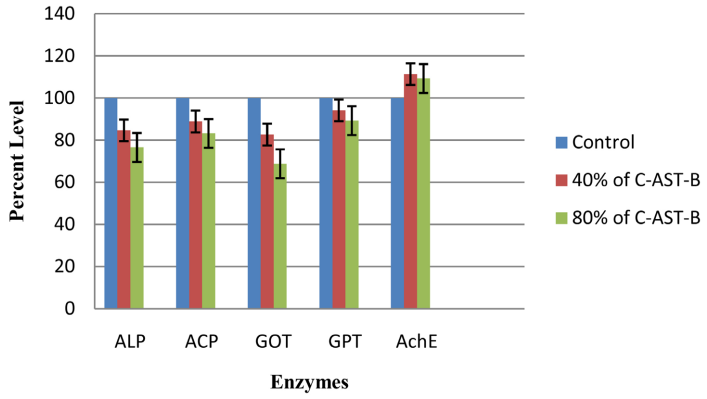

Figure 8. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-AST-B mixture at 16 h.

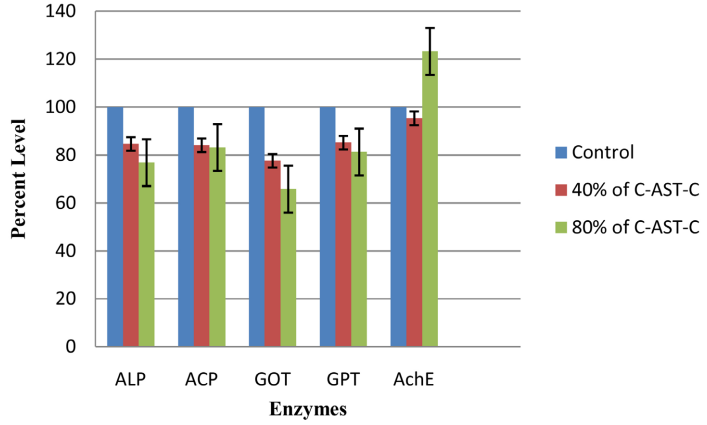

Figure 9. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of C-AST-C mixture at 16 h.

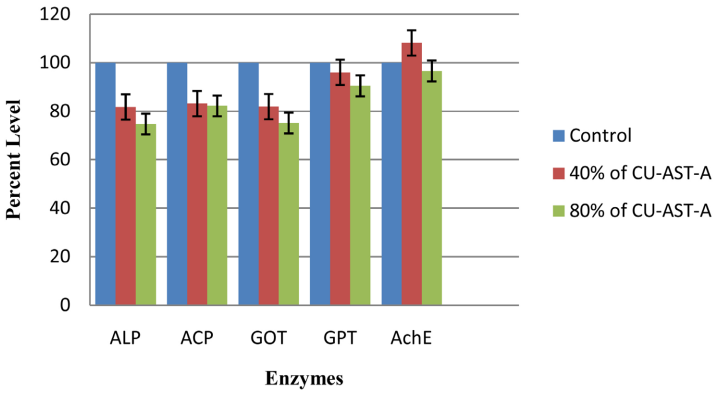

Figure 10. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-AST-A mixture at 16 h.

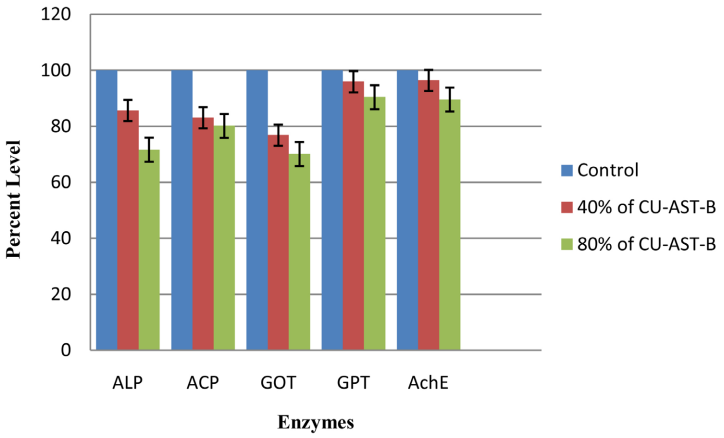

Figure 11. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-AST-B mixture at 16 h.

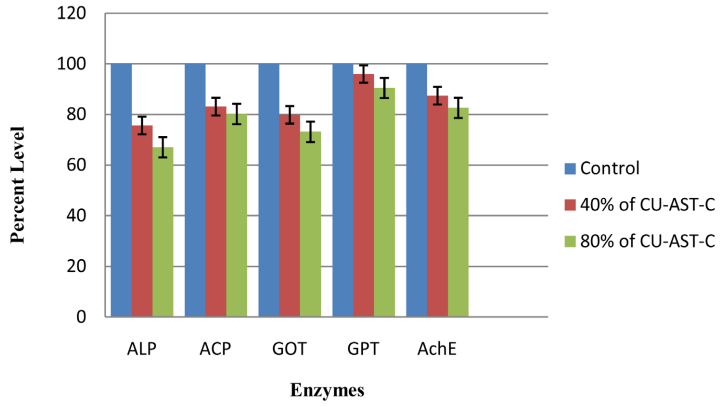

Figure 12. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of CU-AST-C mixture at 16 h.

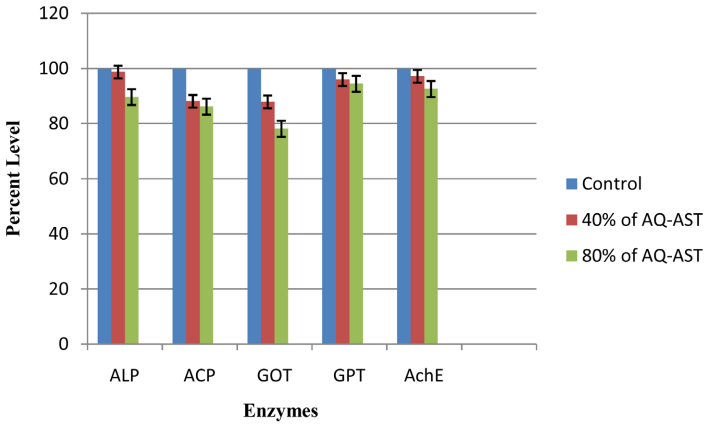

Figure 13. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of AQ-AST mixture at 16 h.

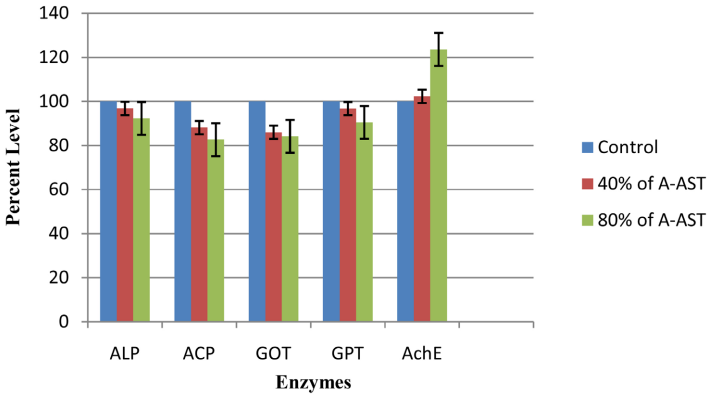

Figure 14. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of A-AST mixture at 16 h.

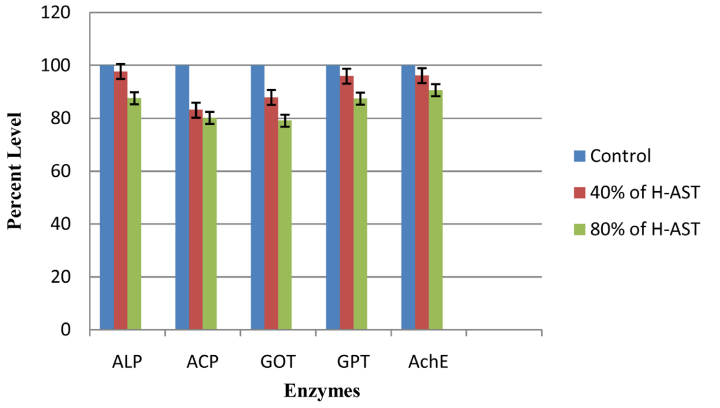

Figure 15. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of H-AST mixture at 16 h.

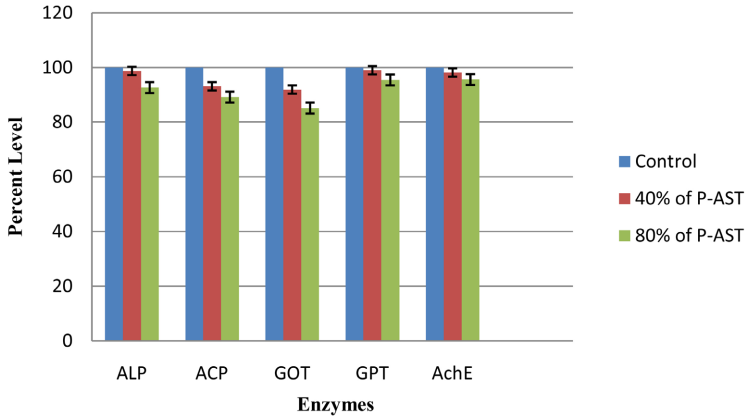

Figure 16. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of P-AST mixture at 16 h.

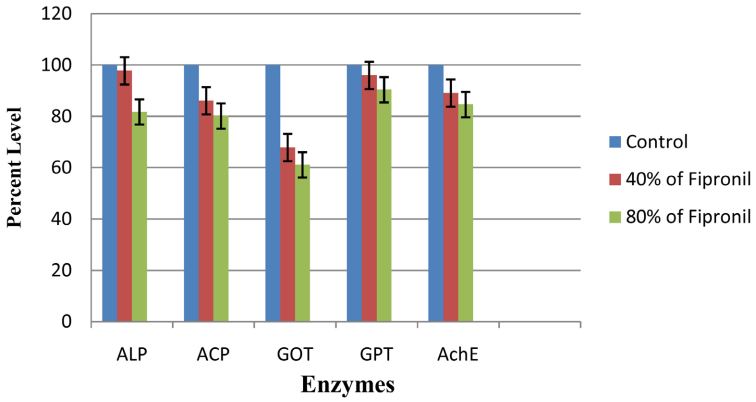

Figure 17. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of Fipronil at 16 h.

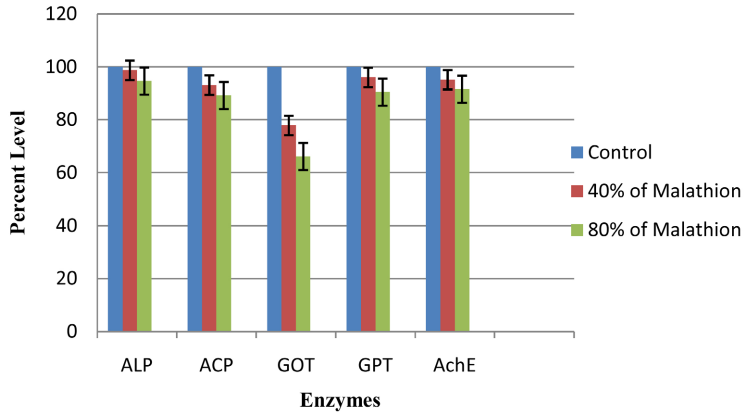

Figure 18. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of Malathion at 16 h.

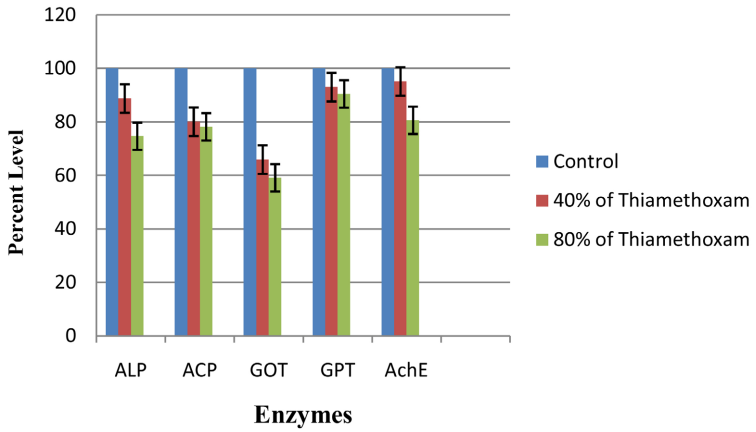

Figure 19. Comparison of alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and acetylcholinesterase in termites treated with 40% and 80% of LD_{50} of Thiamethoxam at 16 h.

40% and 80% of LD_{50} of Combinatorial mixture S-AST-A, S-AST-B and S-AST-C caused significant ($p > 0.05$) decrease in glutamate oxaloacetate transaminase *i.e.* 87.92%, 80.17% and 89.30% & 79.17%, 81.92% and 73.17% respectively at 16 h treatment (Figures 1-3). Similarly, 40% and 80% of LD_{50} of combinatorial mixture B-AST-A, B-AST-B and B-AST-C caused a significant decrease in acid phosphatase level at 16 h treatment in comparison to control, the level recorded 93.15%, 89.20% and 94.15% & 89.20%, 84.15% and 80.02% respectively (Figures 4-6).

When termites were treated with 80% of LD_{50} of C-AST-A, C-AST-B and C-AST-C, combinatorial mixtures, these caused a maximum significant ($p > 0.05$) increase in acetylcholinesterase level at 16 h of treatment *i.e.* 103.02%, 109.34% and 113.60% in comparison to control respectively (Figures 7-9), but in CU-AST-A, CU-AST-B and CU-AST-C level was found to be significantly ($p > 0.05$) decreased in comparison to control *i.e.* 82.21%, 80.20% and 84.20% respectively (Figures 10-12). Similarly, acetylcholinesterase level was found significantly ($p > 0.05$) decreased at 16 h of treatment in 80% of AQ-AST, H-AST and P-AST which were 92.62%, 90.69% and 95.62% respectively and slightly increased in A-AST *i.e.* 123.62% in comparison to control (Figures 13-16). Alkaline phosphatase levels in 80% of synthetic pesticides fipronil, malathion and thiamethoxam were 81.80%, 94.69% and 74.69% respectively after 16 h treatment (Figures 17-19). Glutamate pyruvate transaminase enzyme level was slightly decreased in all tested combinatorial mixtures as well as synthetic pesticides (Figures 1-19).

4. Discussion

For investigation of the anti-termitic activity of *Tagetes erecta* and its various combinatorial mixtures, the level of various enzymes such as alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate

transaminase and acetylcholinesterase were determined in termites. In this study levels of various enzymes were found to be altered that clearly indicate an obstruction in their chemical pathways. These obstructions in their chemical pathways led to the formation of abnormalities in the insect's metabolism and make insects unable to survive. For this study termites were treated with 40% and 80% of LD_{50} of *Tagetes erecta* and its various combinatorial mixtures and also with some synthetic pesticides (**Table 1** & **Table 2**; **Figures 1-19**). Effects on enzyme level alteration were measured in whole-body extracts of termites. The combinatorial mixtures S-AST-A, S-AST-B, S-AST-C, B-AST-A, B-AST-B and B-AST-C have shown significant reduction in alkaline phosphatise and acid phosphatase levels, in a range between 94.69% to 89.20% and 96.80% to 89.70% respectively after 16 h treatment (**Figures 1-6**). While an elevation was measured in acetylcholinesterase levels after 16 h treatment of 80% of C-AST-A, C-AST-B and C-AST-C *i.e.* 104.30%, 109.30% and 123.30% respectively (**Figures 7-9**). More specifically, in another experiment a similar dose of 80% of CU-AST-A, CU-AST-B and CU-AST-C mixture caused slightly decrease in acetylcholinesterase levels after 16 h treatment in comparison to control termites *i.e.* 96.62%, 89.30% and 82.62% respectively (**Figures 10-12**). Alkaline phosphatase is an important membrane-bound enzyme found in all body tissues. This is a lysosomal enzyme that may have a role in autophagy. It also plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis [31]. It mediates the transport of metabolites across the membrane and plays an important role in protein synthesis [32]. Its level may be increased due to the intoxication of body tissues and lysosomal disintegration that leads to the liberation of enzymes from the membrane from where it reaches into circulation [33]. Another reason for the increase in the level of hemolymph acid phosphatase is toxicant-induced hypoxia [34]. Its inhibition may retard the protein synthesis in tissues and release excess free amino acids into the circulation, thereby, increasing amino acid levels in the hemolymph.

In vivo exposure of 40% and 80% of LD_{50} of AQ-AST and A-AST mixtures caused 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 acetylcholinesterase levels after 16 h all tested treatments in comparison to control (**Figure 13** and **Figure 14**). Contrary to this, a similar dose of H-AST and P-AST caused a significant reduction in all the test enzymes i.e. alkaline phosphatase, acid phosphatase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase including acetylcholinesterase levels in comparison to control at 16 h treatment (**Figure 15** and **Figure 16**).

Alanine aminotransferase (ALT), also known as glutamic–pyruvic transaminase (GPT), is a cytosolic enzyme involved in gluconeogenesis that catalyzes the amination of α-ketoglutarate from alanine to produce pyruvate and glutamate [35]. Both enzymes glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT) are found in the liver, heart cells, muscle tissue, pancreas and kidney. Alteration in levels of these enzymes displays damage

Table 1. *Tagetes erecta* extract 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.

to these tissues [36]. Glutamic oxaloacetic transaminase (GOT) and glutamine pyruvic transaminase (GPT) are also known as aspartic transeferase (AST) and alanine transaminase (ALT) respectively. The transaminases are key enzymes in the formation of non-essential amino acids, gluconeogenesis and metabolism of the nitrogen compound, and are associated with protein metabolism [37].

Table 2. Different combinatorial mixture data analysis of LD_{50} values.

S.N.	Name of Extract/ Combinatorial Mixture	LD_{50} µg/gm	0.95 Confidence Limit UCL-LCL	Chi-Square	Slope Function	Degree of Freedom	heterogeneity	LD_{40} µg/gm	LD_{20} 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	−0.684713	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	-	-
17	Malathion*	67.02	95.511 - 52.907	2.083	−0.875488	4	0.519	26.87	13.34
18	Fipronil*	50.18	57.861 - 18.107	11.829	−0.715311	4	2.9567	11.19	5.54
19	Thiamethoxam*	26.89	63.309 - 41.843	2.824	−0.872117	4	0.709	20.15	10.09

*Synthetic pesticides.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are hydrolytic enzymes, which hydrolyze phosphomonoesters under acid or alkaline conditions, respectively. ACP is a lysosomal enzyme [38]. That plays an important role in catabolism, pathological necrosis, autolysis and phagocytosis [39]. Alkaline phosphatase is an important membrane-bound enzyme found in all body tissues. It mediates the transport of metabolites across the membrane and plays an important role in protein synthesis [40]. Its level may be increased due to the intoxication of body tissues and lysosomal disintegration that leads to the liberation of the enzyme from the membrane and it's into the circulation from muscle [41]. Another reason for the increase in the level of serum acid phosphatase is toxicant-induced hypoxia [42]. Its inhibition may retard the protein synthesis in tissues and release excess free amino acids into the circulation, thereby, increasing amino acid levels in the serum. Level of acid phosphatase (ACP) activity get increased in the haemolymph of the desert locust, *Schistocerca gregaria* 3rd day

after inoculation with the entomopathogenic fungus *M. anisopliae var acridum* [43]. ACP increasing also indicates breakdown of haemocytes and a reduction in the proportion of plasmatocytes and coagulocytes [43]. Similarly, phosphatase and transaminase activity are also disturbed due to exposure to certain insecticides in the grubs of the red palm weevil *Rhynchophorus ferrugineus* [44]. But see no effect in lufenuron-treated *Helicoverpa armigera* [45]. This elevated level of detoxification enzymes can possibly lead to an increase in resistance development against synthetic chemical insecticides [45]. And show the decomposition of tissues and organs subjected to cytolysis [46].

Few natural insecticides such as pyrethroids stop phosphatase (Ishaaya, I. and Casida, 1980) [47]. And elevated levels of lactic dehydrogenase also create oxygen deficient stage and cause tissue necrosis. Rising levels of alkaline phosphatase also display extremely high lysosomal activity in cells, which puts insects under metabolic stress) [48]. Cry toxins in lepidopteran pests cause reductions in ALP activity [49]. Toxic substances also affect ALP in *Leptinotarsa decemlineata* [50] and *Culex tarsalis* Houk [51]. Alkaline phosphatase activity also gets increased in *Lamellidens Marginalis* in response to heavy metal toxicity [52]. Heavy metal toxicity also imposes adverse health effects and its exposure causes hepatic and hematological toxicity [52]. Similarly, microbial toxins also damage cells of the gastro-intestinal tract, impairment of kidney function, as well as neurotoxicity and hepatocellular toxicity. This damage in tissues results in an increase in ALT, AST, GGT and ALP levels. ALT and AST levels are often more than 100 times the normal level [53]. Similarly industry workers after 10 years of exposure to plastic showed significantly increased ($p < 0.0001$) levels of ALP, ALT, AST and GPT [54]. These enzymes are also found in the blood, plasma and intestine of human beings. Their elevated levels indicate metabolic disorder [55].

Aqueous extracts of *Gloriosa superba* [56], *Paronia emodi* [57], *Corydalis incise* [58], *Artemisia annua* [59], *Teucrium royleanum* [60], *Andrache cardifolia* [61], *Angelica archangelica* and *Geranium sylvatica* efficiently altered various enzymes levels such as acetyl cholinesterase, alkaline phosphatise and amino transferase of insects [62]. The 40% of LD_{50} of synthetic pesticides fipronil, malathion and thiamethoxam caused a significant decrease in glutamate oxaloacetate transaminase levels *i.e.* 67.52%, 77.92% and 65.92% respectively at 16 h treatment, while 80% of LD_{50} of fipronil, malathion and thiamethoxam caused a decrease in all the enzymatic parameters at 16 h treatment in comparison to control (**Figures 17-19**). Similarly, certain alkaloids found in *Amaryllidaceae* family plants inhibit acetylcholinesterase levels in various insect pests [63]. A large number of alkaloids are found abundantly in plants [64].

Above enzyme activity of *Tagetes erecta* essential oil and its combinatorial mixtures were evaluated with comparison to a few inorganic insecticides like malathion, fipronil and thiamethoxam. It was found that *Tagetes erecta* essential oil and its combinatorial mixtures have shown more significant termiticidal effi-

cacy than synthetic pesticides. The oil extricated from marigold (*T. erecta*) is a great repellent against *O. obesus* [65]. A. Phenolic compounds like phosphorus oxychloride showed acetylcholinesterase inhibition at sub-lethal dose in termite *C. formosanus* [66]. Contrary to this malathion also potentially inhibits acetylcholinesterase activity more than malaxon and isomalathion [67]. Organophosphate (OP) pesticides are known as nerve agents. These are major neuroinhibitors of acetylcholinesterase (AChE) and decrease its level in animals [68]. These insecticides also cause genotoxicity both *in vivo* and *in vitro* treatments. Similarly, diazinon poisoning also influences acetylcholinesterase and butyrylcholinesterase level in affected animals and man [69].

The liver has a significant role in metabolism, digestion, detoxification, and elimination of substances from the body. It contains acid and alkaline phosphatase [70] [71], Alanine transaminase (ALT) and aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), are biomarkers of hepato-toxicity [72]. In the presence of certain toxicants transamination of amino acids gets increased, and the level of glutamate pyruvate transaminase, glutamate oxalo acetate transaminase also gets altered [72]. 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 different *Tagetes erecta* essential oil and its combinatorial mixtures. However, elevation or reduction in enzyme levels is associated with metabolic alterations in insects. However, *Tagetes erecta* essential oil and its combinatorial mixtures significantly change phosphatases, transaminase and esterase levels, which indicate very high toxic effects on the body tissues of termites.

5. Conclusion

To prove the anti-termitic activity of *Tagetes erecta* essential oil and its combinatorial mixtures, the level of certain enzymes was determined in whole-body extract of termites. In the present investigation levels of various enzymes were found to be altered/generally reduced that indicate an obstruction in their chemical pathways. More specifically, *Tagetes erecta* essential oil and its combinatorial mixtures have shown significant alterations in enzyme activity. This led to the formation of abnormal states in the insects and make insects unable to survive. These essential oil-based combinatorial mixtures could be used as an alternative to synthetic pesticides for termite control in a sustainable and eco-friendly manner.

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Authors' Contributions

Ravi Kant Upadhyay and Susheel Kumar 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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