Assessing the Biochemical Implication of using *Stachytarpheta cayennensis* Essential Oil as an Alternative to Synthetic Pesticides in Calabar Nigeria.

Okonkwo, Chibuzor Onyinye (oko210love@gmail.com)
University of Calabar https://orcid.org/0000-0002-9702-1283
Uyang, Glory Akomaye
University of Calabar
Okonkwo, Sunday Nnamdi
University of Calabar Teaching Hospital

Research Article

Keywords: Biopesticide, Stachytarpheta cayennensis, insecticide, essential oil, botanicals, biochemical, toxicity, environment

Posted Date: November 28th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3664402/v1

Abstract

This effect of essential oil from the leaves of *Stachytarpheta cayennensis* on some biochemical indices of Wistar rats was analyzed. Twenty-eight (28) albino Wistar rats weighing between 150-180g were divided into seven groups of four rats each. Group A served as negative control, B_1, B_2, and B_3 were exposed to 5000mg, 3000mg and 1500mg/kg body weight of a synthetic insecticide (BNC) while S_1, S_2, S_3 were exposed to 5000, 3000 and 1500 mg/kg body weight of *Stachytarpheta cayennensis* essential oil respectively. After twenty-eight days, blood samples were collected by cardiac puncture for biochemical analysis. Liver function markers (Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), were analyzed as well as antioxidant enzymes, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), as well as hydrogen peroxide concentration. Results showed that liver enzymes (AST, ALT and ALP) were higher in the BNC exposed rats compared to the control and essential oil exposed groups ($p < 0.05$). Superoxide dismutase (SOD) and catalase (CAT) activities decreased significantly, ($p < 0.05$) while glutathione peroxidase and hydrogen peroxide concentrations increased in the BNC exposed groups relative to the essential oil exposed and control groups. The synthetic household insecticide BNC clearly induced more toxicity, as seen in the increase in the activities of liver enzymes and decrease in activities of catalase and SOD. The botanical oil may hold interesting promises as a potential pesticidal agent for use by farmers and agriculturists and for the preservation of the environment and ecosystem.

INTRODUCTION

Plant essential oils have a long history of human use. Recent attention on essential oils have focused on their broad range of bioactivities on pests, and disease-causing organisms, thus their potential use as alternatives to synthetic chemical insecticides for crop protection and other pest management practices. It is interesting to note that the molecules within the oils that cause these effects are usually non-toxic for mammals (Baser, 2010). Essential oils also have medicinal applications due to their therapeutic properties, as well as agro-alimentary uses because of their antimicrobial and antioxidant effects. Recently, the value of essential oils in human medicine, as phytopharmaceuticals and in aromatherapy has increased and together with their widespread use in foods and beverages have defined their relative safety through both empirical practice and more rigorous experimental evaluations using animal models (Isman, 2020).

Insecticides are chemicals used to kill insects, and can cause poisoning, if swallowed, inhaled, or absorbed through the skin (Sousa et al., 2020). The properties that make insecticides deadly to insects can sometimes make them poisonous to humans. Most serious insecticide poisonings result from the organophosphate and carbonate types of insecticides. Pyrethrins and pyrethroids which are other commonly used insecticides derived from flowers, are usually not very poisonous to humans. Synthetic insecticides have been associated with short term and long-term effects on human health, including elevated cancer risks and potential disruption of the body’s metabolic functioning as well as the reproductive, immune, endocrine, and nervous systems, and the human cardiovascular health (Nicolopoulou-Stamati et al., 2016).

Human poisoning by pesticides has long been seen as a severe public health problem. As early as 1990, a task force of the World Health Organization (WHO) estimated that about one million unintentional pesticide poisonings occur annually, leading to approximately 20,000 deaths (Dunn et al. 2021). The scientific literature published between 2006 and 2018, supplemented by mortality data from WHO revealed that about 385 million cases of Unintentional Acute Pesticide Poisoning (UAPP) occur annually world-wide including around 11,000 fatalities. Based on a worldwide farming population of approximately 860 million, this means that about 44% of farmers are poisoned by pesticides every year (Dunn et al., 2021). These insecticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin (Shayeghi et al., 2012; USEPA, 2023). Therefore, the introduction of alternative; plant-based insecticides, has become a serious issue of national interest.

Sustainable chemistry advances innovations across all chemistry fields to design new and safe chemicals through efficient production processes, which address the challenges of protecting public health (Hao et al., 2021). Modern agriculture is negatively affected due to the rapid exploitation of natural resources, indiscriminate use of pesticide application, and frequent weather events influenced by climate change. Bio-control action is a significant tool for integrated pest management (IPM), it offers alternative and positive management techniques that are safer for humans and the environment (Rani et al., 2021). Natural pesticide products are available as alternatives to synthetic chemical formulations, but their safety or toxicity to humans is one issue that the current research seeks to investigate. Botanical pesticides are efficacious in managing different crop pests, inexpensive, easily biodegraded, have varied modes of action; their sources are easily available and have low toxicity to non-target organisms (Lengai et al., 2020). Moreover, conventional insecticides are based on a single active ingredient, while the botanicals contain mixtures of chemical compounds that can affect both behavioral and physiological processes. Thus, the chance of pests developing resistance to such botanicals is very low. It seems that seeking bio-insecticides which are efficient, as well as being suitable and adaptive to ecological conditions, is essential in obtaining sufficient insect control (Cheraghi Niroumand et al., 2016). This has led to this research on botanical insecticides with comparatively less toxicity than the synthetic insecticides conventionally used in the management and control of pests.

The species *Starchytarpheta cayennesis* is an erect, branching somewhat angular fibrous sub-shrub that is very resistant to traction. It usually has opposite, ovate leaves with a distinct petiole and serrated and indented edges, a slightly wrinkled appearance, green color terminal inflorescence with linear stalks, sessile flower with a gamosepalous calyx, pilose on the dorsum, a corolla with five petals welded at the base, of a lilac coloring androecium with two fertile stamens and two staminodes (Adeneye et al, 2006). It is distributed throughout Brazil, Africa and Asia. (Adeneye et al, 2006). It belongs to the *verbena* family and known by many common names, including blue snakeweed, rough leaf vervian (English name).In Nigeria, it is known as *Truamure, (Yoruba), Aranumon (Ibibio/Efik), Wulsigi (Hausa), Okeanwundeohia*

(Igbo), Anano (Obudu) (Encyclopedia, Science News &Research Reviews, 2023). Okonkwo and Onyeji (2018) had reported the insecticidal efficacy of this plant against some insect pests, thus the current research seeks to validate its use especially with regards to non-target organisms in the environment where they are applied. The aim of this research work was to determine the safety of using essential oil from the leaves of *Stachytarpheta cayennensis* as a pesticide by analyzing its effect on some toxicity indices of Wistar rats.

MATERIALS AND METHOD

Materials

Clavenger apparatus, Centrifuge (Surgifield Medical,SM80-2,England), weighing balance model (Metter-Teledo PL303), ALT, ALP, AST, Catalase, Creatine, Urea assay kits (Agappe Diagnostics Ltd, India), exposure chambers (Timber market, Calabar Nigeria), Spectrophotometer (Drawell DU-8200,China), BNC insecticide (Yuanlong Commodity Co. Ltd, China), Ketamine hydrochloride injection (Rotex Medicals, Germany),Semi-automatic Chemistry Analyzer (Medsinglong MSLBA50, China), Refrigerator (Haier Thermocool).

Method

Fresh leaves of *Stachytarpheta cayannesis* were harvested from a farmland behind University of Calabar Teaching Hospital, Calabar, Cross River State. The plant was identified by Effa, Effa A. (PhD) of the Department of Botany, University of Calabar, with herbarium number BOT/HERB/UCC/351. Harvested plants were pulverized and subjected to hydro distillation with Clavenger apparatus. One thousand (1000) grams of plant sample (fresh leaves) yielded 7.5% essential oil. After extraction, oil was stored in an amber glass vial at ambient temperature until used. Essential oil yield was estimated by volume and weight using the following equations

$$\text{Essential oil (\% V/W)} = \frac{\text{Volume of oil collected (ml)}}{\text{Weight of the sample (g)}} \times 100 \text{ (Ashokkumar, } et\ al\text{, 2021)}$$

This study was approved by the animal ethics committee of the Faculty of Basic Medical Science, College of Medical Sciences, University of Calabar. A total of thirty-one (31) albino Wistar rats of both sexes weighing between 150 and180g were obtained from the animal house of the Department of Biochemistry, University of Calabar under normal environmental conditions of room temperature and relative humidity. Acute toxicity of essential oil was carried out on 3 animals to determine the dose levels of exposure for the experimental animals using Lorke's method (1983), LD_{50} was observed to be greater than 5000mg/kg, this influenced the choice of treatment doses for the subacute toxicity test. Subsequently, twenty-eight (28) Rats were divided into seven (7) groups of four rats each. The control group (A) was not exposed to any treatment, groups B_1, B_2 and B_3 were exposed to 5000, 3000 and

1500mg/kg body weight of BNC insecticide, while groups C_1, C_2, C_3 were exposed to 5000, 3000 and 1500mg/kg body weight of oil. Animals were exposed to treatments in a closed chamber for 5 hours, once a day for 28 days.

Experimental design

Group	Number of animals	Treatment	Dose/body weight
A	4	Distilled water	5000mg/kg
B_1	4	BNC Insecticide	5000mg/kg
B_2	4	BNC Insecticide	3000mg/kg
B3	4	BNC Insecticide	1500mg/kg
S1	4	*S. cayennesis* oil	5000mg/kg
S2	4	*S. cayennesis* oil	3000mg/kg
S3	4	*S. cayennesis* oil	1500mg/kg

On the 28th day, animals were fasted overnight, but with access to water. The rats were then anaesthetized using ketamine injection and sacrificed. Blood samples were collected via cardiac puncture using sterile syringes and needles. Blood was put into plain bottles and allowed to stand for 2 hours, before it was centrifuged at 3000rpm for 10 minutes to recover the serum from cells. Serum was separated with Pasteur pipettes and stored in a refrigerator until used for biochemical analysis.

Alanine amino transferase (ALT) and Aspartate amino transferase (AST) activities were estimated using Agappe assay kits based on Reitman and Frankel's method (1957). Alkaline Phosphatase (ALP) activity was estimated using the Agappe kit based on the method of Schlebusch et al (1974). Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine by the increase in absorbance at 480nm as described by Sun and Zigma (1978). Catalase activity was determined according to (Sinha,1972). Estimation of glutathione peroxidase (GPx) was done by the method of Ahmed et al (2021).

Statistical analysis

Data obtained was analyzed for statistical significance using the one-way analysis of variance (ANOVA) with a post-hoc Dunnet (to compare intra and inter differences between groups) at p <_0.05 using SPSS statistical package (2020).

Results was expressed as mean ± SEM for n = 4

RESULTS

Comparative effect of esssential oil and BNC Insecticide on some serum liver enzymes (AST, ALT and ALP) are shown in Fig. 1. BNC Insecticide treated groups had significantly higher activities of liver enzymes when compared with the groups treated with the essential oil of S.C leaves. A significant ($p < 0.05$) increase in AST activity was observed in all treated groups, relative to the control. BNC treated groups had significantly higher AST activities relative to the S.C treated groups. There was no significant change ($p > 0.05$) in ALT and ALP activity in S.C treated groups relative to the control. However, group B_1 which was exposed to the highest concentration of the BNC insecticide, had significantly higher ALT and ALP activities relative to the S.C. treated and control groups. Result obtained from the analysis of antioxidant enzymes and oxidative stress makers are presented in Figs. 4 and 5, the result showed a significant ($p < 0.05$) decrease in the activities of superoxide dismutase (SOD), and catalase (CAT) especially in group B_1 relative to the control and S_2 group. Glutathione peroxidase (GPX) increased significantly ($p < 0.05$) in B_1 and B_2 relative to the control and extract treated groups. No significant difference ($p > 0.05$) was observed in the H_20_2 concentration of groups S_2 and S_3 relative to the control. However, there was significant decrease in H_20_2 concentration in all the groups treated with synthetic pesticides relative to the control group.

Discussion

Pesticides are among the most extensively used chemicals in the world today and they are also among the most hazardous compounds to humans. A report from Aljazeera (2017) indicates that over 200,000 people are killed due to the toxicity of these dangerous chemicals every year. Although pesticides are intended to harm only the target pests, if not used correctly, they can also cause harm to people or the environment. Pesticide intoxication may be caused either by swallowing accidently, or by inhalation of fumes or by skin contact or accidental eye exposure (Lee et al., 2011; Mostafalou and Abdollahi, 2012; Makris et al., 2019).This has led to the search for more botanical insecticides with comparatively less toxicity than the synthetic insecticides conventionally used in the management and control of pests (Bernays and Chapman, 2012).

Herbal medicines have been used for thousands of years as sources of bioactive and therapeutic substances with industrial and agricultural purposes (Dayan et al. 2009). Medicinal plants, and their derivatives or extracts, have also been evaluated for different pest control strategies. Following inhalation, toxicants are transported by the blood to various organs including the liver and kidney where they may eventually cause harmful effects. Studies have shown that excessive free radical production resulting in oxidative stress could be an important mechanism of insecticide toxicity (Sule et al., 2022). Oxidative stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidant defenses (Alía et al. 2003). Antioxidants are compounds that delay or inhibit oxidative damage to target molecules. The activities of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were found to be decreased significantly accompanied by an increase in the activity of GPx and H_2O_2 concentration, following exposure to the highest dose of BNC insecticide in this study. The decrease in these parameters following exposure to insecticide has also been reported by Sharma and Singh, (2012); Ojo et al. (2014). However, in this study, these alterations were observed to be minimal in the groups exposed to *S. cayennensis* essential oil. SOD is the primary key factor in the defense mechanism of the antioxidant system against oxidative stress by catalyzing the dismutation of two superoxide radicals (O_2-) into molecular oxygen (O_2) and H_2O_2. Hydrogen peroxide is neutralized by the combined action of catalase and glutathione peroxidase in all vertebrates (Mates and Sayed, 2007). These enzymes act in coordination and the cells may be pushed to oxidative stress state if any change occurs in the activity of these enzymes. In the present study, a significant decrease in the specific activity of SOD and CAT observed in BNC Insecticide treated group after exposure for 28 days, suggests an increased superoxide radical production and other reactive oxygen species (ROS) thereby inducing oxidative damage (Repetto et al., 2010). The antioxidant enzyme CAT protects SOD against inactivation by hydrogen peroxide. Reciprocally, the SOD protects the CAT against inhibition by superoxide anion that could be formed during the exposure to the insecticide. Similarly, glutathione provides protection to the tissues by catalyzing conjugation reactions that occur with xenobiotics (Labarrere and Kassab, 2022). There are many cellular and molecular targets of ROS, and as such, measurements of oxidative stress and damage in humans are quite complex. Regarding oxidative stress biomarkers. Sapbamrer et al. (2019) found no difference in 8-Oxo-dG among pesticide applicators pre- and post- pesticide application seasons. In contrast, both Kahl et al. (2018) and Lozano-Panigua *et al*. (2018) found that agricultural workers occupationally exposed to pesticides had significantly increased levels of oxidative stress biomarkers, including increased thiobarbituric acid reactive substances (TBARS) and total equivalent antioxidant capacity (TEAC), ferric reducing ability of serum (FRAS) and total thiol groups (SHT). These inconsistent results make it difficult to draw conclusions about the relationship between agricultural work and biomarkers of oxidative stress. Regarding antioxidant enzyme activity, Cattelan et al. (2018) observed that farmers who used pesticides had significantly reduced levels of SOD, GPx, and glutathione reductase (GSH) compared to farmers who did not use pesticides, this agrees reasonably with our findings with SOD, and may represent a reduced antioxidant defense system in response to an increase in ROS. Other recent studies suggest that occupational pesticide exposure is associated with elevations, rather than reductions, in antioxidant enzyme activity. Sapbamrer and colleagues (2019) found significantly increased SOD activity post-pesticide application relative to pre-pesticide application season. In addition, Lozano-Paniagua et al. (2018) found an elevated but non-significant increase in paraoxonase-1(PON1) activity in greenhouse workers compared to controls. This increase in antioxidant enzymatic activity may represent an adaptive response to an increase in the generation of free radicals.

The significant (p 0.05) decrease in the activities of SOD and catalase especially in the groups exposed to the synthetic insecticide as observed in this study, is indicative of the fact that BNC insecticide may have generated free radicals that reacted with membrane lipids and induced oxidative break down of membrane structure thus decreasing the enzymatic and non-enzymatic antioxidants including H_2O_2 concentration. Conversely, the exposure of Wistar rats to *S. cayennensis* essential oil did not cause any significant changes in the activities of SOD, CAT and GPx. On this basis, it is obvious that *S. cayennensis* essential oil may be far safer on animals than its synthetic counterpart. It doesn't seem to elicit oxidative stress in animals and may even help to scavenge reactive oxygen species (ROS). This further indicates that *S. cayennensis* essential oil could serve as a safer, environmental-friendly and affordable alternative to synthetic pesticides (Okonkwo and Onyeji, 2018).

The liver is the organ most commonly involved in the metabolism of endogenous and foreign compounds. Blood is transported to the liver through the portal vein which carries blood containing digested nutrients from the gastrointestinal tract, and the hepatic artery which carries oxygenated blood from the lungs (Kanu et al. 2016). Liver enzymes AST and ALT are frequently used as biomarkers of liver injury because they are released by hepatocytes into the extracellular space in higher concentrations when there is a threat or injury to the liver (Ozer et al. 2008).

Results from this study indicate that exposure to BNC Insecticide significantly elevated the activities of liver enzyme markers namely AST, ALT and ALP in rats. AST and ALT are very sensitive markers that indicate either acute and/or chronic hepatocellular injury (Barth et al., 2005). Hence elevated activities of these enzymes in the plasma are a reflection of the condition of hepatic function (Konan et al. 2007). Thus, the elevated concentration of serum ALT is indicative of likely hepatotoxicity. In the same way, Moriles and Azer (2022) observed that the elevation of ALT enzyme in the plasma is not unconnected to the fact that the enzyme may have leaked into the blood stream from the liver cytosol which is a reflection of liver dysfunction. On the other hand, according to Lowe et al. (2023), ALP is employed many a times for the assessment of the integrity of the plasma membrane, so on such premise, elevation of serum ALP activity in this study following exposure to these pesticides by inhalation may be associated with liver plasma membrane distortion. However, these observable alterations in liver function parameters were insignificant ($p > 0.05$) for serum ALT and ALP activities in the groups exposed to the different doses of *S. cayennensis* essential oil, and significant ($p > 0.05$) only in serum AST levels of rats exposed to the highest dose of the essential oil indicative of mild hepatic impairment at very high concentration. However, the synthetic pesticide caused significant ($p > 0.05$) increase in the activities of liver enzymes at all dose levels tested.

Conclusion

The results obtained from this study suggest that the oil may possess mild effects on metabolic and oxidative stress markers of rats at very high concentrations. Exposure to synthetic pesticides by inhalation as observed in this study caused adverse effects on biochemical indices and subsequent impairment to various organs especially the liver as evidenced by the increased activities of liver enzymes in groups B_1, B_2 and B_3. The generation of reactive oxygen species and the corresponding decrease in antioxidant enzymes (SOD and catalase) may be indicative of oxidative stress induced by the synthetic pesticide BNC, while biopesticides which are natural occurring compounds present an-eco-friendly, affordable, less toxic alternative that may not be pernicious to non-target organisms but still efficacious for pest management.

Therefore, the use of bio-pesticides should be encouraged and perhaps use of safety wears to minimize exposure, if the outcome of this research can be extrapolated to man. Most essential oil are relatively non-toxic to mammals and fish in toxicological tests, and meet the criteria for “reduced risk” pesticides. Some of these oils and their constituent chemicals are widely used as flavoring agents in foods and beverages and are even exempt from pesticide registration. This special regulatory status combined with the wide availability of essential oils from the flavor and fragrance industries, has made it possible to fast track commercialization of essential oil-based pesticides. Though well received by consumers for use against home and garden pests, these “green pesticides” can also prove effective in agricultural situations, particularly for organic food production. Further, while resistance development continues to be an issue for many synthetic pesticides, it is likely that resistance will develop more slowly to essential-oil-based pesticides owing to the complex mixtures of constituents that many of these oils contain. Ultimately, it is in developing countries which are rich in endemic plant biodiversity that these pesticides may ultimately have their greatest impact in the near future. Integrated pest management (IPM) programs which combine natural and synthetic pesticides for the protection of crops is an option which has come to stay and have helped to reduce the application and circulation of synthetic pesticides in the environment. Further research study is needed to determine the safety and efficacy of the oil in humans.

Declarations

The authors declare that no funds, grants or other support were received during the preparation of this manuscript

Funds

The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Uyang, G.A and Okonkwo, C.O. The first draft of the manuscript was written by Uyang, G.A and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Adeneye**,** A., Benebo, A., & Agbaje, E (2006).Protective Effect of the Aqueous Leaf and Seed Extract of Phyllanthus amarus on Alcohol-Induced Hepatotoxicity in Rats**.** West African Journal of Pharmacology and Drug Research 22 & 23(1):42-50. DOI:10.4314/wajpdr.v22i1.14767.
2. Ahmed, A.Y., Aowda, S.A. & Hadwan, M.H (2021). A validated method to assess glutathione peroxidase enzyme activity. *Chem. Pap.***75**, 6625–6637. https://doi.org/10.1007/s11696-021-01826-1

3. Alía, M., Horcajo, C., Bravo, L., & Goya, L. (2003). Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats. Nutrition Research, 23(9), 1251-1267.
4. Aljazeera (2017). UN: 200,000 die each year from pesticide poisoning https://www.aljazeera.com /economy/2017/3/8.UN: 200,000 die each year from pesticide poisoning | Health | Al Jazeera. Accessed November 2023.
5. Ashokkumar K, Vellaikumar S, Murugan M, Dhanya M.K, Ariharasutharsan G, Aiswarya S, Akilan M, Warkentin T.D and Karthikeyan A (2021). Essential Oil Profile Diversity in Cardamorn Accessions from Southern India.Fron.Sustain.Food Syst. 5:639619. Doi;10.3389/fsufs.2021.639619.
6. Barth, C. Bosses A. Andus T. (2005). Severe acute cholestatic hepatitis by infiltration of monoclonal plasma cells multiple myeloma. 43:1129-1132.
7. Baser, K.H. C and Buchbaker (2010). Handbook of essential oils science Technology and application CRC press
8. Bernays, E. A., & Chapman, R. F. (2007). *Host-plant selection by phytophagous insects* (Vol. 2). Springer Science & Business Media.
9. Cattelan MDP, Maurer P, Garcia F, Berro LF, Machado MM, Manfredini V et al (2018). Occupational exposure to pesticides in family agriculture and the oxidative, biochemical and hematological profile in this agricultural model. *Life sciences*; 203:177–83. doi: 10.1016/j.lfs.2018.04.038.
10. Cheraghi Niroumand M, Farzaei MH, Karimpour Razkenari E, Amin G, Khanavi M, Akbarzadeh T, Shams-Ardekani MR (2016). An Evidence-Based Review on Medicinal Plants Used as Insecticide and Insect Repellent in Traditional Iranian Medicine. Iran Red Crescent Med J. Feb 13; 18(2):e22361. doi: 10.5812/ircmj.22361.
11. Dayan FE, Cantrell CL, Duke SO (2009). Natural products in crop protection. Bioorg Med Chem.;17(12):4022–34.
12. Dunn SE, Reed JE, Neumann C (2021). BMC Public Health. Oct 27;21(1):1944. doi: 10.1186/s12889-021-11940-0.
13. Encyclopedia, Science News & Research Reviews (2023). https://academic-accelerator.com/ encyclopedia/*Stachytarpheta cayennensis*. Accessed November 2023.
14. Hao R, Roy K, Pan J, Ramin S, Mraz J (2021). Critical review on the use of essential oils against spoilage in chilled stored fish: A quantitative meta-analyses, Trends in Food Science & Technology, volume 111 pg. 175-190.(https://www.sciencedirect.com/science/article/pii/S092422442100162X)
15. Isman, M. (2020). Bioinsecticides based on plant essential oils: a short overview. *Zeitschrift für Naturforschung C*, *75*(7-8), 179-182. https://doi.org/10.1515/znc-2020-0038.
16. Labarrere CA, Kassab GS (2022). Glutathione: A Samsonian life-sustaining small molecule that protects against oxidative stress, ageing and damaging inflammation. Front Nutr. 2022 Nov 1;9:1007816. doi: 10.3389/fnut.1007816. PMID: 36386929.
17. Lee, S. J., Mehler, L., Beckman, J., Diebolt-Brown, B., Prado, J., Lackovic, M.,& Calvert, G. M. (2011). Acute pesticide illnesses associated with off-target pesticide drift from agricultural applications.

18. Lengai, G.M.W, Muthomi J.W, Mbega E.R (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific AfricanVolume 7, March 2020, e00239.
19. Lorke D. (1983). A new approach to practical acute toxicity testing. 54; 275-28
20. Lowe D, Sanvictores T, Zubair M, and John S (2023). Alkaline Phosphatase. [Updated 2023 Oct 29]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459201/. Accessed November 2023.
21. Lozano-Paniagua D, Parron T, Alarcon R, Requena M, Gil F, Lopez-Guarnido O et al (2018). Biomarkers of oxidative stress in blood of workers exposed to non-cholinesterase inhibiting pesticides. *Ecotoxicol Environ Saf*, 162:121–8. doi: 10.1016/j.ecoenv.2018.06.074.
22. Kahl VFS, da Silva FR, Alves JDS, da Silva GF, Picinini J, Dhillon VS et al (2018). Role of PON1, SOD2, OGG1, XRCC1, and XRCC4 polymorphisms on modulation of DNA damage in workers occupationally exposed to pesticides. *Ecotoxicol Environ Saf*, 159:164–71. doi: 10.1016/j.ecoenv.04.052
23. Kanu, K. C., Ijioma, S. N., &Atiata, O. (2016). Hematological, biochemical and antioxidant changes in Wistar rats exposed to dichlorvos based insecticide formulation used in Southeast Nigeria. Toxics, 4(4), 28.
24. Konan, N. A., Bacchi, E. M., Lincopan, N., Varela, S. D., and Varanda, E. A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardium occidentale* L.). Journal of ethnopharmacology, 110(1): 30-38
25. Makris, K. C., Konstantinou, C., Andrianou, X. D., Charisiadis, P., Kyriacou, A., Gribble, M. O., &Christophi, C. A. (2019). A cluster-randomized crossover trial of organic diet impact on biomarkers of exposure to pesticides and biomarkers of oxidative stress/inflammation in primary school children. *PLoS One*, *14*(9), e0219420.
26. Mates A. K, Sayed A. K, (2007). Products of the Escherichia coli acid fitness island attenuate metabolite stress at extremely low pH and mediate a cell density dependent acid resistance. 189, 2759-2768.
27. Moriles KE, Azer SA (2022). Alanine Amino Transferase. [Updated 2022 Dec 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559278/. Accessed November 2023.
28. Mostafalou, S., &Abdollahi, M. (2012). Concerns of environmental persistence of pesticides and human chronic diseases. *Clinical and Experimental Pharmacology and Physiology*, *2*, 3.
29. Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P and Hens L (2016) Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front. Public Health 4:148. doi: 10.3389/fpubh.2016.00148
30. Ojo, A. O., Oyinloye, B. E., Ajiboye, B. O., Ojo, A. B., Akintayo, C. O., &Okezie, B. (2014). Dichlorvos induced nephrotoxicity in rat kidney: protective effects of *Alstonia boonei* stem bark extract. Indian J Pharmacol, 1, 429-437

31. Okonkwo, C., &Onyeji, C. (2018). Insecticidal potentials and chemical composition of essential oils from the leaves of *Phyllanthus amarus* and *Stachytarpheta cayennensis* in Nigeria. International Journal of Biochemistry Research & Review, 22(3), 1-16.
32. Ozer, J., Ratner, M., Shaw, M., Bailey, W., &Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. Toxicology, 245(3), 194-205.
33. Rani A T, Kammar V, Keerthi MC, Rani V, Majumder S, Pandey K K, Singh J (2021). Biopesticides: An Alternative to Synthetic Insecticides. In: Bhatt P, Gangola S Udayanga D, Kumar G(eds) Microbial Technology for Sustainable Environment. Springer, Singapore. Pg 439-466. https://doi.org/10.1007/978-981-16-3840-4-23
34. Reitman S. and Frankel S.(1957). A colorimetric method determination of serum GOT (Glutamic Pyruvic Transaminase) activity. American Journal of Clinical Pathology. 28, 56-63.
35. Repetto MG, Ossani G, Monserrat AJ, Boveris A (2010). Oxidative damage: the biochemical mechanism of cellular injury and necrosis in choline deficiency. Exp Mol Pathol ;88(1):143-9. doi: 10.1016/j.yexmp.2009.11.002. Epub 2009 Nov 12.
36. Sapbamrer R, Khacha-Ananda S, Sittitoon N, Wunnapuk K, Seesen M, Sidthilaw S et al (2019). A longitudinal follow-up study of oxidative stress and DNA damage among farmers exposed to pesticide mixtures. *Environmental science and pollution research international* ;26(13):13185–94. doi: 10.1007/s11356-019-04650-z.
37. Schlebusch H, Rick W, Lang H, Knedel M (1974). Standards in the activities of clinically important enzymes. Dtsch Med Wochenschr.12; 99(15);765-766. Doi:10.1055/s-0028-1107840.
38. Sharma, P., & Singh, R. (2012). Dichlorvos and lindane induced oxidative stress in rat brain: Protective effects of ginger. Pharmacognosy research, 4(1), 27.
39. Shayeghi M, Khoobdel M, Bagheri F, Abtahi M, Zeraati H (2012). Organophosphorous residue in *Liza aurata* and *Cyprinus carpio*. Asian Pac J Trop Biomed. Jul;2(7):564-9. doi: 10.1016/S2221-1691(12)60098-7. .
40. Sinha, A.K. (1972) Colorimetric Assay of Catalase. Analytical Biochemistry, 47, 389-394. http://dx.doi.org/10.1016/0003-2697(72)90132-7.
41. Sousa, S *et al* (2020). Chemistry and Toxicology Behind Insecticides and Herbicides. In: K.R., R., Thomas, S., Volova, T., K., J.(eds) Controlled Release of Pesticides for Sustainable Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-23396-9-3
42. Sule R.O, Condon L, Gomes A.V (2022). A common Feature of Pesticides: Oxidative Stress-The Role of Oxidative Stress in Pesticide-Induced Toxicity “Oxidative Medicine and Cellular Longevity, vol.2022, https://doi.org/10.1155/2022/5563759
43. Sun M, Zigma S.(1978). An improved spectrophotometer Assay of Superoxide Dismutase Based on Epinephine Antioxidation Analytical Biochemistry. 90, 81-89.
44. **United States Environmental Protection Agency (2023). Exposure Assessment Tools by Chemical Classes – Pesticides.**https://www.epa.gov/expobox/exposure-assessment-tools-chemical-classes-pesticides. Accesses November 2023.

Tables

Table 1: Comparison of concentration of serum liver enzymes in the different experimental groups

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	115.6 ± 2.88	66.99 ± 4.71	38.96 ± 1.99
B1	177.3 ± 5.27^{*abc}	105.6 ± 9.29^{*cd}	59.22 ± 3.02^{*abc}
B2	154.0 ± 6.06^{*bcd}	88.02 ± 7.75	49.35 ± 2.52*
B3	147.9 ± 5.82^{*bcd}	84.50 ± 7.44	47.38 ± 2.41^{d}
S1	148.4 ± 3.34*	83.39 ± 7.34	46.75 ± 2.38
S2	119.1 ± 2.96^{a}	73.02 ± 5.13	42.47 ± 2.16
S3	119.1 ± 2.96^{a}	69.00 ± 4.85	40.13 ± 2.05

Table 2**:** Comparison of Oxidative stress indices in the different experimental group

Groups	SOD	CAT	GPx	H202
Control	45.61 ± 3.21	48.30 ± 3.30	4.39 ± 0.19	12.92 ± 0.32
B1	30.01 ± 2.12^{*,c}	31.78 ± 2.17^{*,c}	6.67 ± 0.29^{*,a,b,c}	8.50±0.21^{*,a,b,c}
B2	36.01 ± 2.54	38.13 ± 2.61	5.56 ± 0.24^{*,d}	10.20±0.25$^{*.b,c,d}$
B3	37.51 ± 2.64	39.72 ± 2.72	5.34 ± 0.23^{d}	10.63 ± 0.27^{*,c,d}
S1	38.01 ± 2.68	40.25 ± 2.75	5.27 ± 0.23	10.97 ± 0.27*
S2	41.85 ± 2.95	44.31 ± 3.03	4.78 ± 0.21	11.86 ± 0.29
S3	36.01 ± 2.54	46.00 ± 3.15	4.61 ± 0.20	12.31 ± 0.31^{a}

B- synthetic insecticide

S-stachytarpheta cayannesis

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= significant different from Control at p<0.05

a= significant different from B1 at p<0.05

b= significant different from S1 at p<0.05

c= significant different from S2 at p<0.05

d= significant different from S3 at p<0.05

Figures

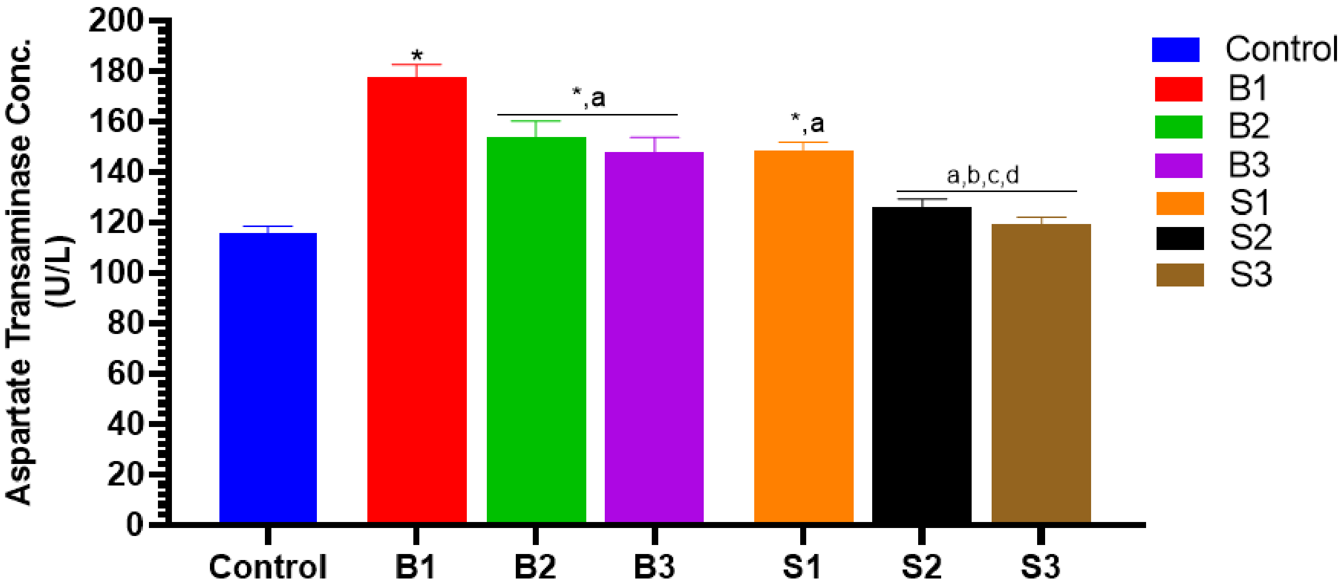

Figure 1

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of Aspartate transaminase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at p<0.05

a= Significantly different from B1at p<0.05

b= Significantly different from B2 at p<0.05

c= Significantly different from S1 at p<0.05

d= Significantly different from S2 at p<0.05

e= Significantly different from S3at p<0.05

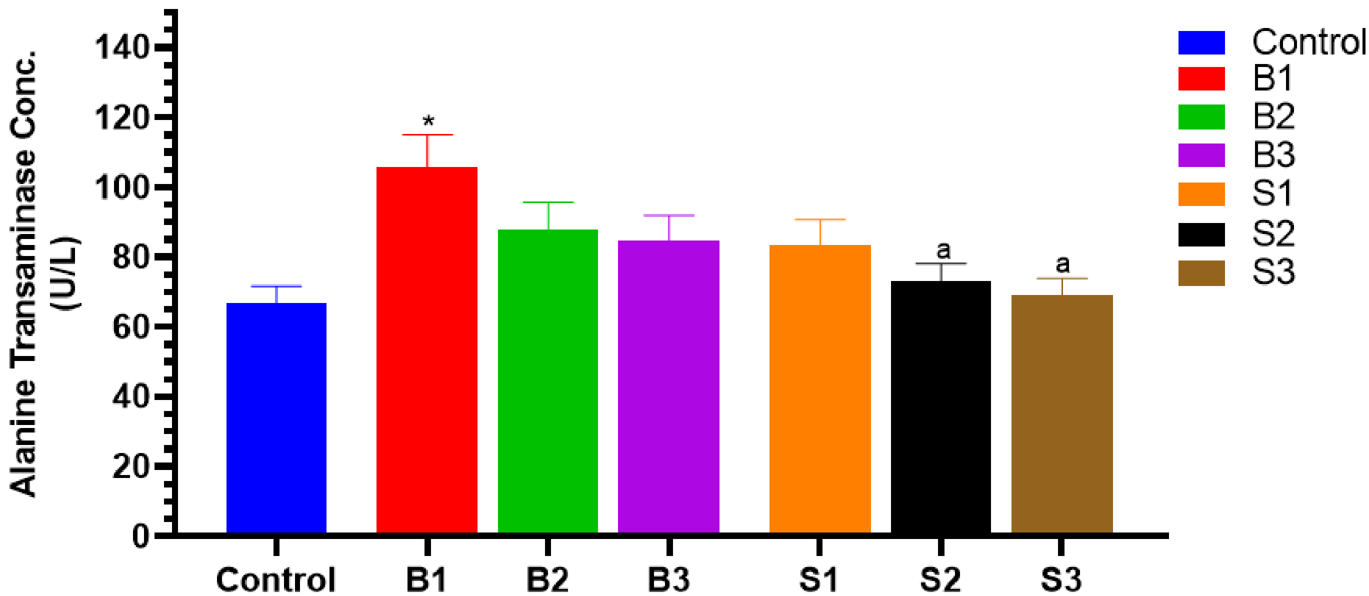

Figure 2

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of Alanine transaminase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at p<0.05

a= Significantly different from B1 at p<0.05

b= Significantly different from B2 at p<0.05

c= Significantly different from S1 at p<0.05

d= Significantly different from S2 at p<0.05

e= Significantly different from S3 at p<0.05

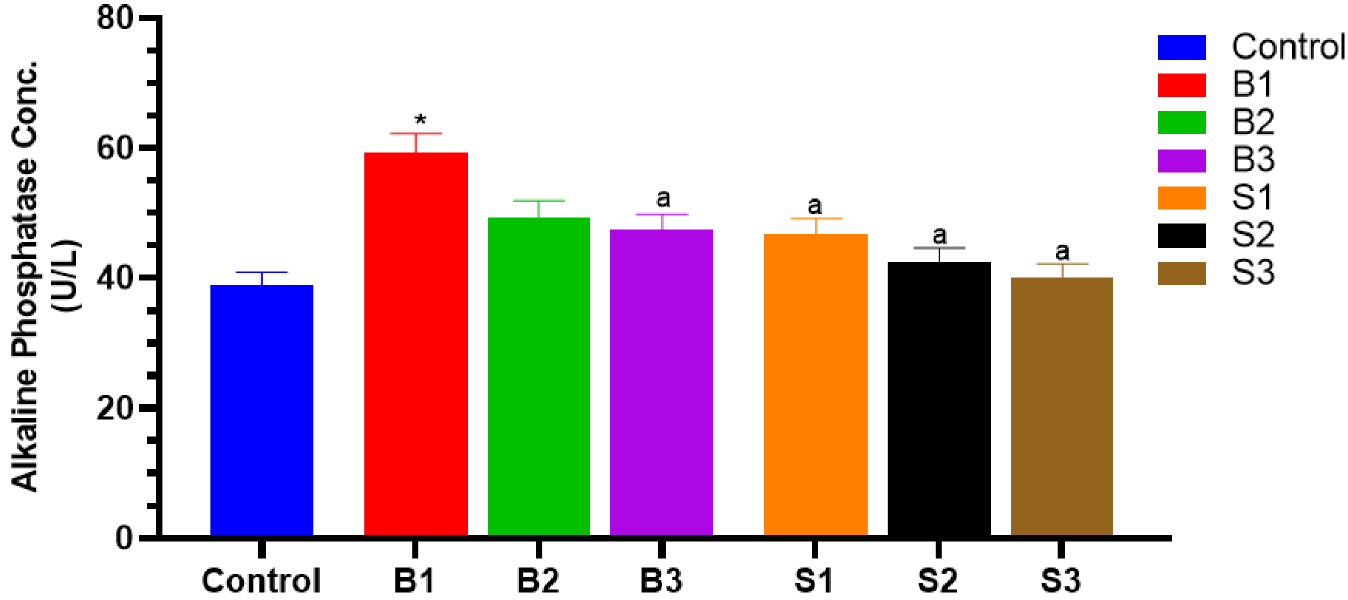

Figure 3

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of Alkaline Phosphatase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at p<0.05

a= Significantly different from B1 at p<0.05

b= Significantly different from B2 at p<0.05

c= Significantly different from S1 at p<0.05

d= Significantly different from S2 at p<0.05

e= Significantly different from S3 at p<0.05

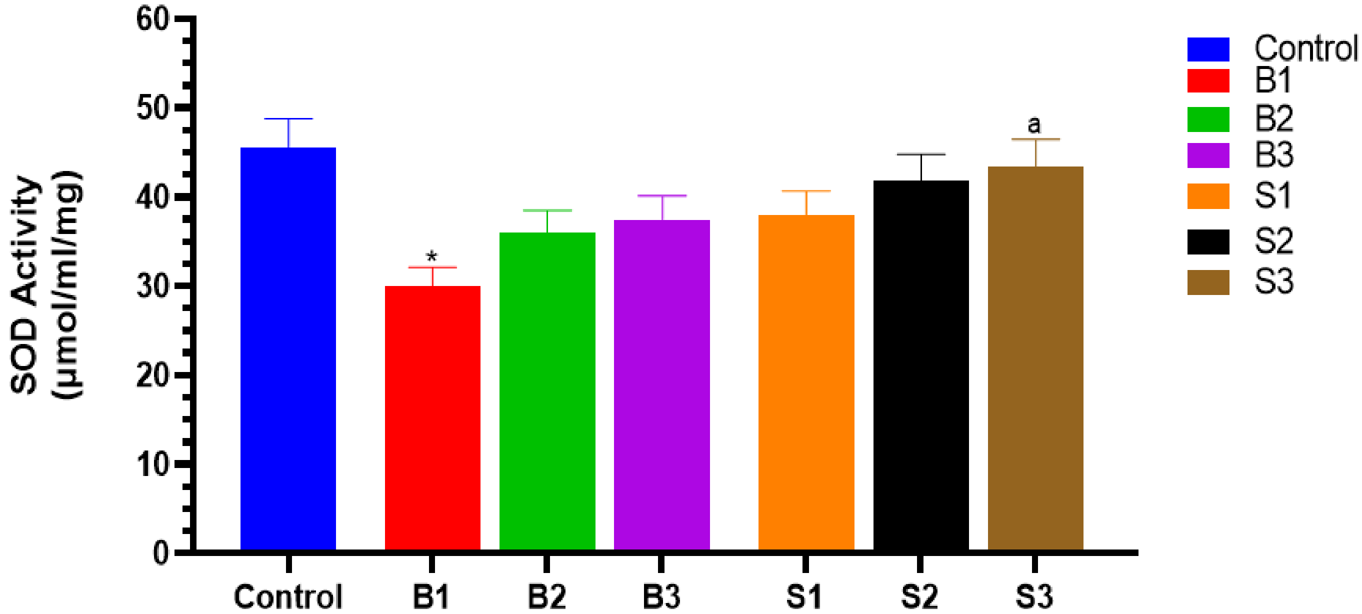

Figure 4

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of superoxide dismutase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at $p<0.05$

a= Significantly different from B1 at $p<0.05$

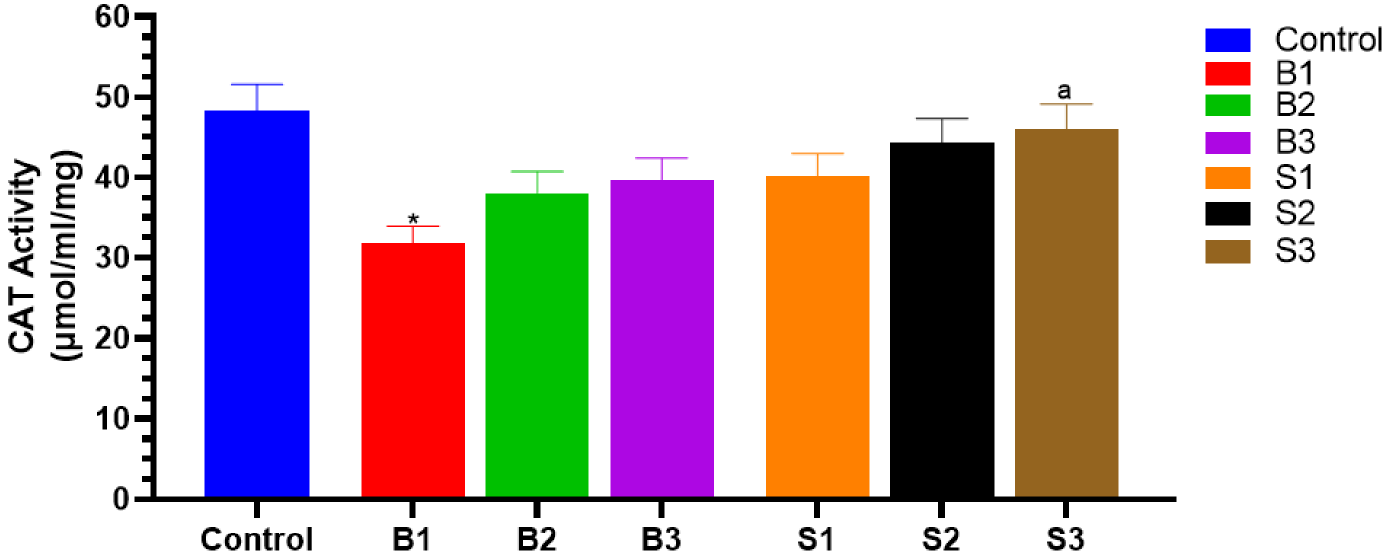

Figure 5

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of catalase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at p<0.05

a= Significantly different from B1 at p<0.05

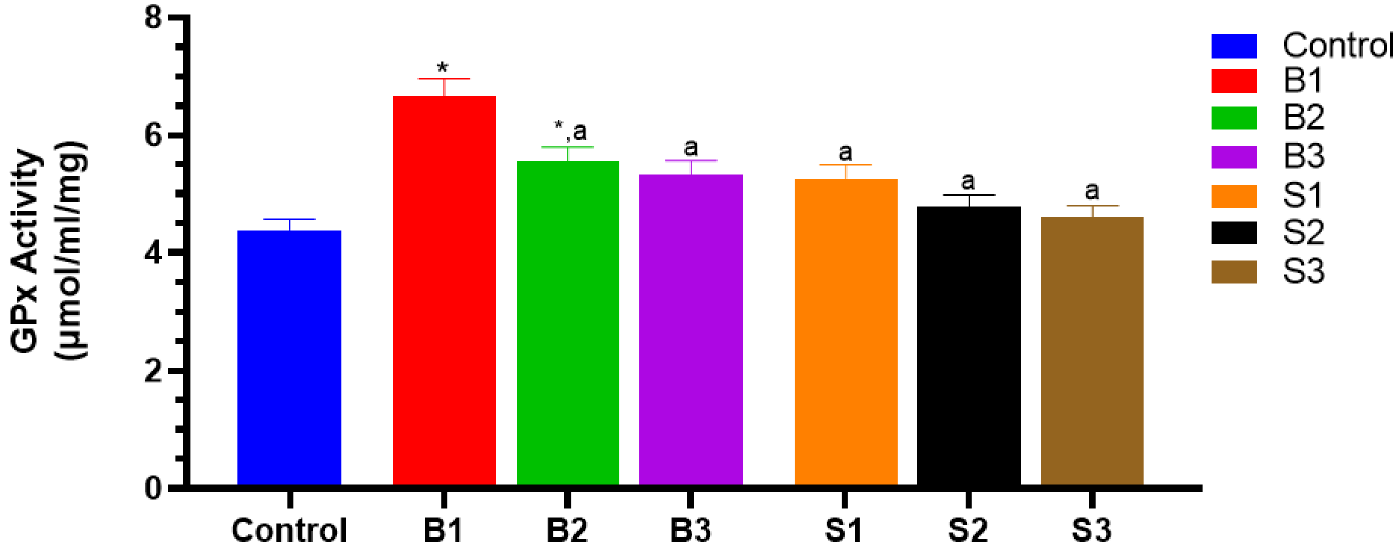

Figure 6

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of Glutathione peroxidase activity in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at $p<0.05$

a=Significantly different from B1 at $p<0.05$

b=Significantly different from S1 at $p<0.05$

c= Significantly different from S2 at $p<0.05$

d= Significantly different from S3 at $p<0.05$

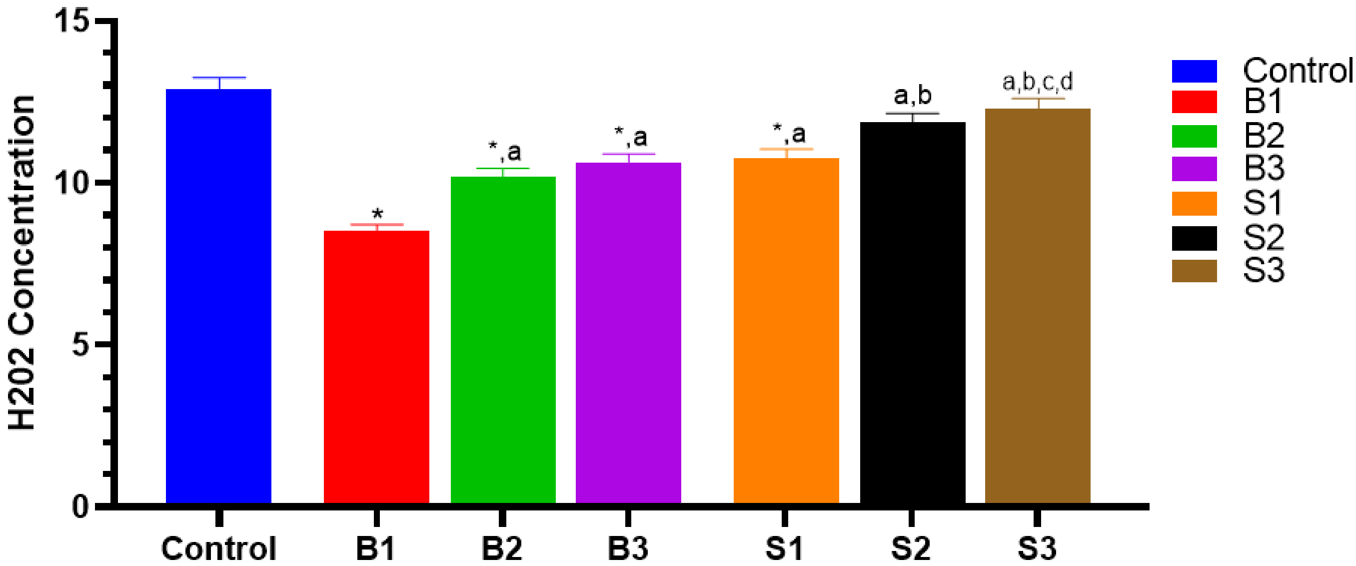

Figure 7

B-Synthetic insecticide

S-stachytarpheta cayennesis

Comparison of H_2O_2 concentration in the different experimental groups

Values are expressed as Mean ± SEM, n=4 (Using One way Analysis of Variance).

*= Significantly different from Control at $p<0.05$

a=Significantly different from B1 at $p<0.05$

b=Significantly different from S1 at $p<0.05$

c= Significantly different from S2 at $p<0.05$

d= Significantly different from S3 at $p<0.05$