Activation of the Oxidative Stress in *Culex quinquefasciatus* by the Augmented Production of Reactive Oxygen Species (ROS) in response to *Stachytarpheta jamaicensis* Exposure

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**Introduction:** Plant-based knowledge has been used for generations for personal protection from various mosquito species. The notion of applying such traditional perspectives in vector control research has received extensive attention in recent years. Unlike other common patterns, the present investigation has tried to explore the augmented production of reactive oxygen species (ROS) in response to *Stachytarpheta jamaicensis* exposure with special inference on larvicidal potential, mode of action of phytochemical compounds, and oxidative stress.

**Methods:** The larvicidal potential was determined as per the WHO protocol. Ultraviolet-visible spectroscopy was used to determine the excessive production of ROS. GC-MS was employed to characterise the phytochemical constituents. The statistical analysis was done by using SPSS version 24.0.0.

**Result:** The acetone extract has been found to exhibit a maximum range of toxicity in terms of larvicidal potential and reactive oxygen species formation. Among the 40 phytochemical elements characterised, Cyclopropane, 1,1,2,2-Tetramethyl; Phenyl-Acetonitrile; Pyranone; Tetradecene; Neophytadiene; Mome Inositol; Monocrotaline; and Squalene may be responsible for the augmented production of ROS in the *Culex quinquefasciatus*.

**Conclusion:** The phytochemical elements in *Stachytarpheta jamaicensis* displayed extensive toxicity and inhibited the normal development of *Culex quinquefasciatus* mosquitoes by augmented production of reactive oxygen species, indicating its prominent role in oxidative stress.

**Keywords:** *Culex Quinquefasciatus*, *Stachytarpheta Jamaicensis*, ROS, Larvicidal, Oxidative Stress
Introduction

A wide proportion of humans from tropics and subtropics worldwide has been seriously threatened by mosquito-borne diseases every year.\textsuperscript{3,12} Mosquito control measures focussing on larval and adult stages have been shown to exhibit maximum potential.\textsuperscript{32} The frequent use of synthetic insecticides is considered the most extensively used tool to fight against mosquito vectors.\textsuperscript{17} However, control of the mosquito-borne disease has entered a new segment of a challenge to the scientific community in the current scenario due to the forecasting threat of resistance in mosquito vectors to routinely used synthetic insecticides.\textsuperscript{27} Hence, currently, the scientific community has drawn attention from natural products since the plants have been a prominent source of traditional medication with the tiniest toxicity to domestic animals and mankind.\textsuperscript{21,22}

An inevitable effect of phytochemical exposure to mosquito larvae is cellular and metabolic damage. The introduction of phytochemicals from plant extract or natural insecticides may augment Reactive Oxygen Species (ROS).\textsuperscript{20} In such cases, it will lead to oxidative stress within a short time. We have to find out the concepts mentioned above in \textit{Culex quinquefasciatus} using the traditionally used medicinal plant \textit{Stachytarpheta jamaicensis} since it allied with the numerous biological functions of mosquito metabolism. Based on the previous literature and research gap, it was clearly evident that the prominent use of compounds that were isolated from traditionally used medicinal plants\textsuperscript{3,4,6} as bioherbicides have been recognised as the backbone of novel vector control strategies and are becoming an effective alternative to synthetic insecticides.\textsuperscript{2,3,7}

There exist a large number of research articles regarding the effectiveness of plant-based insecticides. However, only a few research articles, including our previous studies\textsuperscript{2} have focussed on the probable mode of phytochemical exposure to mosquito vectors with special reference to the Reactive Oxygen Species Generation (ROS). The fact is that none have piloted an investigation in the abovementioned aspects in \textit{Culex quinquefasciatus} using the traditionally used medicinal plant \textit{Stachytarpheta jamaicensis}.

As the aforementioned aspects have played a prominent role in vector control research, this study aimed to investigate the probable mode of action of photochemical constituents from \textit{Stachytarpheta jamaicensis} in \textit{Culex quinquefasciatus} with special emphasis on augmented production of ROS. Furthermore, this investigation also targeted to analyse whether ROS’s excessive production is the prominent mechanism that underlies the death of mosquito larvae.

Materials and Methods

Collection of Plant Material and Extraction Process

Healthy leaves of \textit{Stachytarpheta jamaicensis} were collected from Thirunelly, Wayanad, a part of Western Ghats, Kerala, India. The cleaned plant material was proceeded to being shade dried and milled using an electric grinder. The extraction process was accomplished using the Soxhlet extraction apparatus with acetone, water, methanol, and petroleum ether extract as solvents. The concentrated extracts were then stored at \(-20\) °C for further experiments.

Mosquito Culture and Larval Bioassay

Early fourth instar larvae of \textit{Culex quinquefasciatus} were used for all the executed experiments. The larvae used in this investigation were collected from Communicable Disease Research Laboratory, Department of Zoology, St. Josephs’s College, Irinjalakuda, Kerala, India. The mosquito eggs of \textit{Culex quinquefasciatus} were placed in plastic trays of size 28 L × 39 W × 14 D cm. After egg hatching, the larvae were then transferred into another tray of size 28 L × 39 W × 14 D cm. The following environmental conditions were simulated for rearing the larvae: 55–60% relative humidity, 27 ± 2 °C temperature followed by 12 L:12 D photoperiod cycle. Toxicity studies of the plant extracts on \textit{Culex quinquefasciatus} larvae were performed as per the guidelines given by the World Health Organization.\textsuperscript{31} Twenty early fourth instar larvae of \textit{Culex quinquefasciatus} were transferred to 250 ml glass beakers containing \textit{Stachytarpheta jamaicensis} extracts in different concentrations. Triplicates were maintained for all the executed experiments. The mortality rates were noted after 24 h of exposure.

Phytochemicals Induced Free Radical Generation (ROS)

The plant extracts’ ability to generate augmented production of ROS as a probable mode of action of phytochemicals was investigated using the method suggested by.\textsuperscript{2} Twenty, fourth instar larvae of \textit{Culex quinquefasciatus} treated with different concentrations of \textit{Stachytarpheta jamaicensis} extracts were homogenised as the sample. The control group was maintained with distilled water containing the homogenised \textit{Culex quinquefasciatus} larvae that were not treated, lacking phytochemicals. The augmented production of ROS within the \textit{Culex quinquefasciatus} larvae was determined using ultraviolet-visible spectroscopy.

Identification of Bioactive Compounds

The acetone extract of \textit{Stachytarpheta jamaicensis} was subjected to GC-MS. One ml of the aforementioned sample was injected for GC-MS analysis with the following conditions: film thickness 0.25 μm DB-5MS column, 30 m × 0.25 mm, initial temperature 50 °C (2 min) together with the rate of 20 °C/min to 130 °C; 12 °C/min to a 180 °C; and a final temperature to 280 °C at 3 °C/min. The temperature for the final condition was maintained for 15 min. Helium was used as a carrier gas at one ml/min flow rate.
isolated phytochemical compounds from *Stachytarpheta jamaicensis* were compared with the retention time (RT) values of reference compounds from REPLIB and MAINLIB library for characterisation.

**Statistical Analysis**

The data obtained from all the executed experiments were entered in MS office 2010 and then exported in SPSS version 24.0.0. Probit regression analysis was performed to get the LC50 and LC90 of the plant extracts. The graphs were plotted using SPSS for Windows operating systems.

**Results**

**Phytochemistry**

The acetone extract of *Stachytarpheta jamaicensis* yielded 40 peaks, which specify the presence of 40 phytochemical constituents in the extract (Table 1 and Figure 1). Cyclopropane, 1,1,2,2-Tetramethyl; Phenyl-Acetonitrile; Pyranone; Tetradecene; Neophytadiene; Mome Inositol; Monocrotaline; and Squalene have been previously known for their toxic effects (Figure 2). Previous literature indicates that the phytochemical constituent coumarin has been found to inhibit AChE activity in human beings.

**Toxic Effects of Plants Extracts on Mosquito Larvae**

The toxic effects of phytochemical constituents of *Stachytarpheta jamaicensis* towards *Culex quinquefasciatus* fourth instar larvae. The augmented production of ROS determined in this investigation has been considered as a probable mode of action of the phytochemical constituents that may be recognised as a major reason for oxidative stress that happened in *Culex quinquefasciatus* fourth instar larvae.

The acetone extract of *Stachytarpheta jamaicensis* has been shown to exhibit a maximum amount of reactive oxygen species in the *Culex quinquefasciatus* (Figure 4). The control groups were noted with less amount of free radical generation. As reported in Table 3, water, methanol and petroleum ether extracts of *Stachytarpheta jamaicensis* exhibited a relatively prominent range of free radical generation in *Culex quinquefasciatus*, and this has drawn our concern to discuss the phytochemical specific oxidative stress. We reported that the phytochemical constituents exposure in *Culex quinquefasciatus* caused excessive production of ROS (Table 3), therefore it probably leads to cell damage and oxidative stress.

**Table 1. GC-MS Analysis of Stachytarpheta jamaicensis Acetone Extract**

| Peak# | R.Time | Area    | Area% | Height | Height% | Name                                      | Base m/z |
|------|--------|---------|-------|--------|---------|-------------------------------------------|----------|
| 1    | 7.279  | 13214989| 5.09  | 1933962| 4.21    | CYCLOPROPANE, 1,1,2,2-TETRAMETHYL-       | 55.05    |
| 2    | 8.396  | 4681269 | 1.80  | 904588 | 1.97    | 5H-CYCLOPENTA[B]PYRIDINE #               | 117.10   |
| 3    | 8.450  | 2664035 | 1.03  | 616568 | 1.34    | PHENYL-ACETONITRILE                      | 117.10   |
| 4    | 9.721  | 11007079| 42.37 | 10668374| 23.20   | 1-(6-OXABICYCLO[3.1.0]HEX-1-YL) ETHANONE | 55.05    |
| 5    | 9.758  | 258569  | 0.10  | 338019 | 0.74    | Pyranone                                 | 144.05   |
| 6    | 11.050 | 1167649 | 0.45  | 266453 | 0.58    | Coumaran                                 | 120.10   |
| 7    | 12.694 | 3130970 | 1.21  | 1134799| 2.47    | 2-Methoxy-4-vinylphenol                  | 150.10   |
| 8    | 14.016 | 280712  | 0.11  | 181328 | 0.39    | 1-TETRADECENE                            | 55.05    |
| 9    | 15.131 | 286296  | 0.11  | 138446 | 0.30    | 3-OCTANONE                               | 99.10    |
| 10   | 15.475 | 648359  | 0.25  | 123591 | 0.27    | N-Hexanoyl-DL-homoserine lactone         | 99.10    |
| 11   | 15.642 | 1049688 | 0.40  | 165483 | 0.36    | 2,3-Dimethyl-5-oxohexanethioic acid, S-t-butyl ester | 141.10 |
| 12   | 15.879 | 2852387 | 1.10  | 1483826| 3.23    | Phenol, 2,4-bis(1,1-dimethylethyl)-       | 191.20   |
Table 2. Larvicidal Efficacy of Various Extracts from Stachytarpheta jamaicensis

| Solvents Used          | LC50             | LC90             | Chi-Square | dfb |
|-----------------------|------------------|------------------|------------|-----|
| Acetone               | 121.049 (93.332-161.985) | 393.492 251.223 1320.004 | .964       | 2   |
| Water                 | 134.358 (105.767-184.498) | 419.849 (266.0921-433.834) | .764       | 2   |
| Methanol              | 139.309 (112.798-184.094) | 377.887 (255.441-984.497) | .764       | 2   |
| Petroleum ether       | 140.043 (108.154-205.830) | 489.767 (288.182-2420.791) | .363       | 2   |

Table 3. Determination of Free Radicals from Plant Extract exposed Culex quinquefasciatus
(Experiments were executed in triplicates)

| Sample | Extract Dose (µg/mL) | Absorbance Mean ± SD |
|--------|-----------------------|-----------------------|
| Culex quinquefasciatus Larvae + Distilled water (µg/mL) | 50 | 0.000 ± 0.000 |
| | 100 | 0.001 ± 0.002 |
| | 150 | 0.002 ± 0.002 |
| | 200 | 0.003 ± 0.002 |
| Culex quinquefasciatus Larvae + | 50 | 0.005 ± 0.002 |
| | 100 | 0.013 ± 0.002 |

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Stachytarpheta jamaicensis Acetone extract (μg/mL)  |  150  |  0.018 ± 0.002  
|  200  |  0.021 ± 0.004  

Culex quinquefasciatus Larvae + Stachytarpheta jamaicensis Water extract (μg/mL)  |  50  |  0.003 ± 0.002  
|  100  |  0.006 ± 0.002  
|  150  |  0.014 ± 0.002  
|  200  |  0.019 ± 0.002  

Culex quinquefasciatus Larvae + Stachytarpheta jamaicensis Methanol extract (μg/mL)  |  50  |  0.002 ± 0.02  
|  100  |  0.005 ± 0.002  
|  150  |  0.008 ± 0.004  
|  200  |  0.010 ± 0.002  

Culex quinquefasciatus Larvae + Stachytarpheta jamaicensis Petroleum ether extract (μg/mL)  |  50  |  0.003 ± 0.002  
|  100  |  0.007 ± 0.002  
|  150  |  0.009 ± 0.002  
|  200  |  0.013 ± 0.002  

**Figure 1.** GC-MS Chromatogram of Phytochemical Constituents in *Stachytarpheta jamaicensis* Acetone Extract

**Figure 2.** Predominant Phytochemical Elements responsible for the Augmented Production of ROS in *Culex quinquefasciatus*
Discussion

Although *Culex quinquefasciatus* is an important vector of several mosquito-borne diseases worldwide and is responsible for human lymphatic filariasis in 120 million people worldwide together with common chronic manifestation, the controlling of *Culex quinquefasciatus* mosquitoes using chemical insecticides had certain advantages like easy application and speedy action. Due to the various adverse effects of chemical insecticides, natural insecticides (mainly from plants) are known to draw prominent significance in vector control research in recent years. There exists a huge number of research articles regarding the aforesaid aspects. However, as a follow-up and novel perspective, we have studied the probable mode of action of the phytochemical constituent of the medicinal plant *Stachytarpheta jamaicensis* in relation to ROS production and oxidative stress in *Culex quinquefasciatus*.

The present investigation has reported the following predominant compounds with a prominent range of toxicity in previous studies: Cyclopropane, 1,1,2,2-Tetramethyl; Phenyl-Acetonitrile; Pyranone; Tetradecene; Neophytadiene;
Mome Inositol; Monocrotaline; and Squalene. A study by 28 reported the phytochemical constituent’s presence “Cyclopropane, 1,1,2,2-Tetramethyl” from Moringa Oleifera. The toxic nature of various compounds, including “Phenyl-Acetonitrile” was reported by. 24 The various biological applications of “Pyranone” were previously reported by a recent study. 11 Neophytadiene, a predominant compound in our study, had known to suppress the LPS-induced inflammatory response in Sprague Dawley rats and RAW 264.7 macrophages. 11 A study by 18 verified the various biological activities of Mome Inositol isolated from the medicinal plant Lespedeza bicolor. In agreement with their findings, the current study also verified the presence of aforesaid compounds using GC-MS. The secondary metabolite Monocrotaline, which is predominant in our study, was previously known to possess high acute toxicity in animals as well as in humans. An in vitro–in silico approach by 30 predicted the in vivo acute liver toxicity of this compound to reveal its significant role in hydrolysis, N-oxidation, dehydrogenation, and hydroxylation followed. With the help of morphological, biochemical, and histological studies, 10 verified the various toxic effects of monocrotaline such as endothelial damage of the central vein, severe liver damage, lymphocyte infiltration, sinusoidal haemorrhage followed by hepatocyte necrosis, indicating its prominent role in oxidative stress.

The insecticidal properties of the bioactive compound “squalene” were justified by. 10 In agreement with the previous findings, as discussed in the earlier sections, it was reported that the presence of the abovementioned phytochemical compounds is probably responsible for the larvicidal potential of Stachytarpheta jamaicensis since most of all the compounds have exhibited a prominent range of toxicity in previous findings. The use of environment-friendly approaches and toxic studies to achieve benefits for humans in all aspects has significantly increased in recent years. 5,23,25,26 Altogether, the present status of vector control research urges us to find out cost-effective, environment-friendly, target-specific and biodegradable natural insecticides against Culex quinquefasciatus mosquitoes. 8,24 Previously, 15 reported on the entomocidal potential of five different plants such as Achyranthes aspera, Chenopodium murale, Trianthema portulacastrum, Convolvulus arvensis, and Tribulus terrestris against Culex quinquefasciatus. In agreement with their findings, 20 reported the extensive larvicidal potential of Plectranthus amboinicus with an LC50 of 147.40 mg/L. In addition to this, the studies by 15,29,16,1 also revealed the significant role of various medicinal plant extracts and phytochemical constituents against Culex quinquefasciatus, and emphasised upon the need of further research on plant-based products in vector control. Considering the abovementioned facts, the present study has suggested the phytochemical constituents of Stachytarpheta jamaicensis for mosquito-control research. However, in order to verify the probable mode of action of the phytochemical elements, the present investigation has moved towards the second part, which reveals the augmented production of reactive oxygen species in Culex quinquefasciatus mosquito larvae.

Here we also investigate the role of reactive oxygen species in Stachytarpheta jamaicensis plant extract exposed Culex quinquefasciatus since the excessive production of free radicals has induced oxidative stress in the target insect. One of the major facts is that the ROS allied metabolism intensely influences the fecundity, immune response, and mosquitoes’ vector competence. As reported in a previous study by, 7 it was noted that the augmented levels of free radicals might interrupt various biological activities, including the interruption of redox signalling pathways. Such circumstances will also cause damages to cell organelles, proteins, and nucleic acids as described by. 34 Our study noted that the ROS generated by Stachytarpheta jamaicensis plant extracts cause oxidative stress and is principally recognised as the major reason behind the death of Culex quinquefasciatus mosquito larvae. The acetone extract has shown maximum level of free radical production as compared to water, petroleum ether, and methanol extract of Stachytarpheta jamaicensis. The toxic effects of various phytochemical constituents reported in previous findings might be the forecasting reason for the augmented production of free radicals in the target insect, the Culex quinquefasciatus mosquitoes. As discussed in the earlier section, most of all the secondary metabolites profiled using GC-MS in this study are known for their toxic effects in previous investigations. This has indicated their forecasting role in generating oxidative stress in the target insecticides by producing excessive free radicals.

**Conclusion**

The present investigation made a first report on the augmented production of reactive oxygen species (ROS) in response to Stachytarpheta jamaicensis exposure in Culex quinquefasciatus. Here we reported that the generation of an excessive amount of free radicals by Stachytarpheta jamaicensis extracts had activated the oxidative stress in Culex quinquefasciatus larvae, and it was probably recognised as the mechanisms behind the mosquito larval death. The presence of various phytochemical constituents such as Cyclopropane, 1,1,2,2-Tetramethyl; Phenyl-Acetonitrile; Pyranone; Tetradecene; Neophytadiene; Mome Inositol; Monocrotaline; and Squalene in Stachytarpheta jamaicensis may be responsible for the rapid formation of free radicals inside the plant extract exposed to Culex quinquefasciatus mosquito larvae. The knowledge of medicinal plant-based approaches for mosquito control is a valuable source for developing novel natural products.
The present investigation calls for researchers’ attention towards vector control research to understand the potential position of *Stachytarpheta jamaicensis* in a free radical generation, oxidative stress, and analysing probable mode of action of phytochemical constituents.

**Availability of Data and Material**

The data analysed during the present investigation are available with the corresponding author, and can be accessed on reasonable request.

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**Conflict of Interest:** None

**References**

1. Adday WA, Annon MR. Biological Effective of organic solvent extracts of Datura innoxia Leaves in the Cumulative and Non-cumulative for mortality of Immature insect Culex quinquefasciatus Say (Diptera: Culicidae). Int J Res Pharma Sci. 2020;11(1):452-9.

2. Anoopkumar AN, Aneesh EM, Sudhikumar AV. Exploring the mode of action of isolated bioactive compounds by induced reactive oxygen species generation in Aedes aegypti: a microbes based double-edged weapon to fight against Arboviral diseases. Int J Trop Ins Sci. 2020;40:573-85. [Google Scholar]

3. Anoopkumar AN, Puthur S, Rebello S, Anoopkumar EM. Screening of a few traditionally used medicinal plants for their larvicidal efficacy against Aedes aegypti Linn (Diptera: Culicidae), a dengue fever vector. SOJ Microbial Infect Dis. 2017;5:1-5. [Google Scholar]

4. Anoopkumar AN, Puthur S, Varghese P, Rebello S, Anoopkumar EM. Life cycle, bio-ecology and DNA barcoding of mosquitoes Aedes aegypti (Linnaeusae) and Aedes albopictus (Skuse). J Commun Dis. 2017;49(3):32-41. [Google Scholar]

5. Anoopkumar AN, Rebello S, Anoopkumar EM, Sindhu R, Binod P, Pandey A, Gnansounou E. Use of Different Enzymes in Biorefinery Systems. Biorefinery Production Technologies for Chemicals and Energy. In: Kulia A, Mukhopadhyay M, editors. Biorefinery Production Technologies for Chemicals and Energy. Wiley Online Library; 2020. p. 357-68. [Google Scholar]

6. Anoopkumar AN, Rebello S, Devassy E, Raj KK, Puthur S, Anoopkumar EM, Sindhu R, Binod P, Pandey A. Phytoextraction of Heavy Metals. In: Inamuddin, Ahamed MI, Lighthouse E, Asiri AM. Methods for Bioremediation of Water and Wastewater Pollution. Springer; 2020. p. 267-76. [Google Scholar]

7. Anoopkumar AN, Rebello S, Sudhikumar AV, Puthur S, Anoopkumar EM. A novel intervention on the inhibiting effects of Catunaregam spinosa induced free radical formation and DNA damage in Aedes aegypti (Diptera: Culicidae): a verdict for new perspectives on microorganism targeted vector control approach. Int J Trop Ins Sci. 2020;40:989-1002. [Google Scholar]

8. Anoopkumar AN, Puthur S, Rebello S, Aneesh EM. Molecular characterization of Aedes, Culex, Anopheles, and Armigeres vector mosquitoes inferred by mitochondrial cytochrome oxidase I gene sequence analysis. Biologia. 2019;74:112-38. [Google Scholar]

9. Asiri BMK, Al-Ansari SQ, Edrees NO. Evaluation of Toxicological Effects of Some Plant Oils and Diesel Oil on Some Biological Aspects of Culex pipiens (Diptera: Culicidae) larvae. Life Sci J. 2020;17(9):57-70. [Google Scholar]

10. Benavides-Cordoba V, Silva-Medina M, Varela MX, Gomez MP. Subchronic toxicity of the pulmonary hypertension model due to low-dose monocrotaline in rats. J Pharm Pharmacog Res. 2020;8:308-15. [Google Scholar]

11. Bhardwaj M, Sali VK, Mani S, Vasanthi HR. Neophytadiene from Turbinaria ornata Suppresses LPS-Induced Inflammatory Response in RAW 264.7 Macrophages and Sprague Dawley Rats. Inflammation. 2020;43(3):937-50. [PubMed] [Google Scholar]

12. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. Climate change and vector-borne diseases: a regional analysis. Bull World Health Organ. 2000;78(9):1136-47. [PubMed] [Google Scholar]

13. Kadhim MI, Husein I. Pharmaceutical and Biological Application of New Synthetic Compounds of Pyranone, Pyridine, Pyrmidine, Pyrazole and Isoxazole Incoporating Neophytadiene from Turbinaria ornata. Syste Rev Pharm. 2020;11(2):679-84. [Google Scholar]

14. Kodrik D, Bednarova A, Zemanova M, Krishnan N. Hormonal regulation of response to oxidative stress in insects—an update. Int J Mol Sci. 2015;16(10):25788-816. [PubMed] [Google Scholar]

15. Kulia A, Mukhopadhyay M, editors. Biorefinery Production Technologies for Chemicals and Energy. Wiley Online Library; 2020. p. 357-68. [Google Scholar]

16. Muturi EJ, Dunlap C, Caceres CE. Microbial communities of container aquatic habitats shift in response to Culex restuans larvae. FEMS Microbiol Ecol. 2020;96(7). [PubMed] [Google Scholar]

17. Nauen R. Insecticide resistance in disease vectors of public health importance. Pest Manag Sci. 2007;63(7):628-33. [PubMed] [Google Scholar]

18. Oh KK, Adnan M, Cho DH. Network pharmacology approach to bioactive chemical compounds identified from Lespedeza bicolor lignum methanol extract by GC–MS for amelioration of hepatitis. Gene Rep.
20. Paramasivam D, Balasubramanian B, Park S, Alagappan P, Kaul T, Liu W, Pachiappan P. Phytochemical profiling and biological activity of Plectranthus amboinicus (Lour.) mediated by various solvent extracts against Aedes aegypti larvae and toxicity evaluation. Asi Pac J Trop Med. 2020;13(11):494. [Google Scholar]

21. Puthur S, Anoopkumar AN, Rebello S, Aneesh EM. Hypericum japonicum: a Double-Headed Sword to Combat Vector Control and Cancer. Appl Biochem Biotech. 2018;186(1):1-11. [PubMed] [Google Scholar]

22. Puthur S, Anoopkumar AN, Rebello S, Aneesh EM. Synergistic control of storage pest rice weevil using Hypericum japonicum and deltamethrin combinations: a key to combat pesticide resistance. Environ Sustain. 2019;2:411-7. [Google Scholar]

23. Puthur S, Anoopkumar AN, Rebello S, Aneesh EM, Sindhu R, Binod P, Pandey A. Toxic Effects of Pesticides on Avifauna Inhabiting Wetlands. In: Inamuddin, Ahamed MI, Lichtfouse E, editors. Sustainable Agriculture Reviews. Vol. 47. Springer; 2021. p. 335-49. [Google Scholar]

24. Puthur S, Raj KK, Anoopkumar AN, Rebello S, Aneesh EM. Acorus calamus mediated green synthesis of ZnONPs: A novel nano antioxidant to future perspective. Adv Powd Tech. 2020;31(12):4679-82. [Google Scholar]

25. Rebello S, Anoopkumar AN, Aneesh EM, Sindhu R, Binod P, Kim SH, Pandey A. Hazardous minerals mining: Challenges and solutions. J Hazard Mater. 2021;402:123478. [PubMed] [Google Scholar]

26. Rebello S, Anoopkumar AN, Aneesh EM, Sindhu R, Binod P, Pandey A. Sustainability and life cycle assessments of lignocellulosic and algal pretreatments. Bioresour Technol. 2020;301:122678. [PubMed] [Google Scholar]

27. Sharma SK, Upadhyay AK, Haque MA, Tyagi PK, Kindo BK. Impact of changing over of insecticide from synthetic pyrethroids to DDT for indoor residual spray in a malaria endemic area of Orissa, India. Indian J Med Res. 2012;135(3):382. [PubMed] [Google Scholar]

28. Shunmugapriya K, Venilla P, Kanchana S, Vellaikumar S. Identification of Volatile Flavour Compounds in Moringa Oleifera Powder and Soup Mixes. Chem Sci Rev Lett. 2020;9(33):162-70. [Google Scholar]

29. Sukumaran S, Maheswaran R. Larvicidal Activity of Elytraria acaulis against Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). J Arthropod Borne Dis. 2020;14(3):293-301. [PubMed] [Google Scholar]

30. Suparmi S, Wesseling S, Rietjens IMCM. Monocrotaline-induced liver toxicity in rat predicted by a combined in vitro physiologically based kinetic modeling approach. Arch Toxicol. 2020;94(9):3281. [PubMed] [Google Scholar]

31. Umar AB, Dankaka AH, Shah MM. Larvicidal activity of some selected medicinal plant extracts against the vector of filariasis. J Experim Sci. 2020;11:41-43. [Google Scholar]

32. Walker K, Lynch M. Contributions of Anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. Med Vet Entomol. 2007;21(2):2-21. [PubMed] [Google Scholar]

33. World Health Organization [Internet]. Guidelines for laboratory and field testing of mosquito larvicides. 2005. Available from: https://apps.who.int/iris/handle/10665/69101 [Google Scholar]

34. Xu X, Shan S, Wang W, Liu H. Analysis of the Components in Moxa Smoke by GC-MS and Preliminary Discussion on Its Toxicity and Side Effects. Evid Based Compl Altern Med. 2020;2020. [PubMed] [Google Scholar]