Involvement of Cholinergic and Redox Impairments in Insecticidal Properties of Essential Oils from Fertility Tree and Horseradish Tree Leaves in Fruit Fly (Drosophila melanogaster)

Akomolafe Seun Funmilola¹*, Ogunsuyi Opeyemi Babatunde², ³, Ojo Victor¹, Asaolu Segun¹, Oladapo Iyabo Folake¹, and Oboh Ganiyu³

¹ Department of Biochemistry, Ekiti State University, Ado-Ekiti, Private Mail Bag 5363, NIGERIA
² Department of Biomedical Technology, School of Health and Health Technology, Federal University of Technology Akure, Private Mail Bag 704, Akure 340001, NIGERIA
³ Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology Akure, Private Mail Bag 704, Akure 340001, NIGERIA

Abstract: This study reports the biological activity of essential oils from fertility tree and horseradish tree leaves, and the mechanisms by which these oils promote insecticidal activity using Drosophila melanogaster as a model organism. Adult D. melanogaster were exposed to these essential oils at a final concentration of 10 µL/g for 24 hours. The exposure of flies to the essential oil resulted in significant decrease in their survival and fecundity. In addition, the essential oils produced significant reduction in acetylcholinesterase activity and induced oxidative stress in the flies as evidenced by an increase in reactive oxygen species, thiol and thiobarbituric reactive substance levels, as well as catalase activity. The essential oils were also characterized by gas chromatography coupled with mass spectrometry. Constituents such as terpenoids, Bis (2-ethylhexyl) phthalate, benzeneacetaldehyde, phytol, octadecane, 9,12-octadecadienoic acid (Z,Z)-methyl ester, heneicosane, eicosane and others were characterized. Therefore, our results point out to the potential application of fertility tree and horseradish tree leaf essential oils as natural alternatives to synthetic insecticides in agricultural and pest control practices, especially against dipterans.

Key words: anticholinesterase, Drosophila melanogaster, essential oil, oxidative stress

1 Introduction

Dipterans, otherwise known as the ‘true flies’ are of significant importance to medicine, veterinary medicine and agricultural practices for a number of reasons; many dipterans serve as human and animal vectors, as well as plant pests¹. In Africa, dipterans such as Neosilba perezi, Trirhithrum coffeae, Dacus sp. and Drosophila sp. serve as pest of major valuable crops including cassava, coffee, fruits and citrus respectively⁶. Although Drosophila melanogaster as a dipteran is not considered a major field-pest, it has been used widely as a model to study biological activities and mechanisms of action of insecticidal agents against dipterans, as well as possible resistance to such agents, due to highly conserved genetic properties in this insect order¹, ². In addition, D. melanogaster has been used to study some human diseases due to conservation in disease causing genes between the two species, with many drugs showing efficacy in D. melanogaster having applications in humans⁷. Therefore, D. melanogaster as a model organism is not only valuable as dipteran pest-model, but also can relate (to some extent) to humans.

Apart from being known as volatile oils, essential oils are also referred to as etheric oils. Naturally, they are found in aromatic plants, produced as secondary metabolites, as a mixture of volatile compounds⁸. Etheric oils are essential in plant defense system against microorganisms, insects, herbivores and allelopathic interactions⁹. They are extracted from plants through distillation which could either be thermal or hydro-distillation. Generally, essential oils consist of several single compounds which together give

*Correspondence to: Akmolafseun Funmilola, Department of Biochemistry, Ekiti State University, Ado-Ekiti, Private Mail Bag 5363, NIGERIA
E-mail: purposefulseun@yahoo.co.uk
Accepted April 4, 2020 (received for review September 29, 2019)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs
them their physical, chemical and biological properties.

One of the important roles of essential oils in food production is its ability to serve as global pest control\(^6\). According to Ayvaz et al.\(^5\), the botanical insecticide represents 1% of global insecticide market. This class of insecticides are preferred to most synthetic ones which have deleterious effects on the environment and human health. Studies have shown that there are about 17,500 aromatic plant species growing across the world in tropical environments and more than 3,000 constituents have been identified\(^7\). Out of these essential oils, 300 are used commercially by the pharmaceutical industry for drugs, as well as the cosmetics\(^6\) and pesticides industries\(^5\).

*Neumboldtia laevis* (Bignoniaceae) (*N. laevis*) commonly known as tree of life, fertility tree (English), Ogilisi, Egbo (in Igbo), Aduruuku (in Hausa), Akoko (in Yoruba), Ikimi (in Edo) can be used in the treatments of some of diseases. For instance, Burkill\(^8\) affirms that stem bark decoctions of *N. laevis* are used in treating epileptic cases and convulsions among children in Nigeria and Cote d’Ivoire. Apart from this, after pounding the stem bark, the paste can be used in the treatment of rheumatic cases\(^7\). More so, Burkill\(^8\) affirms that the decoctions made from the boiling of the leaves and roots are used in treating cases of breast tumors and male infertility in Nigeria. Egba et al.\(^3\) reported that the aqueous extract of *N. laevis* leaves showed significant increase in serum concentrations of testosterone, follicle stimulating and luteinizing hormones when compared to the control and they concluded that the plant can promote hormonal imbalances in males. The essential oil from *N. laevis* was found to have anthelminthic activity and no cytotoxic effect on Vero cells\(^10\).

*Moringa oleifera* (*M. oleifera*), is a plant which belongs to the mono-generic family, Moringaceae, and it is also referred to as horseradish tree (English). Different ethnic groups have different native names for it in Nigeria. In Northern Nigeria, The Hausa people call zogalle (in Hausa), in the Eastern Nigeria, it is known as okweoyibo (in Igbo), while the Yoruba people call it eweigbake (in Yoruba)\(^9\). It is generally known for its richness in nutritional and medicinal values. Its leaf can be eaten raw, used as spices in food preparation or used as part of soup ingredients. Its medicinal value is affirmed by Ezeamuzie et al.\(^12\) who found out that it is rich in activities such as anti-ulcer, anti-inflammatory, and anti-hypertensive activity. Similarly, Oommachan and Khan\(^13\) affirm that it is effective in the treatment of-abortifacient cases, and infertility problems. Apart from this, Varsha et al.\(^14\) show that the plant is effective in improving sexual activities in male rats. Also, as shown by Tatiana et al.\(^15\), the etheric oils extracted from *Moringa oleifera* leaf have low free radical scavenging ability and presents antimicrobial properties. On its antifungal activity, Chuang et al.\(^16\) affirm that essential oil of *M. oleifera* has antifungal activity against *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton xoccusum*, and *Microsporum canis*.

Despite the richness of literature on the benefits etheric oils from *N. laevis* and *M. oleifera* leaves, there is a paucity of literature on their insecticidal activities. Hence, this study evaluates the biological activity of the essential oils from *N. laevis* and *M. oleifera* leaves, and investigate the mechanism by which these oils promote insecticidal activity using *D. melanogaster* as the model organism\(^17\). Insecticidal activity was evaluated by rate of survival and fecundity. In addition, the effect of the oils on oxidative stress markers and acetylcholinesterase (AChE) activity were determined in order to search for potential mechanisms of insecticidal activity induced by the essential oils in *Drosophila*.

### 2 Materials and Methods

#### 2.1 Plant samples collection and identification

The fertility tree and horseradish tree leaves samples were acquired in fresh form during raining season from Akure, the capital town of Ondo State, Nigeria. They were verified and validated at the Department of Plant Science, Ekiti State University, and voucher specimen numbers given were UHAE 336 and UHAE 337 respectively.

#### 2.2 *Drosophila melanogaster* stock culture

*Drosophila melanogaster* (Wild-type, Harwich strain) from the National Species Stock Centre (Bowling Green, OH, USA), were kind gift from DR. Amos Abolaji of the Drosophila Laboratory, Biochemistry Department, University of Ibadan, Nigeria. These flies were preserved and nurtured at the Drosophila Research Laboratory, Functional Foods and Nutraceutical Unit, Federal University of Technology, Akure. They were placed on standard diet which consists of corn meal medium. At room temperature, under 12-hr dark/light cycle conditions, the cornmeal medium contains about 1% w/v brewer’s yeast and approximately 0.08% v/v nipagin. The experiments also took place at the same laboratory, using the same *D. melanogaster* strain.

#### 2.3 Reagents

The reagents used include N,N-dimethyl-para-phenylendiamine, acetylthiocholine iodide, reduced glutathione, sulphanilamide, ferrous sulfate, semicarbazide, sodium acetate, trichloroacetic acid, hydrogen peroxide, sodium dodecyl sulfate methanol, acetic acid, hydrochloric acid, aluminum chloride, potassium acetate, iron(II) sulfate, potassium ferricyanide, ferric chloride, starch and ascorbic acid. These were bought from Sigma-Aldrich Co. (St. Louis, Missouri, USA). All the chemical reagents that were used were of analytical grades, except otherwise stated.
2.4 Solvent-free microwave extraction

To extract the essential oil, solvent-free microwave extraction method was used. Briefly, this was conducted in a milestone Dry DIST (2008) microwave apparatus. The reactor used has a twin magnetron (2,800 W, 2,450 MHz) of which the maximum power capacity was 1000 W with an increment of 10 W. The importance of this microwave diffuser is to make sure that there is a uniform distribution in the entire plasma-coated PTFE cavity (35 cm × 35 cm × 35 cm). Also, a shielded thermocouple (ATC-300) which is directly placed into the corresponding container is used to monitor the temperature. At the same time, the power regulator of the microwave helps in regulating the temperature. Typically, in the SFME procedure, a fixed quantity (about 50g) of the dried powdered sample is placed in the multimode microwave reactor. The essential oil is then collected after the extraction time which lasted for about 30 minutes.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS of essential oil was carried out using an Agilent 7890 GC. This was attached to an Agilent 5977 MSD and a Zebcon-5MS column (ZB-5MS 30m × 0.25 mm × 0.25 μm) (5%-Phenyl)-methylpolysiloxane. At a flow rate of 2 mL/min, the carrier gas used was GC grade Helium. Also, splitless 1 μL injector was utilized. The temperature of the Injector, Source and Oven were 280°C, 280°C and 70°C respectively. These were ramped at 15°C/minute to 120°C, 10°C/minute to 180°C and 20°C/minute to 270°C and also held for about 3 minutes. The chemical composition of the essential oil of the fertility leaves and horseradish trees was determined according to their retention time, and data was gathered with Chem station.

2.6 Exposure of D. melanogaster to fertility tree and horseradish tree leaves essential oils

The flies (both genders, 3 to 5 days old) were categorized into three different groups. Each category contained 60 flies each. The first group (Control) was placed on yeast diet alone while the second (10 μL FTL) and third (10 μL HTL) groups were placed on yeast diet which contained the essential oil of fertility tree and horseradish tree leaves to a final concentration of 10 μL/g of yeast. These flies were exposed to the treatments for a time-period of twenty-four hours. All experiments were conducted in five replicate.

2.7 Survival and fecundity assay

After exposing the flies to the essential oils in yeast media as described above, their rate of mortality was monitored 0-24 hr, and thereafter, the survival rate was analysed as percentage of flies that survived after 24 hrs. Subsequently, to determine the flies’ fecundity, the number of viable third instar larva that emerged from eggs were assessed. To accomplish this, equal numbers of male and female flies were exposed to the treatment as described above and the adult flies were excluded from the media after 24 hr. The media was thereafter monitored for 7 days for emergence of third instar larva. The number of viable third instar larva that emerged from laid eggs in the diet containing the oils were counted, 7 days after the treatment period.

2.8 Preparation of tissue homogenate

Ice was used to immobilize the flies. They were also homogenized in 0.1 M phosphate buffer which has a pH of 7.4. The homogenates which resulted from this were centrifuged at 10,000 × g and a temperature of 4°C for about 10 min in a Kenxin refrigerated centrifuge Model KX3400C (KENXIN Intl. Co., Hong Kong). Consequently, the pellet was separated from the supernatant and extracted into labeled Eppendorf tubes, which were then used for the various biochemical assays.

2.9 Biochemical analysis

Catalase Shina\textsuperscript{19} and acetylcholinesterase (AChE)\textsuperscript{20} activities as well as total thiol (T-SH)\textsuperscript{21}, reactive oxygen species (ROS)\textsuperscript{22}, thiobarbituric acid reactive substance (TBARS)\textsuperscript{23} levels were determined. The total fly homogenates’ protein content was also determined\textsuperscript{24}.

2.10 Data analysis

The replica readings result were pooled and presented as averages ± standard deviation. To analyze the result, One-way analysis of variance was used. This was followed by Tukey’s post hoc test. The accepted level of significance was \( p < 0.05 \). Software Graph pad PRISM (V.5.0) was used in conducting all statistical analysis.

3 Results

3.1 Chemical Composition of fertility tree and horseradish tree leaf essential oils

The essential oil yields were: 1.38%, w/w, fertility tree leaf, pale yellow with burnt odour and refractive index of 0.82; and 1.40%, w/w, horseradish tree leaf, colourless with

| Table 1 | Physicochemical properties of essential oil from fertility tree and horseradish tree leaves. |
|---|---|---|
| | Fertility tree leaf | Horseradish tree leaf |
| Percentage yield (Weight/dry weight) | 1.38% | 1.40% |
| Physical appearance | Pale yellow | Colourless |
| Odour | Burnt | Burnt |
| Refractive index | 0.82 | 0.99 |

---

*J. Oleo Sci. 69, (8) 941-950 (2020)*
burnt odour and refractive index of 0.99 (Table 1). The five most abundant compounds in horseradish tree leaf essential oil are octadecane (27.56%), heneicosane (21.67%), eicosane (16.65%), benzeneacetaldehyde (14.27%), and phytol (11.72%) while five most abundant compounds in fertility tree leaf essential oil are bis(2-ethylhexyl) phthalate (63.62%), phytol (8.14%), 9,12-octadecadienoic acid (Z,Z)-methyl ester (7.93%), hexadecanoic acid methyl ester (6.55%) and 7-octen-2-one (6.12%), as demonstrated by the GC-MS analysis (Table 2, Figs. 1 and 2). The total percentage of monoterpenoids, oxygenated diterpenoids and oxygenated sesterterpenoids present in fertility tree leaf essential oil were higher compared to horseradish tree leaf essential oil, whereas the total percentage of aliphatic and aromatic hydrocarbons were higher in horseradish tree leaf essential oil compared to fertility tree leaf essential oil.

### 3.2 Effects of fertility tree and horseradish tree leaf essential oils on survival of *D. melanogaster*

We measured the rate of survival of fruit flies exposed to essential oils from fertility tree and horseradish tree leaves. Our preliminary results (data not shown) revealed that both essential oils at doses ≥ 20 µL/g caused 100% mortality of flies after 24 hours of exposure. However, at 10 µL/g exposure of *D. melanogaster* to the essential oil after 24 h, significantly (at 5% level of significance) decreased the rate of survival of flies as against those of the untreated group (Table 3). Our data showed that essential oil from fertility tree and horseradish tree leaves is toxic to *Drosophila melanogaster*, whereas horseradish tree leaf essential oil showed a more potent insecticide action when compared with fertility tree leaf essential oil. Therefore, 10 µL/g exposed flies were used for further studies.

### 3.3 Effect of fertility tree and horseradish tree leaf essential oils on fecundity

Effect of fertility tree and horseradish tree leaf essential oils on reproduction ability were investigated since reproductive capacity is essential in pest control. The average number of third instar larva 7-days post treatment was significantly (at 5% level of significance) reduced by exposure to the essential oils as against those of the untreated group (Table 3). Interestingly, fertility tree leaf essential oil drastically reduced the average number of larva when compared with horseradish tree leaf essential oil.

### 3.4 Effect of fertility tree and horseradish tree leaf essential oils on acetylcholinesterase activity and Oxidative Stress Markers

In order to elucidate probable mechanisms by which *D. melanogaster*
Insecticidal Properties of Essential Oils in Fruit Fly (Drosophila melanogaster)

J. Oleo Sci. 69, (8) 941-950 (2020)

378 melanogaster is affected by the fertility tree and horseradish tree leaf essential oils, the acetylcholinesterase activity as well as oxidative stress markers were determined (Figs. 3 to 8) after 24 h after they were exposed to the essential oil. The result of the effect of exposure of the flies to the essential oils on acetylcholinesterase (AChE) activity in D. melanogaster is shown in Fig. 3, revealing that flies which were exposed to the essential oils exhibited lower AChE activity against those who were not and this was significant at $p < 0.05$. However, the activity of AChE in flies which were given diet with fertility tree leaf essential oil was not significantly different ($p > 0.05$) contrary to the horseradish tree leaf essential oil. There was also an increase in ROS formation after twenty-four hour of exposure to the essential oil (as shown in Fig. 4) through feeding method against

|                | Survival  | Fecundity |
|----------------|-----------|-----------|
| Control        | 100.00 ± 0.00 | 47.50 ± 5.00 |
| 10 µL FTL      | 82.5 ± 6.74*  | 0.12 ± 0.01*  |
| 10 µL HTL      | 55.84 ± 12.51*# | 11.60 ± 3.86*# |

Values represent mean ± SD. *Values are significantly different at $p < 0.05$ from control; #Values are significantly different at $p < 0.05$ from 10 µL FTL group.
the control, this was also significant at \( p < 0.05 \). The results revealed a higher level of TBARS after 24 h of exposure which indicated that lipid peroxidation occurred (as shown in Fig. 5). The levels of total protein (TP) and total thiol (TSH) significantly \( p < 0.05 \) decreased when compared with control after 24 h of exposure (Figs. 6 and 7). There was a significant increase at \( p < 0.05 \) in the activity of CAT when compared to the control after 24 h (Fig. 8).

4 Discussion

The major mechanisms behind the insecticidal properties of several synthetic insecticides are their anticholinergic and pro-oxidant potentials\(^{25-27}\). However, these synthetic pesticides often have their ecological and toxicological concerns\(^{28}\). Hence, the potential environment-
Insecticidal Properties of Essential Oils in Fruit Fly (Drosophila melanogaster)

In this study, we demonstrate the insecticidal activity of the essential oils against fruit flies (D. melanogaster). The exposure of flies to the essential oils significantly decreased their survival and fecundity. As a matter of fact, in our preliminary study (data not shown), we recorded 100% mortality at doses above 20 µL/g at 24 hours post-exposure. The significant insecticidal activity of the essential oils against fruit flies (D. melanogaster) may probably be due to the high concentration of several terpenes in horseradish tree leaf and Bis (2-ethylhexyl)phthalate in fertility tree leaf. According to Regnault-Roger et al., secondary metabolites such as alkaloids, tannins and terpenes are often used as a means of defence against external hostility by plants.

In correlation with the induced reduction in the rate of survival of flies exposed to the essential oils, exposure to the oils also induced a decrease in AChE activity. This result may be linked to a potential interaction between oil components and flies neurotransmitters pathway. Many terpenes has been shown to be inhibitors of AChE.

Interestingly, fertility tree leaf essential oil showed to have more abundance of mono-, di- and sesterterpenoids compared to horseradish tree leaf essential oil; this could thus, support the higher anticholinesterase effect reported for fertility tree leaf essential oil compared to the other sample. Similarly, Olney et al. found out that oxidative stress, which is a result of protracted decrease in AChE activity in the flies, could increase the reduction rate in their survival rate.

The accumulation of lipid peroxidation by-products (TBARS) and the increased production of reactive oxygen species (ROS) observed in this study also suggests the induction of oxidative stress following exposure of the flies to the oils. In addition, it could be suggested that the oil-exposed flies exhibited adaptive response to oxidative stress, since it was possible to observe the initiation of antioxidant signalling pathways and increased activity of key cellular antioxidant enzyme (catalase). It has been reported that compounds from plants can cause toxicity to various insects and may directly hampers the growth stages of cockroaches and fruit fly.

According to Mungli et al., the major component of cellular antioxidant systems are thiols which play an important role in ameliorating free radical assaults and oxidative stress. Despite this, Osburn et al. reported that often, the overall reaction of thiols to oxidative cellular assaults results from the interactions between thiol oxidation as well as its synthesis. Therefore, the connection between the reaction of thiol consumption when ROS is present and its replenishing which results from adaptive response under acute exposure could be the reason why there was a significant reduction in thiol content observed in the treatment groups.

Primarily, endogenous antioxidant molecules helps to protect cellular macromolecules from the assault of free radicals. Catalase catalyzes the reduction of H₂O₂ to H₂O.
and O₂, and thus, helps to protect cell tissues from the harmful peroxidative impact of H₂O₂\(^{[40]}\). Therefore, the induction of ROS and TBARS production in the flies by essential oils exposure could be linked with an increase in catalase activity in the oil treated groups as a means of adaptive response to the surge in free radical generation. It is important to note that terpenoid-rich plant essential oil has shown insecticidal and toxicological effects mediated by oxidative stress and impaired antioxidant systems\(^{[41]}\). Hence, we hypothesize that the abundance of terpenoids in both essential oil used for this study, could contribute majorly to their oxidative stress-mediated insecticidal properties. Specifically, the fact that fertility tree leaf essential oil showed to have more abundance of mono-, di- and sesterterpenoids compared to horseradish tree leaf essential oil could further support the higher oxidative stress indices, and subsequently insecticidal effect observed in flies exposed to fertility tree leaf essential oil.

5 Conclusions

In conclusion, our data suggests that the essential oils from fertility tree and horseradish tree leaves were able to induce significant level of ROS and TBARS production in *D. melanogaster*, coupled with reduced AChE activity and impaired antioxidant system. These observation could be part of the biochemical mechanisms of action by which these essential oils exhibited insecticidal effect and impaired significantly the fecundity of the flies. Therefore, our results point out to the potential application of fertility tree and horseradish tree leaf essential oils and/or their compounds as natural alternative to synthetic insecticides in agricultural and pest control, especially against dipterans.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Statement

This study does not involve any human or vertebrate animal testing that requires ethical approval.

Author Contributions Statement

ASF was involved in project design, data analysis and preparation of the manuscript; OV and AS were involved in data collection; OOB was involved in project design and manuscript proofreading; OG was involved in project design. The authors have read and approved the final manuscript.

References

1. Zolfaghari Emameh, R.; Syrjänen, L.; Barker, H.; Supuran, C.T.; Parkkila, S. *Drosophila melanogaster*: A model organism for controlling Dipteran vectors and pests. *J. Enzyme Inhib. Med. Chem.* **30**, 505-513 (2015).
2. Perry, T.; McKenzie, J.A.; Batterham, P. A Da6 knock-out strain of *Drosophila melanogaster* confers a high level of resistance to spinosad. *Insect Biochem. Mol. Biol.* **37**, 184-188 (2007).
3. Christaki, E.; Bonos, O.; Giannenas, I.; Florou-Paneri, P. Aromatic plants as a source of bioactive compounds. *Agriculture* **2**, 228-243 (2012).
4. Mossa, A.H. Green pesticides: Essential oils as biopesticides in insect-pest management. *J. Environ. Sci. Technol.* **9**, 354-378 (2016).
5. Ayvaz, A.; Sagdic, O.; Karaborklu, S.; Ozturk, I. Insecticidal activity of the essential oils from different plants against three stored-product insects. *J. Insect Sci.* **10**, 21 (2010).
6. Rassem, H.A.; Nour, A.H.; Yunus, R.M. Techniques for extraction of essential oils from plants: A review. *Aust. J. Basic & Appl. Sci.* **10**(16), 117-127 (2016).
7. Burkhill, H.M. *The Useful Plants of West Tropical Africa*. 2nd ed. vol. 1 (Families A-D), Royal Botanic Gardens, Kew, London (1985).
8. Burkhill, H.M. *The Useful Plants of West Tropical Africa*. 2nd ed. vol. 4 (Families M-R), Royal Botanic Gardens, London (1997).
9. Egba, S.I.; Sunday, G.I.; Anaduaka, E.G. The effect of oral administration of aqueous extract of Newbouldia laevis leaves on fertility hormones of male albino rats. *J. Pharm. Biol. Sci.* **16** (2016).
10. Olounlade, P.A.; Azando, E.V.; Houzangbe-Adote, M.S.; Ha, T.B.; Leroy, E.; Moulis, C.; Fabre, N.; Magnaval, J.F.; Hoste, H.; Valentin, A. *In vitro* anthelmintic activity of the essential oils of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* against *Strongyloides ratti*. *Parasitol. Res.* **110**, 1427-1433 (2012).
11. Fahey, H. *Moringa oleifera*, A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Trees for Life Journal* **1**, 5 (2000).
12. Ezeamuzie, T.C.; Amberkedeme, A.W.; Shode, P.O.; Ekwebelem, S.C. Anti-inflammatory effects of *Moringa oleifera* root. *International Journal of Pharmacognosy* **34**(93), 207-212 (1996).
13. Oommachan, M.; Khan, S.S. Plants in aid of family planning. *Ancient Science of Life* **1**(1), 64-66 (1981).
14. Varsha, S.Z.; Dinesh, K.D.; Vaibhao, G.T.; Shital, R.P.
Insecticidal Properties of Essential Oils in Fruit Fly (Drosophila melanogaster)

Efect of aqueous extract of Moringa oleifera seed on sexual activity of male albino rats. Biological Forum 5, 129-140 (2013).

15) Tatiana, M.; Filomena, N.; Emilia, M.; Florinda, F.; Raffaele, C.; Laura, D.; Adelaide, B.A.; Vincenzo, D. Chemical composition and biological activity of the essential oil from leaves of Moringa oleifera Lam. cultivated in Mozambique. Molecules 18, 10989-11000 (2013).

16) Chuang, P.H.; Lee, C.W.; Chou, J.Y. Anti-fungal activity of crude extracts and essential oil of Moringa oleifera Lam. Bioresour. Technol. 98, 232-236 (2007).

17) Zemolin, A.P.; Cruz, L.C.; Paula, M.T. Toxicity induced by Prascoli crispa to fruit fly Drosophila melanogaster and cockroach Nauphoeta cinerea: Evidence for bioinsecticide action. J. Toxicol. Environ. Health. 77, 115-124 (2014).

18) Ogunsuyi, O.B.; Oboh, G.; Ohuokun, O.O.; Ademiluyi, A.O.; Ogunrрук, O.O. Gallic acid protects against neurochemical alterations in transgenic Drosophila model of Alzheimer’s disease. Oriental Pharmacy and Experimental Medicine 20, 89-98 (2020).

19) Shina, A.K. Colorimetric assay of catalase. Anal. Biochem. 47, 389-394 (1972).

20) Ellman, G.L.; Courtney, K.D.; Andres Jr., V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7 (2), 88-95 (1961).

21) Akomolafe, S.F. The effects of caffeine, caffeic acid, and their combination on acetylcholinesterase, adenosine deaminase and arginase activities linked with brain function. J. Food Biochem. 2017, e12401 (2017).

22) Hayashi, I.; Morishita, Y.; Imai, K.; Nakamura, M.; Nakachi, K.; Hayashi, T. High-throughput spectrophotometric assay of reactive oxygen species in serum. Mutat. Res. 631, 55-61 (2007).

23) Jentzsch, A.M.; Bachmann, H.; Fürst, P.; Biesalski, H.K. Improved analysis of malondialdehyde in human body fluids. Free Radical Biol. Med. 20, 251-256 (1996).

24) Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254 (1976).

25) Fetoui, H.; Makni, M.; Garoui, E.M.; Zeghal, N. Toxic effects of lambda-cyhalothrin, a synthetic pyrethroid pesticide, on the rat kidney: involvement of oxidative stress and protective role of ascorbic acid. Exp. Toxicol. Pathol. 62, 593-599 (2010).

26) Lukaszewicz-Hussain, A. Role of oxidative stress in organophosphate insecticide toxicity—Short review. Pestic. Biochem. Physiol. 98, 145-150 (2010).

27) Ghohivand, K.; Valmoozi, A.A.E.; Sahli, M.; Taghpiour, F.; Torabi, E.; Ghadimi, S.; Ghadamyari, M. Bisphosphoramide derivatives: Synthesis, crystal structure, anti-cholinesterase activity, insecticide potency and QSAR analysis. Journal of the Iranian Chemical Society 14, 427-442 (2017).

28) Carvalho, F.P. Pesticides, environment, and food safety. Food Energy Secur. 6 (2), 48-60 (2017).

29) de Carvalho, N.R.; Rodrigues, N.R.; Macedo, G.E.; Bréstot, I.J.; Bolgion, A.A.; de Campos, M.M.; Posser, T. Eugenia uniflora leaf essential oil promotes mitochondrial dysfunction in Drosophila melanogaster through the inhibition of oxidative phosphorylation. Toxicol. Res. 6, 526-534 (2017).

30) Wu, Y.; Guo, S.; Huang, D.; Wang, C.; Wei, J.; Li, Z.; Du, S. Contact and repellant activities of zerumbone and its analogues from the essential oil of Zingiber zerumbet (L.) Smith against Lasioderma serricorne. J. Oleo Sci. 66, 399-405 (2017).

31) da Rocha Voris, D.G.; dos Santos Dias, L.; Lima, J.A.; Lima, K.D.S.C.; Lima, J.B.P.; dos Santos Lima, A.L. Evaluation of larvical, adultical, and anticholinesterase activities of essential oils of Illicium verum Hook. f., Pimenta dioica (L.) Merr., and Myristica fragrans Houtt. against Zika virus vectors. Environ. Sci. Pollut. Res. 25, 22541-22551 (2018).

32) Regnault-Roger, C.; Philogène, B.J.R.; Vincent, C. Biopesticides d’origine végétale, 2nd ed. Lavoisier, Paris (2008).

33) Loizzo, M.R.; Tundis, R.; Conforti, F. Salvia leriifolia Benth (Lamiaceae) extract demonstrates in vitro anti-oxidant properties and cholinesterase inhibitory activity. Nutr. Res. 30, 823-830 (2010).

34) Olney, J.W.; Collins, R.C.; Sloviter, R.S. Exotoxic mechanisms of epileptic brain damage. Adv. Neurol. 44, 857-877 (1986).

35) Ravi, K.; Sita, D.; Janardhan, R. Bioactivity of essential oils and sesquiterpenes of Chloroxylon swietenia DC against Helicoverpa armigera. Curr. Sci. 93, 544-548 (2007).

36) Miyazawa, M.; Ishikawa, Y.; Kasahara, H.; Yamanaka, J.I.; Kameoka, H. An insect growth inhibitory lignan from flower buds of Magnolia fargesii. Phytochemistry 35, 611-613 (1994).

37) Mungli, P.; Shetty, M.S.; Thalak, P.; Anwar, N. Total thiols: Biomedical importance and their alteration in various disorders. Online J. Health Allied Sci. 8 (2), 2 (2009).

38) Osburn, W.O.; Wakabayashi, N.; Misra, V.; Nilles, T.; Biswal, S.; Trush, M.A.; Kensler, T.W. Nrf2 regulates an adaptive response protecting against oxidative damage following diquat-mediated formation of superoxide anion. Arch. Biochem. Biophys. 454, 7-15 (2006).

39) Rand, M.D. The growing potential for Drosophila in neurotoxicology. Neurotoxicol. Teratol. 32, 74-83 (2010).
40) Abolaji, A.O.; Olayi, C.O.; Oluwadahunsi, J.O.; Farombi, E.O. Dietary consumption of monosodium L-glutamate induces adaptive response and reduction in the life span of *Drosophila melanogaster*. Cell Biochem. Funct. 35, 164-170 (2017).

41) da Cunha, F.A.B.; Wallau, G.L.; Pinho, A.I.; Nunes, M.E.M.; Leite, N.F.; Tintino, S.R.; Pereira, A.B. *Eugenia uniflora* leaves essential oil induces toxicity in *Drosophila melanogaster*: Involvement of oxidative stress mechanisms. Toxicol. Res. 4, 634-644 (2015).