

Essential Oils From The Leaves Of Euphorbia Milieu Exert Insecticidal Activity Through Disruption In Ionic Composition.

¹Okonkwo C.O ; ²Ohaeri O.C

¹Department of Biochemistry, University of Calabar, Cross Rivers State, Nigeria

² Michael Okpara University of Agriculture, Umudike Abia State Nigeria.

Corresponding: Kwo C.O

Abstract: Fat bodies and haemolymph were extracted using standard methods from *Periplaneta americana* and *Tettigonia virridissima* exposed for 24 hours to 600mg of essential oil extracted via soxhlet apparatus with hexane from the leaves of *Euphorbia milii*. Biochemical evaluation of fat bodies and haemolymph was carried out to ascertain the possible routes of insecticidal action of the oil. Changes in ionic composition (Mn^{2+} , Mg^{2+} , Na^{2+} , K^{2+} , Ca^{2+}), were analyzed in the fat body while possible changes in the activity of metabolic enzymes (Acetyl cholinesterase, Catalase, Glutathione-S-transferase,) and other biochemical parameters including; reduced glutathione, total protein and glucose concentrations were evaluated in the haemolymph. The results of ionic composition showed a significant decrease ($p < 0.05$) in Magnesium ion in the fat bodies of *P.americana* exposed to *E. milii* oil as compared to the negative control, and a significant increase in Sodium ion when compared to the positive control. However, the oil did not cause any significant change ($p < 0.05$) in the ionic composition of *T.virridissima*, it also did not cause any change in biochemical parameters analysed in the haemolymph of both insects when compared to the control groups. Overall, data from this study provides strong evidence to show that essential oils from *E.milii* may exert their insecticidal efficacy through physiological disruption of ionic composition; they may therefore be classified as organo-chlorine class of insecticides.

Keywords: Essential, Insecticidal, Activity, Disruption, Ionic composition.

Date of Submission: 20-07-2018

Date of acceptance: 04-08-2018

I. Introduction

Insects are major enemies of human, livestock and agricultural crop yield all over the world while insecticides are important tools used for the control of insects and minimizing the damages they cause to agricultural crops as well as improve the quality of life for humans, domestic animals and livestock. For a very long time, farmers have been plagued by insects that make an easy meal of their crops. Aphids, locusts, beetles and caterpillars are some of the insect species that attack crops and cause losses to farmers. Insecticides are therefore employed to reduce the constant assault by these pests and are used by practically all farmers to control a variety of insect pest.

Insects are also chief pathogenic agents that cause many human, animal and plant diseases. They transmit pathogens that cause malaria, dengue fever, yellow fever and leishmaniasis resulting in low life expectancy throughout the world. Nearly half of the world's population is infected with at least one type of insect-borne pathogen or the other. Malaria affects up to 300 million in persons yearly and thrives more in areas of poverty and low economic growth (Sachs and Malaney, 2002). Insects can cause economic damages and losses that can lead to starvation particularly in underdeveloped countries like Nigeria.

In the past, immediate physical reactions such as grooming, swatting or squashing and mud baths were used to combat aggressive insect behaviour until the development of civilization. The earliest insecticides were natural substances derived from minerals or toxic plants. The first known example was the control of insects and mites with ground sulphur compounds by the Sumerians as early as 2500 BC (Pretty and Bharucha, 2015). Extracted compounds from plants have been in use as botanical insecticides for many years. For instance, compounds harvested from *Chrysanthemum* species were used to manage insect pests (Silva-Aguayo, 2009). The history of poisonous plant as agents to control insects started with hellebore by the ancient Romans; it was equally used as a poison for rats. Also, the dried powdered flowers of the pyrethrum daisy have been used in China to control lice (Jide-Ojo *et al.*, 2013). One of the largest groups of modern synthetic insecticides, the pyrethroids is modelled on the pyrethrins (BASF, 2013). A 2009 study has shown that U.S farmers gained a total value of about twenty-one point seven billion dollars each year from the use of insecticides (BASF, 2013).

Plant products have been successfully exploited as insecticides, insect repellents and insect anti-feedants (Mordue., 1998). Higher plants are a rich source of novel natural substances that can be used to develop environmentally safe products for insect control (Arnason *et al.*, 1989). Since the synthetic pesticides used in the

control of insect pests such as organophosphate pesticides and organochlorine insecticides have been associated with various forms of diseases in humans (Mergel., 2010). There is an urgent need to develop safe alternatives that are cost effective, convenient to use and environmentally friendly (Mostafa *et al.*, 2012). These insecticidal effects are manifested through toxicity, anti-feedant, growth inhibition, suppression of reproductive behaviour and reduction in egg production and fertility (Mostafa *et al.*, 2012). Many plant species contain substances that protect them from predators; these substances can be extracted and used to produce effective, natural insecticides. They are believed to possess many advantages over the synthetic pesticides (Muhammed, 2015). They are also more accessible in less developed countries, environment-friendly and are also becoming increasingly effective and affordable. They consist more than 10% of the pesticide market in the United States and come in different formulations like; dust, liquid and aerosol. (Ecoworld Nature and Technology, 2004).

The synthetic pesticides currently in use such as the organophosphate and organochlorine insecticides have been associated with various forms of cancer, neurological disorders and lung irritations in humans (Mergel, 2010). Agriculturists who apply these insecticides in farms come in contact with these dangerous pesticides and may be prone to nervous system damages. "Pesticide drift" may also occur as pesticides are sometimes carried by wind and water to non-target areas. They penetrate groundwater, pollute streams and harm wildlife. (Nash, 1994). There is therefore need for a credible alternative to the available pesticides particularly in the developing countries where there are inadequate occupational safety standards, protective clothing and washing facilities, insufficient enforcement, poor labelling, illiteracy and insufficient knowledge of pesticide hazards (Pimentel and Greiner, 1996). A large portion of sprayed insecticides are likely to reach destinations other than their target species because they are sprayed across an entire agricultural field or household (George, 2004). Flowing water can also carry pesticides into aquatic environments thereby affecting other species. Other ways of non-target exposure result from low-standard production, transportation and storage conditions (Tashkent, 1998).

A call for agricultural sustainability has led to numerous initiatives by the government to develop environmentally friendly agricultural practices (Lynch *et al.*, 1996). The Government of Canada have initiated a program aimed at providing to providing basic amenities for the development and implementation of lower-risk approaches in the management of pests (Agriculture and Agri-Food Canada., 2003). These sustainability programs continuously emphasize the importance of developing organic insecticides for pest control as they assume that natural insecticides present less risk to the environment than synthetic insecticides, which agrees with the general opinion of the public (James, 1990). Influential scientific papers have also proposed a higher level of sustainability using natural products (Reganold *et al.*, 2001) as the global use of synthetically produced pesticides have resulted in dare consequences. It has become necessary to produce safer, more environmentally friendly and effective alternatives that can displace the synthetically manufactured pesticides and are easier to use. Among the biopesticides, botanicals are presently at the fore-front due to the eco-toxicological properties of the non-botanicals. Many secondary metabolites from various plants are popular for their insecticidal efficacies, and are sometimes used domestically to kill or minimize the impact of insect pests (Kim *et al.*, 2013). Plants are very useful in ecological systems to control pests since they contain a rich source of bioactive chemicals (Pavela, 2008).

Studies have shown that a large number of insects and mite species have developed resistant to conventional pesticides (Harmingway *et al.*, 2002). Incidence of multiple resistances is also on the increase. On the other hand, natural compounds with complex chemistry and structure should effectively combat and overcome the observed resistance, coupled with the additional advantage of rapid environmental degradation and low toxicity to non-target organisms. Plant-based oils have been exploited in this respect for indoor and outdoor pesticidal activities. A major consideration in the use of pesticides is the development of resistance by target organisms. Repeated application of a particular insecticide continuously, increases pest resistance (Damalas, 2011). Resistance results when an insecticide does not completely kill the pest population, surviving pests manage to tolerate the insecticide and after multiple generations of the pest, the pest population will be dominated by these insecticide-tolerant individuals. To avoid this, regular interchange of insecticides with different modes of action on the target pest is important. The availability and sustainability of effective pest management technologies are necessary to meet the growing needs of a growing population. Environmental factors, non-target species, dietary residues, consumer and applicator exposure are issues to be considered when developing an insecticide. (BASF Crop protection 2013). Also, the use of natural pesticides rather than the synthetic ones is likely to result in healthier agricultural soils with more microbial diversity, even though resistance to biological pesticides could occur, it is likely to be less common.

In search of alternatives to synthetic pesticides, scientists have continued to study the efficacy of essential oils from so many aromatic plants. The discovery of bioactive secondary metabolites from plants which are toxins to herbivores that attack them opened the vista for their assessment as insecticides. These secondary compounds represent a large reservoir of chemical structures with biological activity (Duke *et al.*, 2010). Natural products, including insecticides can be described as chemicals, minerals or biological compounds

produced by living organisms. The goal of an insecticide is to kill or reduce to the barest minimum the damaging effects of insect pests and vectors of diseases. Essential oils are one of the most tested natural products against insect pests. They act by affecting some biological processes in the pest such as growth rate, life span and reproduction. (Isikber *et al.*, 2006).

There are presently many mechanisms by which various insecticides control insect pests through the disruption of various specific vital biological processes (BASF Crop Protection, 2013). Based on physiological targets, insecticides can be classified as: neuromuscular poisons, respiratory poisons, gut disruptors and insect growth regulators. (BASF Crop Protection, 2013). Diversity in mode of action is a very important tool for sustaining the ability of an insecticide to control insect pests. By rotating pest control agents that work through different modes of action, insecticide resistance can be reduced or even eradicated completely. Repeated application of insecticides with the same mode of action may contribute to pest resistance. This research aims to discover and explore insecticides with novel modes of action in order to provide the much needed diversity to 'fight' against insecticide resistance.

Euphorbia milii (Crown of thorns) is a plant whose empirical observation/evidence strongly suggests that it possesses chemical compounds with insecticidal properties. It is a low-growing evergreen shrub with very thorny grooved stems and branches. Not much has been reported about its insecticidal activity despite the empirical evidence of its insect repellent activity. However, Kiran *et al.*, (2015) in a preliminary investigation reported that the leaf extract of *E. milii* exhibited certain levels of insecticidal action against Diamond back moth (*Plutella xylostella*). On close and repeated observation, the leaves of *Euphorbia milii* are usually not affected by insect pest attacks even when other plants around it suffer serious attacks from these pests. The plant is known by the common names; crown of thorns, Christ plant or red shrub and is not indigenous to Nigeria, but is believed to have been imported to Nigeria from India (Ombrello., 2015). There are claims by inhabitants of Okwuta in Ibeku Umuahia Abia State Nigeria that the plant may be linked with insecticidal tendencies. However, no research to our knowledge has investigated the possible route of insecticidal or larvicidal activity of this plant. This research aims to investigate the possible route of insecticidal action of essential oils from the leaves of *Euphorbia milii*



Euphorbia milii also known as crown of thorns, Christ plant or Christ thorn is a specie of flowering plants in the spurge family; *Euphorbiaceae*, class: *Magnoliopsida* and Order: *Euphorbiales*, native to Madagascar. The species name commemorates Baron Milius, a one time Governor of Réunion, who introduced the specie to France in 1821 (Ombrello, 2015). It is suspected that the specie was introduced to the Middle East in ancient times, and legends associate it with the crown of thorns worn by Christ (Ombrello, 2015). It is also found in Kerala, India, where an ancient tale suggests that the Jews inhabiting Kerala might have brought the "Christ thorn" from Israel to India. The crown of thorns is now found all over the world as widely grown ornamental specie (Eggle, 2002). *Euphorbia* flowers always have only one stigma (the female part that receives pollen) and one stamen (the male part that releases the pollen). It is a succulent climbing shrub growing to 1.8 m tall, with densely spiny stems (<http://www.arkive.org/crown-of-thorns/euphorbia-milii>). Most crown of thorn varieties have stunning red bracts, although pink, yellow or whitish varieties are also known (Ombrello, 2015).

A characteristic feature of all *Euphorbia* species, including the crown of thorns, is the presence of milky latex, or sap, which is secreted by the plant through broken stems, or damaged roots and leaves (Ombrello, 2015). The latex, which is present in all parts of the plant, is usually poisonous and probably developed in order to protect the plant from herbivores (<http://www.arkive.org/crown-of-thorns/euphorbia-milii>). Ingestion of the plant causes severe irritation of the mouth and digestive systems, and may induce nausea, diarrhoea and swelling, while direct contact with the sap may cause skin irritation, inflammation and blistering (Oldfields, 1997). There are several cultivars and varieties with different coloured bracts (pink, yellow, white, orange) growth habits. (IUCN Red list, 2010). It is generally unpalatable and causes severe blistering when contact is made with the latex. The poisonous principles have been identified as phorbol esters. Phorbol esters activate protein kinase C which in turn increases Protein phosphorylation and may thus alter multiple enzyme

and other protein functions. Effects may result in cyto-skeletal damage and tumour promotion (Veterinary medicine library, 2015). Generally most animals and humans are affected by *Euphorbia* and may experience severe irritation of the mouth and gastrointestinal tract, accompanied sometimes with haemorrhage and diarrhoea (Veterinary medicine library, 2015). Other general signs include blistering, swelling about the eyes and mouth, excessive salivation and emesis, abdominal pain and weakness (Veterinary medicine library, 2015). It has been reported to be effective as a molluscicide (Eduador *et al.*, 2010). In another study, very low concentrations of lyophilized latex was found to be toxic to *planorbidae* snails but less toxic to oligochaeta, planktonic crustacea, fishes, frog and tadpoles. (Oliveira and Paumgarten, 2000).

II. Materials and Methods

The major instruments used in this research include; Soxhlet extractor Manufactured by B.BRAN Scientific and Instrument Company England, Thermo Scientific Rotary evaporator Model R-300 USA, Electric blender, AKAI TOKYO JAPAN, Model No: BDOO11DA-1033M, made in PRC. Echotherm Chilling/Heating dry bath, weighing balance (Symmetry Colle-Parmer Instrument Co, USA).

Collection and Identification of Plant Samples

The leaves of *Euphorbia milii*, was harvested from GPS mobile location Latitude=4.961538, Longitude= 8.349273, No 4 Edim Otop close, off victory way, Satellite town Calabar, Cross Rivers State, Nigeria on the 18th of October 2015. The plant appeared healthy, leaves bright green in colour and flowers intact at the time of the harvest. Prior to screening, the plant was identified by a botanist in the Department of Biological Sciences (Botany), College of Natural Sciences, Michael Okpara University of Agriculture Umudike Abia State while the SWAN all-purpose commercial insecticide was purchased from Whatt market in Calabar, Cross Rivers State. The leaves were carefully selected, washed with distilled water and air-dried for a period of three weeks to reduce moisture content. The dried leaves were pulverized using an electric blender, put in an air-tight container and used subsequently for soxhlet oil extraction. Oil was extracted by continuous extraction in soxhlet apparatus for sixteen hours using n-hexane as solvent according to the method described by A.O.A.C., (1990). Hexane has been reported as the best solvent used to remove oily and fatty materials (non-polar) (Chen *et al.*, 2012)). It also has a lower boiling point (68°C) than most of the other organic solvents, thus making it an ideal solvent especially with respect to heat sensitive compounds.

Fifty grammes (50g) of the ground plant sample was weighed and loaded into a thimble placed inside the soxhlet extractor, six hundred millilitres (600 mls) of n-hexane was added to a round bottom flask which was attached to the soxhlet extractor and condenser. The side arm was lagged with glass wool. The solvent was heated using an isomantle to 70° C and began to evaporate moving through the apparatus to the condenser. The condensate was collected in the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle continued lasting for 16hours. After many cycles the desired compound was concentrated in the distillation flask and solvent was removed using a rotary evaporator. The non-soluble portion of the extracted solid remained in the thimble, and was discarded. Five grams (4g) of oil with pH 3.4 was realised from fifty grams (50g) of ground powder, amounting to 8% oil yield.

Essential oils obtained were used for insecticidal activity test on insects. Cotton lint soaked with 600mg of essential oil was placed inside a cylindrical, perforated empty tin; the test insects (10 adults in each tin) were placed in the tins and covered with a net to prevent escape of insects and to avoid death by suffocation. The insects were monitored for 24 hours to observe mortality. The control passed through similar treatment but with distilled water only (Mohd *et al.*, 1992). All experiments were done in triplicates. Insects from the contact insecticidal test above were used for biochemical studies within 24 hours of conducting the contact insecticidal activity test.

Test insects were identified by an Entomologist at the Department of Zoology and Environmental Biology, Faculty of sciences, University of Calabar. He also assisted with the extraction of fat body and haemolymph from insects. All insects used were of adult stage (except for mosquito whose larva was used), this is because they exhibit the greatest destructive and infectious tendencies at this stage. Insects were healthy and very active as at the time of the experiment, no symptoms of any disease or weakness was observed. Their response to environmental factors and stimuli, movement and general behaviour indicated that they were physiologically sound at the time of the procedure. The adult cockroach was recognised by its reddish brown colour and a pale brown band around the edge of its pronotum, they also have a pair of slender jointed cerci at the tip of the abdomen. *T.virridissima* was distinguished by its very long and thin antennae which can reach up to three (3) times the length of the body differentiating it from grasshoppers which carry short antennae. Larvae are green while Imago are pale green to brown with a thin brown longitudinal stripe on their back, wings are well developed (William, 2005).

Periplaneta Americana (American Cockroach) was trapped from a domestic sewage pit at Satellite town Calabar, in a plastic container perforated at the base and baited with some domestic food particles.

Tettigonia viridissima, (Great Green Bush Cricket) was caught from the grass fields in the University of Calabar Staff quarters. *P.americana* was provided with commercial and domestic household food, while *T.virridissima* was provided with fresh green tiger nut grass throughout the period of the experiment.

The anticoagulant buffer used for fat body extraction was prepared by adding 7.88g of 41mm citric acid, 3.92g of 98mm sodium hydroxide (NaOH), 11.096g of 0.19M sodium chloride (NaCl) and 0.497g of 1.7mM Ethylenediaminetetraacetic acid (EDTA). These were dissolved in five hundred (500) mls of distilled water; the volume was made up to 1 litre using distilled water to arrive at pH of 4.6 (Strand *et al.*, 1997).

Insect hemolymph was extracted according to the method described by Harrison *et al.*, (2013).The inter-segmental membrane was punctured using a sterile needle and the fluid was drained using a micro-syringe, the fluid was transferred immediately to an air-tight tube and capped for analysis.

Biochemical Studies and evaluation of test insects.

Insects were divided into six (6) groups. Groups A and B and C consisted of *Periplaneta americana* treated with distilled water, *E.milii* oil and SWAN commercial insecticide (positive control) , while C, D and E consisted of *Tettigonia virridissima* treated with distilled water, *E.milii* oil and SWAN insecticide respectively. Insects were dissected in ice-cold anticoagulant buffer pH 4.6 (98mM NaOH, 0.19M NaCl, 1.7mM EDTA and 41mM citric acid) for collection of the fat body tissues (Strand *et al.*, 1997). Physicochemical changes in the fat body were evaluated, including ionic composition; Calcium ion (Ca²⁺) was determined by the method of Stern and Lewis (1957), Magnesium ion (Mg²⁺); Rice and Lapara (1964). Manganese ion (Mn²⁺) as described by Pawar *et al* (2001), Sodium ion (Na²⁺) as described by Trinder (1951) and Potassium ion (K⁺) as described by Terri and Sesin (1958)

The activities of some enzymes were investigated in the haemolymph, which include; Acetyl cholinesterase by the method of Ellman (1961), Catalase; Johansson and Borg (1988). GST activity; Habig *et al.*, (1974) with minor modification by Anosike *et al.*,(1991).The 1.0ml in 2% ethanol enzyme assay mixture contained 0.5mM CDNB (0.02ml),1.0mM GSH (0.05ml), 0.68ml of distilled water and 100mM phosphate buffer (K₂HPO₄/KH₂PO₄ ;PH=6.5 (0.2ml). GSH was determined by the The DTNB and GR Recycling method described by Trent and Lynette (2012). Also total protein and blood sugar were also determined in haemolymph according to the Biuret method of Weichselbaum, (1946) modified by Tietz,(1995), and glucose oxidase method as described by Mikac-Devic *et al* (1972) respectively.

III. Results

Electrolyte composition of fat body from *Periplaneta americana* exposed to *E.milii* oil

	Ca ²⁺ (mg/dL)	Mn ²⁺ (µg/dL)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Mg ²⁺ (mg/dL)
Control(A)	9.91	1.00	138.58	3.97	1.98
E.M. oil(B)	9.57 ±0.18	1.00 ±0.04	139.79 ±0.21*	4.05 ±0.04	1.92 ±0.02* ^a
SWAN(C)	9.52 ±0.24	0.99 ±0.01	136.39 ±0.95	3.97 ±0.02	1.97 ±0.01

Values are expressed as mean ±SEM, n = 3

a = significantly different from control at P < 0.05

* = significantly different from SWAN at P < 0.05;

Electrolyte composition of fat body extract from *Tettigonia viridissima* exposed to *E.milii* oil

	Ca ²⁺ (mg/dL)	Mn ²⁺ (µg/dL)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Mg ²⁺ (mg/dL)
Control(D)	9.96 ±0.10	0.99 ±0.04	139.17 ±2.36	3.95 ±0.07	2.02 ±0.10
E.M. oil(E)	9.76 ±0.10	0.95 ±0.02	144.47 ±2.21	3.95 ±0.04	1.97 ±0.02
SWAN(F)	9.70 ±0.25	0.93 ±0.02	139.51 ±1.60	3.82 ±0.07	1.90 ±0.04

Values are expressed as mean ±SEM, n = 3

No significant (P>0.05) change in ionic composition was observed in the fat body of *T.virridissima*

Biochemical indices measured in Haemolymphs of *P. americana* exposed to *E.milii* oil

	AChE activity (IU/L)	Gluthathione-S-transferase activity (IU/L)	Catalase activity (mmol/L)	Protein conc. (g/dL)	Glutathione conc. (mg/dL)	Glucose conc. (mmol/L)
Control(A)	2433.63	1.12	18.47	0.01	3.20	0.13
	±61.80	±0.06	±2.36	±0.00	±0.10	±0.03
E.M. oil(B)	2391.40	1.20	19.90	0.04	3.20	0.13
	±30.80	±0.12	±2.76	±0.01	±0.21	±0.03
SWAN(C)	2426.93	1.25	19.00	0.03	3.27	0.10
	±76.93	±0.06	±1.61	±0.02	±0.12	±0.00

Values are expressed as mean ±SEM, n = 3

There was no significant (P>0.05) change in the above parameters in treated insects when compared to the control,

Biochemical indices measured in Haemolymph of *T. viridissima* exposed to *Emilii* oil

	AChE activity (IU/L)	Gluthathione-S-transferase activity (IU/L)	Catalase activity. (mmol/)	Protein conc. (g/dL)	Glutathione conc. (mg/dL)	Glucose conc. (mmol)
Control(D)	2478.57	1.12	21.57	0.07	3.40	0.23
	±17.25	±0.05	±2.12	±0.01	±0.31	±0.03
E.M oil(E)	2490.23	1.30	19.63	0.06	3.33	0.33
	±60.28	±0.12	±2.51	±0.03	±0.33	±0.19
SWAN(F)	2445.50	1.15	19.27	0.03	3.57	0.17
	±73.65	±0.02	±1.22	±0.02	±0.17	±0.03

Values are expressed as mean ±SEM, n = 3

There was no significant (P>0.05) change in the above biochemical parameters in the treated group when compared to the control.

IV. Discussion

The result of the electrolyte analysis of insect fat body, revealed a significant (P<0.05) increase in sodium ion (Na⁺) as compared to the positive control and decrease in magnesium ion (Mg²⁺) as compared to the negative control in *P.americana* treated with *E.milii* oil extract. This means that *E.milii* oil extract may induce physiological changes in test insects by decreasing Mg²⁺ stores in the insects ‘fat body. However, this was not the case for *T.viridissima*, this shows that different pests may react to a particular insecticide differently probably based on their chemical composition and response to xenobiotics. We also observed in the preliminary study, that *E.milii* oil caused more mortality in *P.americana* than in *T. virridissima* in all the treated groups. *T.viridissima* seemed to fight the invasion of the insecticide (toxin) thus making it less susceptible and easier to develop resistance to pesticides, this is a confirmation of our observation in a previous research using other insecticidal plant extracts on grass hoppers (Okonkwo and Ohaeri, 2013., Okonkwo and Onyeji, 2018).

Electrolyte balance is very important in insects as it controls the transmission of nerve impulses and electrical potential especially Na⁺ and K⁺ ions. For each ATP molecule consumed, the sodium-potassium pump expels three sodium ions from the cell but brings in only two potassium ions, leading to a net expulsion from the cell of one positive charge for each pump cycle, eventually making the interior of the cell negative by 50 to 100 mV (BASF, 2013). Thus any disruption in electrolyte balance will disrupt the action potential causing impaired transmission of nerve impulses, spasm, paralysis and even death.

In carrying out its insecticidal activity on *P.americana*, the plant oil may have altered the concentration and function of some important biochemical electrolytes including; Mg²⁺ and Na²⁺, which may have resulted in great disturbances in nerve transmission, tremors, seizures, irritability, basal ganglia calcifications, cardiac arrest, coma and death of the insects. The oil is likely an ion channel disruptor which belongs to the organo-chlorine class of insecticides. The mechanism of insecticidal action of *E.milii* may be different from that of SWAN (positive control) in that; while *E.milii* reduced Mg²⁺ concentrations in insects as compared to the negative control, SWAN did not have any such effect. Also *E.milii* caused significantly higher concentrations of sodium ion as compared to the positive control (SWAN). SWAN (the positive control) which contains pyrethroids acts by keeping the sodium channels in neuronal membranes open, thus increasing the likelihood of an action potential.

There was no significant (P>0.05) change in acetylcholinesterase (AChE), Glutathione-S-transferase (GST) and catalase activities in *P.americana* treated with *E.milii* oil as compared to the negative control and positive control (SWAN) in both insects. Also no significant (P>0.05) change was observed in protein, GSH and glucose concentrations. This means that *E.milii* oil may not have direct effect on the above mentioned

parameters but may operate through other mechanism like electrolyte disturbances and ion channel modulations etc as observed from the result.

Unlike SWAN, which functions as a pyrethroid (class II) neurotoxin in insects and also causes genetic damage and chromosomal abnormalities (Amer *et al.*, 1993), as well as kills beneficial insects and animals together with the targeted insects (Pascual and Peris, 1992) with the development of resistance in insects exposed frequently rendering it ineffective (Martinez *et al.*, 1991), *E.milii* oil which seem to belong to the organochlorine class of insecticides, being a natural compound may not pose such harm to non target species. Also, resistance to this natural oil is not likely to occur very easily given the complexity of natural chemicals that combine together to make this oil effective. They may thus serve as good alternatives to the synthetic insecticides.

V. Conclusion

E.milii oil seem to function basically as an organo-chlorine insecticide specifically; ion channel disruptors, by disrupting important biochemical electrolyte and thus obstructing the transmission of nerve impulses causing spasm and eventual death. Its mode of action is quite different from that of SWAN which functions by opening up ion channels and causing action potential as well as DNA damages.

References

- [1]. Agriculture and Agri-Food Canada (2003). Pesticide risk reduction and minor use programs: improving ways to manage pests with new technology. Government of Canada. www.agr.gc.ca/prmup. Accessed Nov 10, 2016.
- [2]. Amer, S.M., Ibrahim, A.E.S and El-Sherbeny, K.M. (1993). "Induction of chromosomal aberrations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin ". *Journal of Applied Toxicology* **13** (5): 341–345.
- [3]. Anosike, E.O., Uwakwe, A.A., Monanu, M.O and Ekeke G.I (1991). Studies on human erythrocyte glutathione-S transferase from HbAA, HbAS and HbSS subjects. *Biochem.Biomed.Acta* **50**:1051-1055.
- [4]. Arnason, J. T., Philogene, B. J .R and Morand, P. (1989) Insecticides of plants origin. American Chemical Society Symposium Series Vol. **387**. Washington.
- [5]. Association of Official Analytical Chemists (AOAC) (1990). Extraction of oil content, standard method, <https://archive.org/stream/gov.law.aoac>. Accessed December 2016.
- [6]. Baden Aniline and Soda Factory (BASF) Crop Protection (2013). Insecticide mode of Action. Technical Training manual Pg4. <http://www.agproducts.basf.com>. Accessed February 2015.
- [7]. Chen, Min., Tianzhong, Liu., Xiaolin, Chen., Lin, Chen., Wei Zhang, Junfeng .Wang., Lili Gao, Yu.Chen and Xiaowei Peng (2012). Subcritical co-solvents extraction of lipid from wet microalgae pastes of *Nannochloropsis sp.* *Eur Journal of Lipid Science and Technology* **114**:205-212.
- [8]. Damalas, C.A and Eleftherohorinos I.G (2011). "Pesticide exposure, Safety Issues and Risk assessment Indicators" *Int Journal of Environmental Rsch and Pub Health* **8**(12):1402-19.
- [9]. Duke, S.O., Cantrell, C.L., Meepagala, K.M., Wedge, D.E., Tabanca, N and Schrader, K.K (2010). Natural toxins for use in pest management. *Toxins* **2**:1943-1962.
- [10]. Ecoworld Nature and Technology in harmony (2004). Organic farming in India. Available online at www.ecoworld.com>pollution and Toxins> Chemicals, Accessed August 2017.
- [11]. Eduardo, C., Oliveira, Filho., Barbara, R., Geraldino, Deise., Coelh, R., Rosangela, R., De-Carvalho, Francisco., Paumgarten, J.R (2010). Comparative toxicity of *E.milii* latex and Synthetic molluscides to *Biomphalaria glabrata* embryos. *Chemosphere* **81**(2):218-227.
- [12]. Egli, U (2002) Illustrated Handbook of Succulent Plants: Dicotyledons. Springer-Verlag, Berlin, Germany: 301-305.
- [13]. Ellman, Diane. K., Courtney, Valentino Andres jr and Robert, M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* **7**(2): 91-95.
- [14]. George, Tyler Miller (2004). Sustaining the Earth: An Integrated Approach. Thomson / Brooks/Cole : 211-216.
- [15]. Habis, W.H., Pabst, M.J., Fleischner, G., Gatmaitan, Z., Arias, I.M and Jakoby, W.B (1974). "The Identity of Glutathione S-Transferase B with Ligandin, a Major Binding Protein of Liver". *Proc. Natl. Acad. Sci. U.S.A.* **71** (10): 3879– 82.
- [16]. Harmingway, J., Field, L., Vontas, J (2002). An overview of insecticide resistance. *Science* **289**: 96-97.
- [17]. Harrison, Jon.F., Arthur woods, H., and Stephen, P. Robert (2013). Ecological and Environmental physiology of Insects. *The quarterly review of Biology* vol **88**; No1 P:274. <http://www.arkive.org/crown-of-thorns/euphorbia-milii>. Accessed jult, 2015.
- [18]. Isikber, A.A., Alma, M.H., Kanat, M and Karcia, A (2006). Fumigant toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all life stages of *Tribolium confesum*. *Phytoparasitica* **34**:164.
- [20]. International Union for Conservation of Nature (IUCN) Red List (2010). The IUCN Red List of Threatened Species. <http://www.iucnredlist.org/>. Accessed Nov, 2016.
- [21]. James, K.H (1990). Risk perceptions and food choice: An exploratory analysis of organic- versus conventional-produce buyers. *Risk Analysis* **10**: 367– 374.
- [22]. Jide-Ojo, Constance C., Daniel, T. Gungula and Opeolu O.Ojo (2013). Extracts of *Jatropha curcas* L exhibit significant insecticidal and grain protectant effects against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) *J.of Stored Pcts and Postharvest Research* **4**(3):44-50.
- [23]. Johansson, L.H., Borg, L.A (1988). A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal Biochem* **174**(1):331-6
- [24]. Kim, S.I., Yoon, J.S., Jung J.W., Hong K.B., Ahn Y.J and Kwon H.W (2010). Toxicity and repellency of *origanum* essential oil and its components against *Tribolium castaneum* (Coleoptera: Tenebrionidae) adults. *Journal of Asia-Pacific Entomology*, **13** : 369-373.
- [25]. Kiran, Shehzadi., Munir, Ahmad., Imran, Bodlah and Asim, Grulzar (2015). Susceptibility of Diamond back moth, *Plutella xylostella* (Lepidoptera: plutellidae) to some *euphorbiaceae* Plant extracts under laboratory conditions. *Asian J Agri Biol* **3**(4):145-149.

- [26]. Lynch, S., Greene, C and Kramer-LeBlanc, C (1996) Proceedings of the third national IPM symposium /workshop: Broadening support for 21st century IPM. U.S.Department of Agriculture, Economic Research Service, Natural Resources and Environment Division. www.citeseerx.ist.psu.edu/viewdoc. Accessed; Nov 2016.
- [27]. Martinez-Carrillo, J. L., Schouest Jr L. P and Miller T. A. (1991). "Responses of populations of the tobacco budworm (*Lepidoptera: Noctuidae*) from northwest Mexico to pyrethroids". *Journal of Economic Entomology* **84** (2):363–366.
- [28]. Mergel, Maria (2010). Effects of Pesticides on Human health. (www.toxipedia.org/plugins). Accessed October 2016.
- [29]. Mikac-Deric, Dusanka., Stankoric, H and Wurth, G (1972). Manual and Automated Determination of Glucose in Blood with Glucose oxidase and Molybdate/Iodide as Redox Catalyst. *J. Klin. Chem. Klin. Biochem* **10**: 5372- 373.
- [30]. Mohd, Aspoliah Suraki., Mawardi, Rahmani., Abd, Rahman Manas and Shozo, Takahashi (1992). Toxicity Studies of Plant Extracts on Insects and Fish. *Pertanika* **14**(1):41-44.
- [31]. Mordue A J (1998). Azadirachtin - A review of its mode of action in insects. In: Kleeberg H (Ed.). *Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones* Germany; pp. 1-4.
- [32]. Mostafa, M., Hemayet, Hossain., Anwar Hossain, M., Pizush, Kanti Biswas and Zahurul, Haque. M (2012). Insecticidal activity of plant extracts against *Tribolium castaneum* Herbst. *Journal of Advanced Scientific Research* **3** (3):80-84.
- [33]. Muhammad, Sarwar (2015). The killer Chemicals for Control of Agriculture Insect Pests: The Botanical Insecticides. *Int.j.Chem.Biom Sci* **1** (3):123-128.
- [34]. Nash, E.P. (1994). What's a life worth. New York: New York Times. www.nytimes.com. Accessed Nov 2014.
- [35]. Okonkwo, C.O and Ohaeri, O.C (2013). Insecticidal Potentials of some selected plants. *Journal of Chemical and Pharmaceutical Research* **5**(4):370-376.
- [36]. Oldfield, S. (1997) *Cactus and Succulent Plants – Status Survey and Conservation Action Plan*. IUCN/SSC Cactus and Succulent Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK . <https://portals.iucn.org/library/efiles/documents/Chapter1>.
- [37]. Oliveira-Filho and Paumgarten (2000). Toxicity of *E.milii* latex and Niclosamide to snails and non-target aquatic species. *Ecotoxicol Environ Saf* **46**(3):342- 50.
- [38]. Ombrello, D .T (2015). *Crown of Thorns, Plant of the Week, UCC Biology Department*, www.arkive.org › Species › Plants and algae. Retrieved; July 2015, Accessed 11/8.2016.
- [39]. Pascual, J.A. and Peris, S.J (1992). "Effects of forest spraying with two application rates of cypermethrin on food supply and on breeding success of the blue tit (*Parus caeruleus*)". *Environmental Toxicology and Chemistry* **11** (9): 1271–1280.
- [40]. Pavela, R (2008). Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (*Diptera: Culicidae*). *Parasitology Research* **102**,555–559.
- [41]. Pawar, R.B., Padgaonkar, S.B and Sawant, A.D (2001). Spectrophotometric determination of manganese (II) by picolinaldehyde nicotinoylhydrazine. *Indian Journal of Chemical Technology* **8**: 200-203.
- [42]. Pimentel, D and Greiner (1996). Environmental and Socio-economic costs of pesticide use. In D Pimental ed. *Techniques for Reducing Pesticides: Environmental and Economic Benefits*. Chichester, England John Wiley and Sons. In Press.
- [43]. Pretty, Jules and Zareen, Perez Bharucha (2015). Integrated Pest Management for sustainable Intensification of Agriculture in Asia and Africa. *Insects* **6** (1):152-182.
- [44]. Reganold, J.P., Glover, J.D., Andrews, P.K and Hinman, H.R (2001) Sustainability of three apple production systems. *Nature* **410**: 926–930.
- [45]. Rice, E.W and Lapara, C.Z (1964). Rapid ultra micro spectrophotometric determination of Mg. *Clin. Chim. Acta* **10**:360-364.
- [46]. Sachs, Jeffrey and Malaney, Pia (2002). The economic and social burden of malaria. *Nature* **7**:415 (6872):680-5.
- [47]. Silva-Aguayo, G (2009). Botanical Insecticides. In *Radcliffe's IPM World Textbook*, edited by E.B. Radcliffe, W.D. Hutchison, and R.E. Cancelado. <http://ipmworld.umn.edu/chapters/SilviaAguayo.htm>. Accessed Aug, 2015.
- [48]. Stern, J and Lewis, W.H.P (1957). The colorimetric estimation of Calcium in serum with O- cresolphthalein complexone. *Clin. Chim Acta* **2**:576-80
- [49]. Strand, M.R., Witherell, R.A and Trudeau, D (1997). Two microplitis demolitor polydnavirus, mRNAs expressed in hemocytes of pseudoplusia includes contain a common cysteine-rich , domain. *J. Virol* **71**:2146-2156.
- [50]. Tashkent (1998). Part I. Conditions and Provisions for developing a national strategy for biodiversity conservation. <https://www.cbd.int/doc/world> Retrieved; Sept 17, 2007 accessed; May 2016.
- [51]. Terri, A.E., Sesin, P.G (1958). Colorimetric method of K⁺ estimation using Na tetraphenylboron. *Am J. Clin. Path* **29**:86
- [52]. Tietz, N W (1995). *Clinical Guide to Laboratory Tests*. 3rd Edition. WB Saunders Company. Philadelphia. PA : 518-519.
- [53]. Trent, E. Tipple and Lynette, K. Rogers (2012). Methods for the Determination of Plasma or Tissue Glutathione Levels. *Methods Mol Biol.* **889**: 315–324.
- [54]. Trinder, P (1951). A rapid method for the determination of sodium in serum. *Analyst* **76** (907):596.
- [55]. Veterinary Medicine Library (2015). Crown of Thorns (*Euphorbia milii*) Plants toxic to animals. UIUC Library Gateway Homepage. (www.library.illinois.edu/) Accessed July 2016.
- [56]. Weichselbaum, T. E (1946) An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am J Clin Pathol.* **10**:40-9.
- [57]. William H Robinson (2005). *Urban Insects and Arachnids: A handbook of Urban Entomology*, Cambridge University Press. <https://books.google.com.ng>. Accessed July, 2016.

Kwo C.O " Essential Oils From The Leaves Of Euphorbia Milieu Exert Insecticidal Activity Through Disruption In Ionic Composition.." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.4.(2018) PP 46-53