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Research Article
Mechanism of Insecticidal action of Oil Extracted from the Leaves of *Cassia occidentalis*

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Abstract

Background and Objective: Insecticides are agents used to control the harmful effects of insect pests and vectors on agriculture, livestock and human. The danger associated with most synthetic insecticides currently in use, have made it necessary to look for natural alternatives of which botanicals take an upper hand. The research aimed to determine the mechanism of insecticidal action of oil extracted from the leaf of *Cassia occidentalis* by carrying out biochemical investigations on insects exposed to the oil. **Materials and Methods:** Fat bodies and haemolymph were extracted using standard methods from *Periplaneta Americana* (American cockroach) and *Tettigonia virridisima* (Bush cricket) exposed for 24 h to 600 mg of oil extracts from the leaves of *Cassia occidentalis* (Coffee senna). Biological fluids were analyzed for changes in electrolyte composition and other toxicity indices of insects including; acetylcholinesterase, GST and catalase activities. **Results:** A significant decrease ($p<0.05$) in k^+ and Mg^{2+} was observed in *P. americana* as compared to the control, while a significant increase ($p<0.05$) in GST activity and protein and decrease ($p<0.05$) in acetyl cholinesterase activity was observed in *T. virissdisima* as compare to the control. **Conclusion:** Overall, data from this study provides strong evidence to show that oil extract from the leaf of *C. occidentalis* exhibits its insecticidal effects through inhibition of acetyl cholinesterase activity of target pests and disruption of ionic composition making it a potential neuromuscular cum organo-chlorine pesticide for future use.

Key words: Mechanism, insecticidal, *Periplaneta Americana*, GST activity, *Cassia occidentalis*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Insects are major enemies of human, livestock and agricultural crop yield all over the world[1]. They are also chief pathogenic agents that cause many human, animal and plant diseases[2]. They can cause economic damages and losses that can lead to starvation particularly in underdeveloped countries like Nigeria.

Plant products have been successfully exploited as insecticides, insect repellents and insect anti-feedants[3]. 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[4]. Since the synthetic pesticides currently used in the control of insect pests such as organophosphate pesticides and organochlorine insecticides have been associated with various forms of diseases in humans[5]. There is an urgent need to develop safer alternatives. Natural pesticides are believed to possess many advantages over the synthetic ones[6].

Influential scientific papers have proposed a higher level of sustainability using natural products[7]. Among the bio-pesticides, botanicals are presently at the fore-front. 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[8]. Again, studies have shown that a large number of insects and mite species have developed resistance to conventional pesticides[9]. On the other hand, natural compounds with complex chemistry and structure could effectively combat and overcome the observed resistance, coupled with the additional advantage of rapid environmental degradation and low toxicity to non-target organisms. Regular interchange of insecticides with different modes of action on the target pest is important for effective pest management. Also, the use of natural pesticides rather than the synthetic ones is likely to result in healthier agricultural soils with more microbial diversity.

C. occidentalis (Fedegoso) is a plant whose empirical observation/evidence strongly suggests that it possesses chemical compounds with insecticidal properties. It is a shrub which grows about 5-8 m high and found majorly in tropical areas. It is known as coffee senna in English, *Akidi ogbara* in Igbo, *Dora rai* in Hausa and *Aboo rere* in Yoruba Nigeria[10]. It is claimed to scare away insects and reptiles from its environs by indigenes of Akwa Ibom State in Nigeria, thus making it a popular domestic plant in that part of the world. The species give off a foul odour when damaged[11].

The constant assault to man, livestock and agricultural crops calls for action. Since most synthetic insecticides have been associated with various levels of toxicity to man[5], the need for a safer and more wholesome alternative of natural origin believed to be more advantageous than their synthetic counterparts[6] cannot be over emphasized. This research aimed to investigate the possible mechanism by which oil extracted from the leaf of *C. occidentalis* carry out its insecticidal effect as a promising insecticidal agent for future use.

MATERIALS AND METHODS

This research was carried out at the University of Calabar, while biochemical evaluation of biological fluids was done at the University Teaching Hospital located at Eteagbor, Calabar Municipal, Cross Rivers State, Nigeria. The research was an aspect of a PhD research work that lasted for 24 months, starting in October, 2015 and completed in September, 2017.

Equipments: The major equipments 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, weighing balance Symmetry Colle-Parmer Instrument Co, USA.

Collection, identification preparation and oil extraction from plant: The leaves of *C. occidentalis* were 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 at the time of 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 Nigeria. The leaves were washed and air-dried for two weeks. Dried leaves were pulverized into a fine powder which was used for oil extraction. Oil was extracted by continuous extraction in soxhlet apparatus for 16 h using n-hexane as solvent according to the AOAC[12] method. Oils obtained were used for insecticidal activity test according to the method desribed by Sukari *et al.*[13].

Identification and trapping of test insects: 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, healthy and active at the time of the experiment. Their response to environmental factors, movement and general behaviour indicated that they were physiologically sound at the time of the procedure. The adult *P. americana* was recognized by its reddish brown colour and a pale brown band around the edge of its pronotum. *T. virridissima* was distinguished by its long and thin antennae. Imagoes were brown with thin brown stripes on their back, wings were well developed[14]. *Periplaneta Americana* was trapped from a domestic sewage pit at Satellite town Calabar, in a plastic container perforated at the base and baited with boiled rice and stew. *Tettigonia viridissima* (Great Green Bush Cricket) was caught from the grass fields in the University of Calabar Staff quarters and provided with fresh green alfalfa leaves throughout the period of the experiment.

Extraction of biological fluids from test insects: The anticoagulant buffer used for fat body extraction was prepared by adding 7.88 g of 41 mm citric acid, 3.92 g of 98 mm sodium hydroxide (NaOH), 11.096 g of 0.19 M sodium chloride (NaCl) and 0.497 g of 1.7 mM Ethylenediaminetetraacetic acid (EDTA). These were dissolved in 500 mL of distilled water; the volume was made up to 1 L using distilled water to arrive[15] at pH of 4.6. Insect hemolymph was extracted according to the method described by Harrison *et al.*[16]. 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 6 groups. Group A, B and C consisted of *Periplaneta americana* treated with distilled water, *C. occidentalis* oil and SWAN (positive control), respectively, while C, D and E consisted of *Tettigonia virridissima* treated with distilled water, *C. occidentalis* oil and SWAN, respectively. Insects were dissected in ice-cold anticoagulant buffer and fatbody was collected[15]. Physicochemical changes in the fatbody were evaluated, including ionic composition; Calcium ion (Ca^{2+}) was determined by the method of Fischl and Schwartz[17]. Magnesium ion (Mg^{2+}) by the method of Chromy *et al.*[18]. Manganese ion (Mn^{2+}) as described by Pawar *et al.*[19], Sodium ion (Na^{2+}) as described by Jackson[20]. Potassium ion (K^{+}) as described by Castilho and Stradiotto[21].

The activities of some enzymes were investigated in the haemolymph, which include; Acetyl cholinesterase by the method of Khalil and Kasim[22], Catalase; Mahmoud[23], GST activity; Habig *et al.*[24] with minor modification by Anosike *et al.*[25], GSH concentration was determined by the method of Tipple and Rogers[26]. Also total protein and blood sugar were determined by the method of Weichselbaum[27] and modified by Tietz[28] and glucose oxidase method as described by Srikanth *et al.*[29], respectively.

Statistical analysis: Results for the biochemical evaluation of test insects was analysed using; One-way analysis of variance (ANOVA) with *post hoc* test.

RESULTS

In Table 1, *C. occidentalis* caused a significant decrease ($p<0.05$) in potassium (K^{+}) and Magnesium (Mg^{2+}) ions when compared to the control.

In Table 2, a significant increase ($p<0.05$) in, Mn^{2+} was observed in *T. virridissima* treated with *C. occidentalis* oil extract as compared to the positive control (SWAN).

In Table 3, no significant ($p>0.05$) change was observed in all biochemical indices tested when compared to the control.

In Table 4, a significant decrease ($p<0.05$) in AChE activity and increase ($p<0.05$) in GST activity and protein concentration was observed in *T. virridissima* treated with *C. occidentalis* oil extract as compared to the control.

Table 1: Electrolyte composition of fat body extract from *Periplaneta americana* exposed to *C. occidentalis* oils and standard insecticide (SWAN)

Parameters	Ca^{2+} (mg dL^{-1})	Mn^{2+} (µg dL^{-1})	Na^{+} (mmol L^{-1})	K^{+} (mmol L^{-1})	Mg^{2+} (mg dL^{-1})
Control	9.91±0.16	1.00±0.01	138.58±1.14	3.97±0.02	1.98±0.01
SWAN	9.52±0.24	0.99±0.01	136.39±0.95	3.97±0.02	1.97±0.01
C.O. oil	9.97±0.02	1.01±0.02	138.71±0.87	3.83±0.01*[a]	1.91±0.02*[a]

Values are expressed as Mean±SEM, n = 3, *Significantly different from SWAN at $p<0.05$, [a]Significantly different from control at $p<0.05$

Table 2: Electrolyte composition of fat body extract from *Tettigonia viridissima* exposed to *C. occidentalis* oils and standard insecticide (SWAN)

Parameters	Ca^{2+} (mg dL^{-1})	Mn^{2+} (µg dL^{-1})	Na^{+} (mmol L^{-1})	K^{+} (mmol L^{-1})	Mg^{2+} (mg dL^{-1})
Control	9.96±0.10	0.99±0.04	139.17±2.36	3.95±0.07	2.02±0.10
SWAN	9.70±0.25	0.93±0.02	139.51±1.60	3.82±0.07	1.90±0.04
C.O. oil	6.97±2.99	1.03±0.02*	143.77±2.04	4.04±0.15	2.01±0.07

Values are expressed as Mean±SEM, n = 3, *Significantly different from SWAN at $p<0.05$, [a]Significantly different from control at $p<0.05$

Table 3: Biochemical indices measured in haemolymphs of *Periplaneta americana* exposed to *C. occidentalis* oil and SWAN

Parameters	AChE activity (IU L^{-1})	Glutathione-S-transferase activity (IU L^{-1})	Catalase activity (mmol L^{-1})	Protein concentration (g dL^{-1})	Glutathione concentration (mg dL^{-1})	Glucose concentration (mmol L^{-1})
Control	2433.63±61.80	1.12±0.06	18.47±2.36	0.01±0.00	3.20±0.10	0.13±0.03
SWAN	2426.93±76.93	1.25±0.06	19.00±1.61	0.03±0.02	3.27±0.12	0.10±0.00
C.O. oil	2413.13±62.48	1.15±0.04	19.07±2.18	0.06±0.01	3.17±0.23	0.17±0.07

Values are expressed as Mean±SEM, n = 3

Table 4: Biochemical indices measured in Haemolymph of *Teltigonia viridissima* exposed to *C. occidentalis* oil and SWAN

Parameters	AChE activity (IU L^{-1})	Gluthathion-S-transferase activity (IU L^{-1})	Catalase activity (mmol L^{-1})	Protein concentration (g dL^{-1})	Glutathione concentration (mg dL^{-1})	Glucose concentration (mmol)
Control	2478.57±17.25	1.12±0.05	21.57±2.12	0.07±0.01	3.40±0.31	0.23±0.03
SWAN	2445.50±73.65	1.15±0.02	19.27±1.22	0.03±0.02	3.57±0.17	0.17±0.03
C.O. oil	2451.23±26.52[a]	1.61±0.07*[a]	19.00±1.36	0.18±0.04*[a]	3.53±0.28	0.40±0.17

Values are expressed as Mean±SEM, n = 3, *Significantly different from SWAN at $p<0.05$, [a]Significantly different from control at $p<0.05$

DISCUSSION

In Current study, the decrease in acetylcholinesterase activity, K^+ and Mg^{2+} concentrations, suggested that *C. occidentalis* oil may exert its insecticidal effect through disturbances in ionic composition and inhibition of acetylcholinesterase activity in insects. This electrolyte imbalance may cause spontaneous contractions of the insect, which may lead to spasm and eventual death.

The insecticidal efficacy of oil extracts from *C. occidentalis* has been widely studied and reported by many scientific articles, which corroborate with our findings in this research. In 2014, Venkatesan *et al.*[30] reported a moderated level of adulticidal activity of crude leaf extracts of *Cassia occidentalis* against the urban malaria vector; *Anopheles stephensi* Liston. In another study, the adverse effects of proteinous extract obtained from *Cassia occidentlis* on fecundity, longevity, percentage egg hatching and nutritional indices in *Spodoptera litura* was reported[31].

In another study in Senegal, the leaves of *C. occidentalis* was reported to be useful in the protection of cowpea seeds (*Vigna unguiculata)* against *Callosobruchus maculatus*. The study also reported the presence of some fatty acids including; linoleic, oleic and stearic acids in *C. occidentalis* oil suspected to be responsible for the observed toxicity on *Callosobruchus maculatus*[32]. This agrees with a recent study by Okonkwo and Ohaeri[33] which studied the insecticidal efficacy of hexane oil extracted from the leaves of *C. occidentalis*. The study revealed the presence of oleic acid, methyl stearate, hexadecanoic acid and decane amongst others as the active principles likely responsible for the insecticidal action of *C. occidentalis* oil.

C. occidentalis has also been reported to suppress wood damage by termites causing mortality of worker termites within the shortest duration of applicaion[34]. The plant has also been justified as having a realistic mortality result for larvae of filarial vector; mosquito culex quinquefasciatus. It is reported to be a natural weapon for mosquito control[35]. Murugan *et al.*[36] has also reported that *C. occidentalis* evoked mortality rates comparable to a pyrethrin-based positive control.

The increase in GST activity observed in *T. virridissima* shows the response of the insects towards the toxin that has invaded it. However, the effect of the oil still prevailed. High intracellular concentrations of GSTs allow them to function as biomarkers for localizing and monitoring injury to defined cell types. The GSTs are one of the most efficient xenobiotic detoxification systems in all animals[37]. In insects, GST has been recognized for their importance in the metabolic detoxification of insecticides[38]. The significant decrease ($p<0.05$) in acetyl cholinesterase activity observed in the haemolymph of this group (Table 4) when compared to the negative control indicates that *C. occidentalis* oil inhibits acetyl cholinesterase activity in *T. viridissima* thus portraying it as a neuro-toxic insecticide.

Worthy of note is the fact that while *C. occidentalis* oil did not cause any significant change in acetyl cholinesterase (AchE) activity in the haemolymph of *P. americana*, it caused a significant ($p<0.05$) decrease in the same enzyme in the haemolymph of *T. viridissima*. This means that the biological composition and complexity of a particular type of insect may also play a role in determining the course and mechanism of activity of an insecticide.

In carrying out its insecticidal activities, the oils were also found to alter the concentration and therefore function of some important biochemical electrolytes, which may have resulted in alteration in nerve transmission, tremors, seizures, irritability, basal ganglia calcifications, cardiac `arrest, coma and death of the insects.

If the active principles found in these oils are carefully isolated, purified and reconstituted into an industrially active and commercially available form, we may have in

our hands another potent insecticide, with comparative advantage over the synthetic insecticides.

CONCLUSION

Oil extracts from the leaves of *C. occidentalis* evoke insecticidal effects on insect pests through disruption of electrolyte balance and inhibition of acetylcholinesterase activity in haemolymph thus making it a potential organochlorine cum neurotoxic insecticidal agent of botanical origin.

SIGNIFICANCE STATEMENT

This study discovered the mode of insecticidal action of *C. occidentalis* oil that can be beneficial for the development of a novel organochlorine cum neurotoxic insecticide. The study will help researchers to uncover the critical areas of insecticidal mechanism of action of botanical insecticides that many researchers were not able to explore. Thus a new theory on mode of action of a novel natural insecticide of botanical origin may be arrived at.

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