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Formulation Of Biopesticide From Leucasmartinicensis(Jacq.) R.Br. (Whitewort) Extract For Control Of Adult Acanthoscelides Obtectus (Beans Weevil)

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ABSTRACT
In this study the repellent properties of L. Martinicensis on beans weevils were investigated. The oil obtained was purified with the use of a micro column and then characterized using Gas Chromatography Mass Spectrometer (GC-MS) instrument. The characterization revealed the presence of numerous bioactive compounds with their peak area, retention time, and % area which possess strong insect repellent activities. The powder extract was used to formulate a stable insect repellent in the form of a tablet. The formulation was applied to beans that contained 80 weevils at normal room temperature. After 72 hours, 50 weevils were recorded dead inside the beans that contained the formulated tablet while 20 weevils were recorded dead inside the beans that contained the commercial products.Therefore, the efficiency of the formulated product was higher compared to that of the commercial product.The formulation has proven to be very valuable as pests' repellent, which will be a good substitute to synthetic repellents.

Keywords: *Biopesticide, Leucas martinicensis, Adult Acanthoscelides.*

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I. INTRODUCTION

The use of organophosphate pesticide and other synthetic chemicals are getting reduced /being prohibited globally because of their poisonous effects on human beings and its eco-system, residual toxicity, environmental problems, pest out-breaks and drastic effects on beneficial pests. But Bio pesticide that are extracted, for example, from *leucas martinicensis* seed can tackle pests to make it eco-friendlier, economically viable for the farmers. *L. martinicensis*is thought to have originated in South America and West Indies. The *L. martinicensis* seeds contains a thousand of chemical components. It is remarkable the occurrence of the so-called "flavonoid", which are very rare. They appear in *L.martinicensis* in more than two hundred types. By far the most active yet studied is the alkanoid.(Hyde&Wursten, 2009).

Pesticides are the chemical substances that are used to decimate, repulse, prevent, inhibit and control the pests creating environmental nuisances and help to increase the yield in agricultural sector (Kumar et al., 2019). Practically, all chemical pesticides are poisons and pose long term danger to the environment and humans, through their persistence in nature and body tissue. There are mainly two types of pesticides: Bio pesticides and Chemical pesticides. Over the next 20 years, crop production will have to meet the needs of a rising human population. This has to be done without damaging the other public goods – environment and social – that farming brings. There will be no "silver bullet" solution to the impending food production challenge. Rather, a series of innovations and research must be carryout to meet the different needs of farmers according to their local conditions (Bastiaans et al., 2008).

The research on the leaf extracts of *L.martinicensis* plant by (Maramorosch,2007).Reveal that the plant is a quality repellent of adult mosquitoes. The highest repellency on the adult culex mosquitoes was observed to be 0.00 over 5 and 60 minutes at 20.0% concentration and lowest being 1.00 and 0.67 over 5 and 45 minutes respectively at 25% concentration of the extract. Although these results show a very slight activity, the plant *L.martinicensis* is very commonamong the rural people as very active repellent on adult mosquitoes. Another famous mosquito repellent plant called citronella was found to perform poorly in keeping off mosquitoes and

bugs, but there is promising research in the U.S. on the repellent properties of a substance found in tomatoes (Estifanoss, 2003). However, repellency of *L.martinicensis* as shown in the research increases with increased concentration of the extract. This implies that, it is possible that the existence of certain compounds in the plant like alkaloids might be responsible. Alkaloids compounds extracted from the skin of poison frog (dendrobatids) from the Smithsonian Institution, Virginia, were found to repel adult mosquitoes and that very little amount was required to have poisonous effect.

Bio pesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria and certain minerals.Bio pesticide contains carbon and seem to be less toxic to humans, plants and animals. For example, eucalyptus oil, mahogany oil, citronella oil and neem oil have pesticidal applications and are considered as bio-pesticides. Bio-Pesticides are of three types. And they are as follows: micro pesticides, biochemical and plant incorporated protectants pesticides (Ware, 2008).

The active ingredients in micro pesticide are microorganism such as virus, bacteria and protozoa. Micro pesticides have vast application in the control of different pests. The commonly use micro pesticide are sub species and strain of *Bacillus thuringiensis*. Plant incorporated pesticide are produce from the generic material of the plants such as root, leaves, seed oil and bark of plants while biochemical pesticide are naturally occurring pesticides such as insect pheromones (Ware, 2008).

L.martinicensis (Jacq.) R. Br. which is also known as Whitewort belong to the family of Lamiaceae (Hyde and Wursten, 2009), is a vertical muscular aromatic annual tree growing up to 1.3m to 1.5m high. It is broadly spread in the tropical parts of Africa, Arabia, Asia and America (Muhammad et al., 2012).The plant has a local name of "Bunsurunfadama" in Hausa part of Nigeria and a common plant in the Northern part of Nigeria. Ethnobotanical survey of *Leucas* showed that the brew of leaves and aerial parts are used for the treatment of kidney disorders, rheumatism and inflammations (Agra et al., 2007). Hot water decoction of the plant is also used for the treatment of cough (Minja, 2011). It has also been used to inhibit and to healdiarrhoea. For the cure of fevers, the new harvested leaves are rubbed on or the whole plant made into an inoculation and used as a mist fumigation (Fowler, 2006). In West Africa, the ash of the plant is used to repulse mosquitoes (Brown, R.2017).

Mosquito plants, they are actually plants that possess minty odor that repel mosquitoes, examples of such plants include *L. martinicensis.*(Melanie, 2007).*L.martinicensis* is an annual herb which is used for repellant of mosquito due to minty odor, it is an erect plant which is usually unbranched, with length of up to 1m, whichare finely hairy. They possess opposites leaves, ovate to ovate lanceolate with the margin coarsely serrate- crenate inflorescence of several space with many flowered verticals having long thistle- like calyx teeth. The flowers they possess are small and white in colour (Hyde, 2009). The stems of *L. martinicensis* are retorse pubescent, having petiole of 0.7 - 1.5 cm, the leaves are reduced to ceneate. The nutlets are dark brown ablong-ovoid and shiny

II. MATERIALS AND METHOD

2.1Materials: Hydrothermal distillation apparatus, Gas Chromatography Mass Spectroscopy (GC-MS), digital weigh balance, column chromatography, tableting machine, analytical balance.

The glass wares include; 500ml, 100ml measuring cylinder, 250ml and 100ml beaker, glass funnel, 250ml separating funnel, 500ml round bottom flask, wash bottle, retort stand, porcelain mortar and pestle glass rod. Acacia, lactose, sample collection and treatment, distilled water, tap water, sodium sulphate, silica gel, light magnesium carbonate.

2.2Sample Collection and Treatment

Fresh shoots of sample of the plant *L.martinicensis* were collected around Dadinkowa, last gate of Jos south LGA of Plateau state. The fruits were air dried for one week. The dried fruits were then crushed and ground into powdered form.

2.3Rotary Evaporator.

After substances get evaporated by heating, the gas phase substances will go into condensing part to get condensed. In this process, gas substances are transferred via the glass tubes. After gas phase substances got condensed into the liquid phase, the liquid substances were transferred into receiving flash, which is the final destination of the extracted substance. The recovered oil was then collected and stored at 40c for further analysis.

2.4 Purification of the Essential Oil

The extracted oil was purified in a micro column prepared with silica gel and sodium sulphate respectively.

The oil was allowed to pass through the column under gentle pressure using a pump and was collected in a beaker and then transferred into an amber storage bottle at 40℃ for further analysis.

2.5 Gas Chromatography (GC-MS) Analysis

The GC-MS analysis was adopted from the method described by(Joseph et al., 2020). It reveals the various compounds and their corresponding spectra.

2.6Formulation

Ten grams of the sample, 3.5 g of Acacia, and 3.0 g of Lactose were weighed accurately into a porcelain mortar and mixed thoroughly until a homogenous mixture wasobtained. The powder mixture was then introduced into the compaction unit of a tableting machine and was compressed by a punch. The tablet was removed and weighed and stored.

III. RESULTS AND DISCUSSION

3.1 Table 1:
GC-MS Analysis of the Essential oil from *L.Martinicensis*

No.of peak	Retention time	Area (%)	Compound	Molecular Formular	Molecular Weight (g/mol)
1	3.434	18.43	Ethylene oxide	C_2H_4O	44.052
2	4.63	1.8	Butane	C_4H_{10}	58.12
3	5.181	25.95	Methylene chloride	CH_2cl_2	84.93
4	5.948	4.05	Acetic acid	CH_3COOH	60.052
5	7.119	3.16	(2E)-1-Hydroxy-2-propanone	$C_3H_7NO_2$	89.09
6	9.148	2.07	1-Methyl-1H-pyrrole	C_5H_7N	81.115
7	14.245	5.4	3-chloro-1,2-propanediol	$C3H_7CIO_2$	110.54
8	14.56	1.24	Phenol	C_6H_6O	94.113
9	15.675	1.31	2-propenamide	C_3H_5NO	71.079
10	17.856	1.55	Oxirane,3-butenyl-	$C_6H_{10}O$	98.143
11	18.742	28.28	1,7,7-Trimethylbicyclo [2.2.1] heptan-2-one	$C_{10}H_{16}O$	152.233
12	19.496	3.03	Benzofuran	C_8H_6O	118.135
13	21.6	1.36	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.177
14	27.831	2.36	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282.5

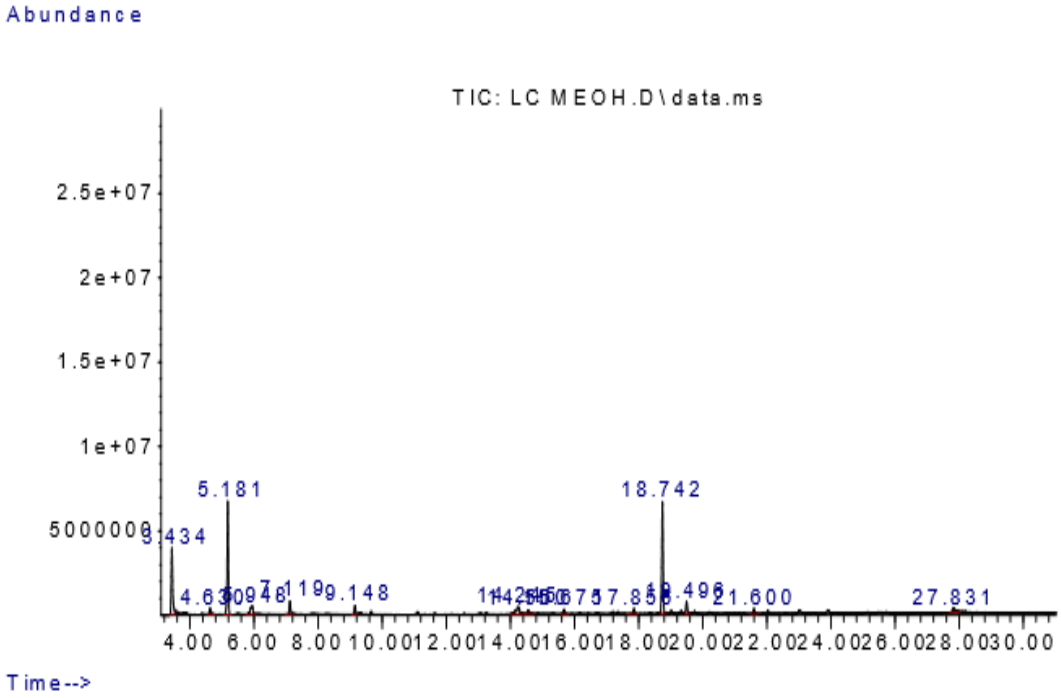

Figure1: GC-MS Mass Chromatogram of*L.Martinicensis*

3.2 DISCUSSIONS

Table 1. shows the breakdown of compounds with insect repellent properties from GC-MS analysis of *L. martinicensis.*

From the results obtained, the most abundant compounds are 1,7,7-Trimethylbicyclo [2.2.1] heptan-2-one with peak area of 28.28%, Methylene chloride with peak area of 25.95% and Ethylene oxide with peak area of 18.43%. These all have been reported by previous studies to have strong repellent properties(Noudogbessi *et al*., 2013).

Furthermore, the following compounds appeared to be less in abundance, though have been reported to have repellent properties 2-propenamide with peak area1.31%,2-Methoxy-4-vinylphenol with peak area of 1.36% and Octadec-9-enoic acid with peak area of 2.36%.

Results from GC-MS analysis of *L.Martinicensis* essential oil (EO) compared with those reported in literature (Estifanos, 2003) confirmed that this EO can have different chemical contents as a function of their origin and habitat where plants are grown as well as of climatic conditions. Endogenous (genetic, development, etc.), botanical source, edaphic and climatic factors could alter the chemical composition of plants.

The essential oil was incorporated into a powder and was later formed into a tablet to enhance the efficiency of the essential oil and the desired product was obtained.

This tablet tends to control the release of the fragrance from the essential oil thereby increasing the shelf life.

IV. CONCLUSION

In this study the repellent properties of *L. Martinicensis* on beans weevils was investigated. The oil obtained was purified with the use of a micro column and then characterized using Gas Chromatography Mass Spectrometer (GC-MS) instrument. The characterization revealed the presence of numerous bioactive compounds list with their peak area, retention time, and % area which possess strong insect repellent activities.

The powder extract was used to formulate a stable insect repellent in the form of a tablet which was applied to beans that contained weevils at normal room temperature. The efficiency was higher compared to that of the commercial products.

This performance is correlated with it being an organic compound enriched with alkanoids, volatileoils and flavonoids with antioxidants properties which are absorbed and desorbed from beans surface thereby limiting the diffusion of oxygen which are also responsible for the repellency of pests

This research reaffirms the possibility of using indigenous Plateau State plants withpesticidal properties for the control of Beans Weevils.

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