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Potentials of Plant Oil Extracts (*Azadirachta indica* and *Ocimum suave*) in Single and Mixed Forms against Adult House Fly (*Musca domestica*)

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Abstract

Aims: To determine the insecticidal potentials of plant oil from neem(*Azadirachta indica*) seeds and wild basil (*Ocimum suave*) leaves applied as individual oils and mixed equal proportion (50:50) against adult house flies (*Musca domestica*), thereby showcasing its attributes and effectiveness in the proper management of house flies.
Study design: The experiment was setup on a completely randomized design using two replicates per concentration's formulation.
Methodology: Oils from the extracts of *Azadirachta indica* and *Ocimum suave* were formulated as emulsifiable concentration at 0.005, 0.010, 0.015, 0.20/50 ml of water, and then sprayed on 15 newly emerged reared adult house flies in cages at an hour interval for six hours.
Results: There was significant difference ($P < 0.05$) in the percentage mortality of adult house flies as the concentration increased from 0.005 – 0.20/50 ml water of both the single formulations (*A. indica* and *O. suave*) and the equally mixed 50:50 ratio. There was also significant difference as the time of exposure increased from the first hour to the sixth hour. The concentration of *A. indica* and *O. suave* required to cause 50% mortality of adult house fly was 0.066/50 ml water at 3.4 hours and 0.072/50 ml at 3.8 hours respectively, while the concentration of the mixed oils at equal proportion (50:50) required to cause 50% mortality of house fly was less (0.065/50 ml water) and at 3.4 hours.
Conclusion: This study has therefore revealed the potency of the plant oil extracts (*A. indica* and *O. suave)* as single and mixed at equal proportion (50:50) against house fly (*Musca domestica*). However, the formulation of the mixed plant oil required to cause 50% mortality of the house fly was less, showing higher potential over the single formulations. Therefore using the mixed plant extracts is recommended for the proper management of house fly.

Keywords: *Azadirachta indica;* formulations; mortality; *Musca domestica; Ocimum suave*; potentials.

1. Introduction

Insects have the largest variety of specimens among the entire animals, according to scientific findings, 80% of the known animal's specimens in the world comprises of the insect class [1, 2]. The house fly *Musca domestica,* is of the suborder cyclorrhapha, it is found all over the world and most widely distributed domestic insect accounting for 98% of all flies in human habitations [3].

House flies are medically important insects worldwide. Their adults are not only pestiferous but portray nuisance attributes, playing roles as reservoirs and mechanical carriers of several pathogens causing disease in man and its livestock and in turn the environment. The larvae stages of these insects are also myasis producing agents in both man and their livestock, thus leading to economic loss in agronomic sector. House flies can carry both human and livestock pathogens on the spongy mouth parts, body, leg hairs (setae) and stick pads on their feet (tarsi) which are coated with sticky substance which allows it adhere while resting or climbing on non-horizontal surfaces. Their exoskeleton have certain electrostatic charges that attract negative and neutral charged particles, their gastrointestinal tract are conducive for the easy access and passage of protozoan parasites (*Ascaris lumbricoides, Cryptosporidium parvum* etc.); bacterial diseases (typhoid, dysentery etc.); viruses (poliomyelitis, viral hepatitis). Alternatively pathogens can be regurgitated on surfaces as regurgitation always precedes feeding causing progressive accumulation of human pathogens both in the environment and the flies' alimentary tract [3, 4, 5].

Previous studies have documented over 50 species of synanthropic flies associated with unsanitary conditions and involved in vectoring human and livestock pathogens in the environment which can be transmitted as airborne particles. When infected persons defecate openly, there are chances that there will be a contact between flies and pathogen positive feacal matter [6, 3].

Chemical insecticides was introduced during World War II, so as to reduce pest population, however, they leave residues on foods (grains, milk, vegetables and fish) [7], accumulate, persists and excessively exposed to the environment [7] as a result of excessive application[8, 9, 10]. Also, their broad-spectrum natural activities, in contrast to pests and advantageous insects equally, made it an ill-fated choice for use in agriculture after World War II [7, 9]. With the incessant usage of insecticides, insect resistance pest species were born pesticide residues were observed to be present in unexpected places [7]. These problems highlights the need for proper management of fly population without leaving residues for further accumulation in the environment, birth of insecticide resistance species, pest resurgence and impotency on non-target pest including natural predators, parasites and pathogens. Plants' extracts such as nicotine, rotenone and pyrethrum as pesticides has been in use for a long time [11]. Therefore the interest on plants as potential source of insect control agent as a safer alternative has been awakened to replace conventional organic chemicals [10, 12], of which Neem, *Azadirachta indica* of the meliaceae family has shown amazing pesticidal activities and is used by both households and farmers to control a wide variety of pest such as mosquitoes [13], house dust mites [14], desert locust [15]bean weevil [16], melon fly [17], fruit fly [18].

In Nigeria it is formally called dogoyaro and used to combat toothache and typhoid serving as remedy to many other illnesses, whereas, the Basil, *Ocimum basilicum*of the lamiaceae is one of the most important herbs to many cultures and cuisines. With over 50 species and more than 60 varieties of *O. basilicum*presently existing of which *O. suave* is derived from. Basil oil has anti-viral, antimicrobial, antioxidant and anti-cancer properties [19, 20].

Several studies have evaluated the potentials of many plant materials against pest insects of which the use of *Azadirachta indica* and *Ocimum suave* has been studied [3, 10, 11, 18, 21, 22, 23]. However, to our knowledge the investigation of the potency of the mixed oil extracts has not been investigated.

Thus, this present investigation was therefore carried out to determine the insecticidal potentials of plant oil from *Azadirachta indica* and *Ocimum suave*applied as individual oils and mixed equal proportion (50:50) against adult house flies (*M. domestica),* thereby showcasing its attributes and effectiveness in the control of house flies in the environment.

2. Materials and Methods

2.1 Preparation of Plant Materials

2.1.1 Collection and preparation of insecticidal plant material

Fresh leaves of *O. suave* (wild basil) were collected from Issele-Azagba in Aniocha North Local Government Area of Delta State (6^0 16' 0' N 6^0 36' 0' E) and were identified as *O. suave* by the Department of Botany, Delta State University Abraka. Fresh leaves were placed in a wooden cabinet (1.0 × 0.5 × 1.0 m) to slowly dry for 2 weeks at room temperature. The leaves were pulverized in a motorized high speed-grinder and then sieved with a 0.1 mm mesh size. The particles were placed in an air tight container to prevent active components from escaping. This method was adopted from [3, 22, 23].

Seeds of *A. indica were* collected from Ebor-Iyede in Isoko North Local Government Area of Delta State (5^0 31 29 N 6^0 14' 90' E) and were identified as *A. indica* by the Department of Botany, Delta State University Abraka. The seeds were sundried for 2 weeks and beaten to remove their endocarp (shell). They were pulverized in a motorized high speed-grinder and then sieved with a 0.1 mm mesh size. The method was adopted from [3] with slight modifications.

The oil was extracted using soxhlet extractor using diethyl ether which was then evaporated by a rotary chamber evaporator at 39^0C. The crude extract was then put in an airtight amber container to avoid sunlight penetration and avoid components from escaping. This method was adopted from [3].

2.1.2 Formulation of insecticidal plant oils

A calibrated beaker was used to measure 50 ml of distilled water into spraying bottles. Plant oils were introduced at different concentrations and the equal 50:50 ratio few drops of sulphonic acid was added to form an emulsifiable solution. Method adopted from [3]

2.2 Insect Culture

The insect cages were built in the size of 15cm x 15cm x 15cm. The cages were built with net and wire gauze. Adult house flies used for the experiment were obtained by placing stool on Petri dishes to attract flies into the cages. The insects were then cultured. Feacal samples were placed in the Petri dishes and kept in the cage for the house fly to feed on. The flies were allowed to lay eggs and mature to adult. This method was adopted from [3].

2.3 Experimental Design

Irrespective of their sexes, 15 newly emerged adults were selected randomly and used for this experiment. They were transferred into new cages and fed with fresh stool samples. The cages and spraying bottles were labeled in duplicates as 0.05, 0.10, 0.15, 0.20 ml and just a single cage and spraying bottle labeled as 0.00 ml serving as the control for individual *O. suave*, *A. indica* and the mixed oils at equal proportion (50:50).

The insect (house flies) were sprayed, and the acute mortality test was carried out for six (6) hours to determine the LC_{50} and LT_{50}. The experiment was duplicated and the mean mortality taken.

2.4 Statistical Analysis

The data calculated were analyzed using Analysis of Variation (ANOVA), Probit analysis [24] and Multiple comparison using Turkey's multiple comparison test.Results were considered significant at the significance level of *($P < 0.05$)*

3. Results and Discussion

Mortality increased with increase in neem (*A. indica)* seed oil extract concentration from 0.05 to 0.20/50 ml of water and also percentage mortality increased from 32% to 100%. There was a higher mortality rate of adult *M. domestica* at the 6^{th} hour indicating that mortality increased as the exposure time increased. However, there was significant difference (*$P < 0.05$)* on the adult mortality resulting from the treatments as the extract concentration increased from 0.05 to 0.20/50 ml of water after every hour within the 6 hours of exposure as shown in Table 1.

The concentration of the extract Neem (*A. indica*) seed oil required to cause 50% mortality of adult housefly as observed is 0.066/50 ml of water at 3.4 hours as shown in Figs. 1 and 2.

As the concentration of the extract Wild basil (*O. suave*) leaf oil increased from 0.05 to 0.20/50 ml of water, there was an increase in the percentage mortality and also the probit mortality from 33.3% to 100% and 4.53 to 8.71 respectively. The high mortality rate at the last hour (6^{th}) also indicates that

mortality increased as the period of exposure increased. There was also significant difference ($P < 0.05$) as the concentration increased and as the exposure time increased as shown in Table 2.

The concentration of wild basil (*O. suave)* leave oil required to cause 50% mortality of adult house fly was 0.072/50 ml of water at 3.8 hours as shown in Figs. 3 and 4.

There was an increase in mortality as the concentration of the mixed oils of neem seed and wild basil leaf at equal proportion (50:50) increased from 0.05 to 0.20/50 ml of water and the probit and percentage mortality increased also from 4.53 to 8.71 and 33.3% to 100% respectively. However the high percentage mortality of adult *M. domestica* at the 6^{th} hour indicates that the mortality increased as the exposure time increased. There was significant difference ($P < 0.05$) from the effect of the mixed oils on adult mortality resulting from the treatments as the mixed extract concentration increased from 0.05 to 0.20/50 ml of water after every hour within the 6 hours of exposure as shown in Table 3.

The concentration of the mixed oils that will cause 50% mortality of adult house fly as observed is 0.066/50 ml of water within 3.4 hours as shown in Figs. 5 and 6.

Findings from this study has demonstrated the potency of oils from Neem (*Azadirachta indica*) seed and Wild basil (*Ocimum suave*) leaf as single and mixed oils at equal proportion (50:50) on adult house fly *(M. domestica*). It portrays the similar topical and contact effects of the plant oils when applied singly and as a mixture of equal proportion (50:50) on adult house fly.

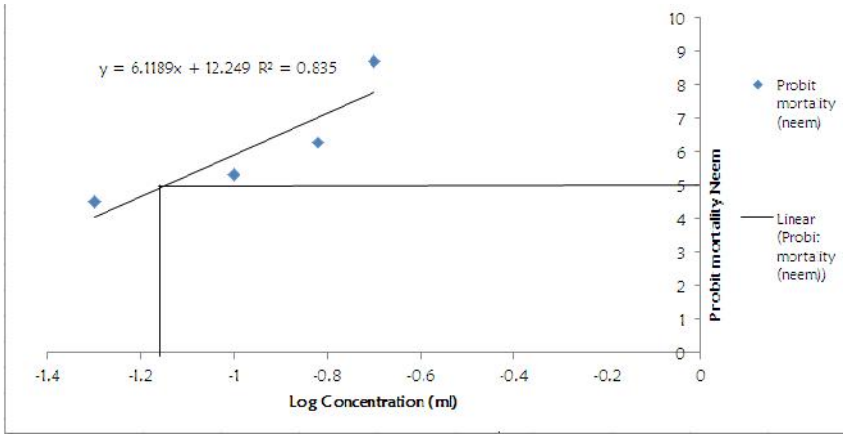

Fig. 1.Probit mortality of adult house fly*(M. domestica)*sprayed with neem(*A. indica)* seed oil

Table 1. Toxicity effect of adult *M. domestica* exposed to neem (*A. indica)* seed oil

*Conc. (ml)	Log Conc. (ml)	**No of insects	Number of hours sprayed						Probit mortality	% mortality	Mean$\bar{x}$
			1	2	3	4	5	6			
0	0	15	0	0	0	0	0	0	0	0	0^{a}
0.05	-1.30	15	1	2	3.5	4	4.5	5	4.53	32	3.33^{ab}±1.54
0.10	-1	15	1	3	5.5	7	9.5	9.5	5.31	62.2	5.9^{2bc}±3.46
0.15	-0.82	15	1.5	4	7	7	11.5	13.5	6.28	90	7.71^{cd}±4.18
0.20	-0.700	15	1.5	6	9.5	10.5	11.5	15	8.71	100	9.0^{d}±5.03
		Mean$\bar{x}$	1^{a}±0.61	3^{ab}±2.24	5.1^{bc}±3.60	5.7^{bc}±3.93	7.4^{bc}±5.03	8.6^{c}±6.18			

*Alphabets denoted as superscript on values denotes variance of the mean time of exposure and different concentrations*All concentrations were mixed with 50 ml of water **Values are means of duplicate observation*

Table 2. Toxicity effect of adult *M. domestica* exposed to wild basil (*O.suave)* leaf oil extract

*Conc. (ml)	Log Conc. (ml)	**No of insects	Number of hours sprayed						Probit mortality	% mortality	Mean$\bar{x}$
			1	2	3	4	5	6			
0	0	15	0	0	0	0	0	0	0	0	0^{a}
0.05	-1.30	15	1	2.0	3.5	3.5	4.0	5	4.56	33.3	3.16^{ab}±1.46
0.10	-1	15	1.5	2.5	5.5	6.0	7.0	8.0	5.08	53.3	5.08bc±2.56
0.15	-0.82	15	1.5	3.5	5.5	6.0	10.5	12.5	5.95	83.3	6.58^{cd}±4.18
0.20	-0.700	15	1.0	4.0	8.5	10.5	10.5	15.0	8.71	100	8.25^{d}±5.03
		Mean$\bar{x}$	1.0^{a}±0.61	2.4ab±1.56	4.6^{abc}±3.13	5.2^{abc}±3.85	6.4^{bc}±4.49	8.1^{c}±5.96			

Source [3] Alphabets denoted as superscript on values denotes variance of the mean time of exposure and different concentrations
*All concentrations were mixed with 50 ml of water**Values are means of duplicate observation*

Table 3. Toxicity effect of adult *M. domestica* exposed to neem and wild basil oil mixture at equal proportion (50:50)

*Conc. (ml)	Log Conc. (ml)	**No of insects	Number of hours sprayed						Probit mortality	% mortality	Mean$\bar{x}$
			1	2	3	4	5	6			
0	0	15	0	0	0	0	0	0	0	0	0^{a}
0.05	-1.30	15	1.0	2.0	2.5	3.5	5.0	6.0	4.75	40	3.3^{ab}±1.89
0.10	-1	15	1.0	3.0	3.0	7.5	9.0	10.0	5.41	66	5.58bc±3.72
0.15	-0.82	15	1.5	3.5	6.0	8.5	11.5	14.5	6.75	96.6	7.58^{cd}±4.90
0.20	-0.700	15	2.0	4.5	8.5	9.5	12.5	15.0	8.71	100	8.67^{d}±4.84
		Mean$\bar{x}$	1.1^{a}±0.74	2.6^{ab}±1.71	4^{abc}±3.30	5.8^{bcd}3.96	7.6^{cd}±5.14	9^{d}±6.16			

Alphabets denoted as superscript on values denotes variance of the mean time of exposure and different concentrations
*All concentrations were mixed with 50 ml of water **Values are means of duplicate observation*

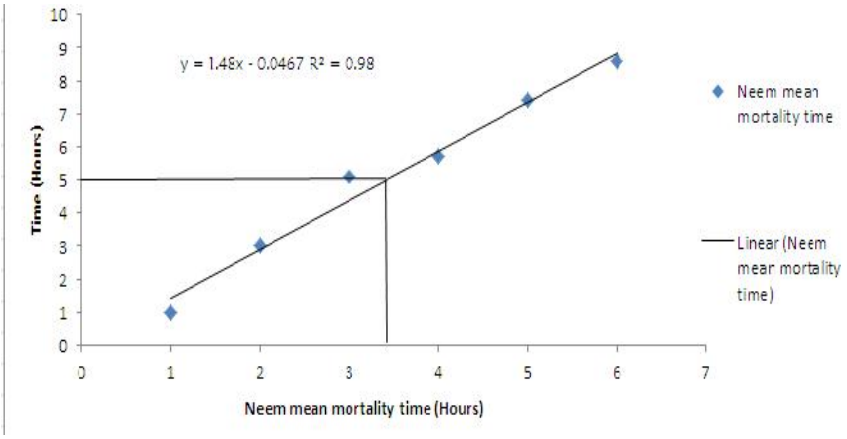

Fig. 2. Mean mortality time for adult house fly *(M. domestica)* sprayed with neem(*A. indica)* seed oil

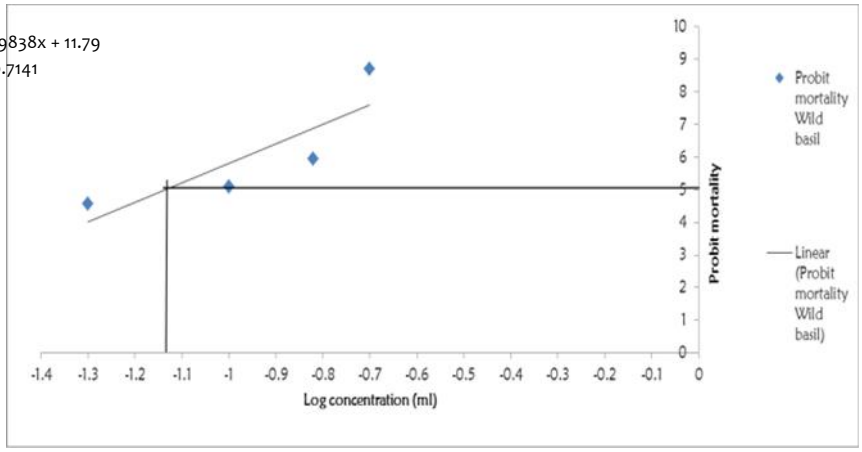

Fig. 3.Probit mortality of Adult house flies (*M.domestica)* sprayed with wild basil (*O. suave)* leave oil

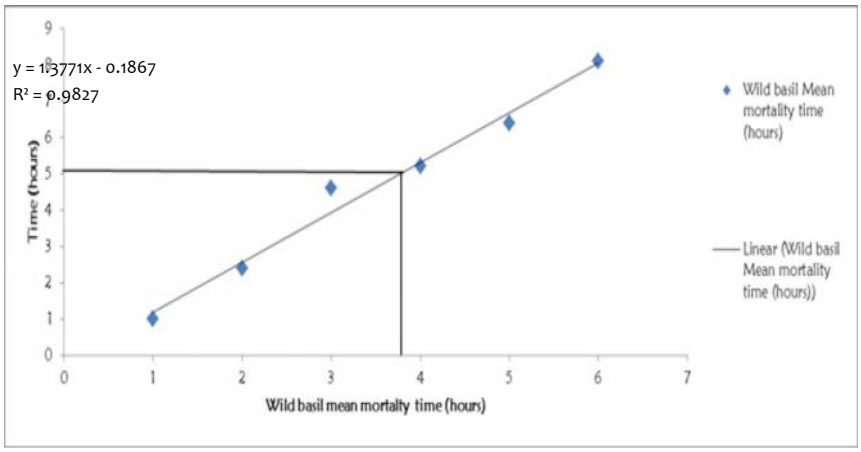

Fig. 4. Mean mortality time for Adult house fly *(M. domestica)* sprayed with wild basil (*O. suave)* leave oil

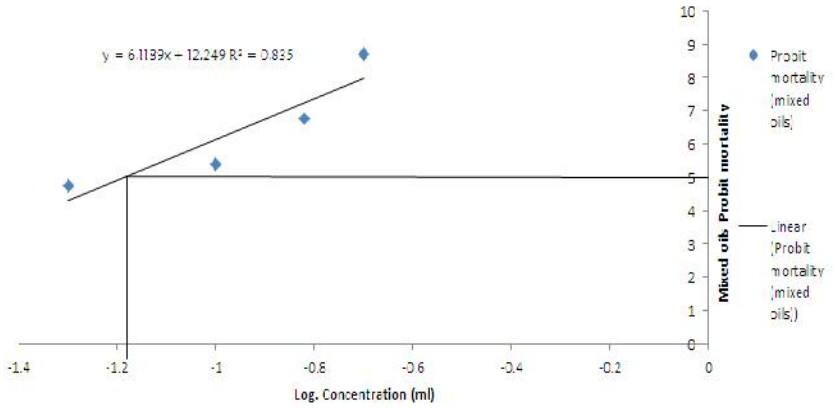

Fig. 5.Probit mortality of Adult house fly(*M. domestica)*sprayed with Neem and Wild basil oil mixture at equal proportion (50:50)

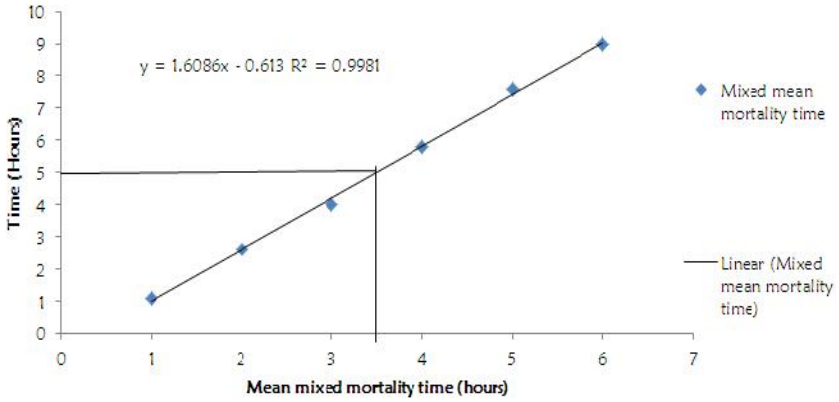

Fig. 6. Mean mortality time for Adult house fly *(M. domestica)* sprayed with Neem and Wild basil oil mixture at equal proportion (50:50)

Generally, the toxicity of the oils of both forms (individual and mixed equal proportions) caused mortality of the adult house fly *(M. domestica*). The toxicity of the leaves and seed extract may be attributed to their constituent compounds which have shown insecticidal activities acting as stomach and contact poison to the insects [3, 25, 26]. However, same observations have also been recorded by [3, 25], their findings revealing the potentials of Lemon Grass (*Cymbopogoncitratus*) and Wild Basil (*Ocimum suave*) which also corresponds with our results as the mortality rate increased the concentration and exposure time increased. According to [26, 27] azadirachtin $(C_{35}H_{44}O)_{16}$ is a nortriterpenoid belonging to the limonoids and a major constituent of *A. indica*, it acts as an insect growth regulator (IGR) by down-regulating the haemolymphecdysteroid level which blocks the prothoracicotropic hormone disrupting moulting, growth and development giving rise to mortality. In addition, its effect on allatropin and juvenile hormone is highly effective against most insect pest [18, 21, 28, 29]. Eugenol ($C_{12}H_{12}O_2$) is a naturally occurring substance belonging to an organic class of molecules called phenylpropanoid in which benzene ring has an allyl group attached [30], it caused significant depression in the fecundity of the *Phthorimaeoperculella*and decrease the percentage of egg hatchability [3,10, 22, 31]. Other components like linalool and thymol are vital constituents of these extracts and are complex mixtures of volatile organic carbons produced as secondary metabolites in plants. They are characterized by a pungent odour and have a generally lower density than water. They interfere with basic metabolic, biochemical, physiological and behavioral functions of insects which inhale ingest or absorb through skin [26].

It was also observed that as the concentration increased from 0.05 to 0.20/50 ml of water of both mixed equal proportion and single oils and as the period of exposure increased from the 1st hour to the 6th hour there was an increase in the mortality rate. The best bit of this study was the 100% mortality recorded by the highest concentration (0.20/50 ml of water) by both formulations (single and mixed equal proportion 50:50) at the 6th hour. This is in accordance with [3] who also recorded 100% mortality of Adult house fly when treated with *O. suave.* [10] also recorded 100% mortality when methane extract of *C. maculatus* was treated with *C. citratus.* Low mortality rate at the early hour along with low concentration may be ascribed to not as much concentration of the volatile compounds reaching the spiracles. The mortality rate can also be attributed to the absence of hard sclerotized thoracic cuticle which increases the physical absorption rate of the constituents through the cuticle. The flies also demonstrated hyperexcitation, muscle spasms and paralysis, which may indicate the possible neurotoxic potential affecting the acetycholinesterase and octopaminengic levels of the sprayed insects as suggested by [10, 20, 32].

The combination of plant extracts equal proportion (50:50) ratio caused LC_{50} of 0.065/50 ml of water which was the least when compared to the single formulations of *A. indica* and *O. suave* with LC_{50} of 0.066/50 ml of water and 0.072/50 ml of water respectively. Although no significant difference ($P>0.05$) recorded in this result, previous studies [22, 13, 33, 34] has shown the insecticidal potency when plant extracts are combined.

The mortality of the adult *M. domestica* by the plant extracts in this study could be attributed to the pungent odours as well as its oily nature of the formulations which could have blocked or inferred with the insect's spiracles thus causing the respiratory impairment. Previous authors [35, 30], also noted that the insecticides as oil formulation blocks the spiracles of insects which may affect the metabolism and alter other body system of the insects leading to suffocation and death.

As an emulsifiable concentration the plant extracts greatly reduced size particles and were evenly mixed within the water column. The spread of the fine particles probably increased the efficacy of the formulation. Since the mortality was not recorded in the control, it is certain that the mortality was due the plant extracts.

4. Conclusion

Plants such as that *A. indica* and *O. suave* can serve as alternative source for insect pest control as they are bioactive and environmentally friendly, the use of these will not only reduce the cost production of synthetic insecticides but as well reduce the pest itself.

A. indica and *O. suave* are found throughout Nigeria with a myriad of uses. Oils forms can be obtained through cold press and an additional surfactant and solvent to ensure equal distribution. There could however be a need for re-application to obtain the desired results since plant products volatilize quickly in the environment and do not persist for longer duration unlike synthetic insecticides.

Manufacturing of these plant based insecticides can be stimulated through local businesses and don't require importation from outside Nigeria.

Competing Interests

Authors have declared that no competing interests exist.

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