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Effect of Neem, Siam Weed and Vetiver Oils on Physiological Reactions and Fitness of House Fly, *Musca domestica* L

[1]*SOYELU, OJ; [1]OLUWAMAKINDE, BA; [2]OKONJI, RE

*[*1]Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife 220005, Osun State, Nigeria.*
[2]Department of Biochemistry and Molecular Biology, Faculty of Science, Obafemi Awolowo University, Ile-Ife 220005, Osun State, Nigeria.
*Corresponding Author Email: jlekan2001@yahoo.co.uk; soyelu@oauife.edu.ng

ABSTRACT: Insecticidal activities of hexane extracts of leaves and roots of siam weed and vetiver, and roots of neem were assessed against house fly, *Musca domestica* L. Mortality test was conducted using serial concentrations 20%, 10%, 5% and 2.5% of extracted oils while behavioural orientation of house fly to oil odour, antioviposition effect of oil toward the insects, biochemical reactions in treated flies and fitness of offspring were determined using 20% oil concentration. House fly mortality varied significantly with plant species and part of plant extracted ($P < 0.001$), concentration applied ($P < 0.001$) and time post-exposure ($P < 0.001$). All tested plant extracts showed potential as good control agents with average mortality ranging from 59-74%. However, significantly lower median lethal values (LC_{50} and LT_{50}) separated vetiver as the most toxic plant against the insect pest. The plant oils repelled house flies (93-100%), reduced the number of larvae that hatched from laid eggs, lowered adult emergence and caused a significant reduction in size and weight of offspring. On the contrary, exposure to plant oils did not alter offspring sex ratio. In comparison to untreated house flies, plant oils induced biochemical stress in poisoned cohorts as evidenced in significant deviation of digestive enzyme (α- and β- amylases, lipase) activity and concentrations of detoxifying enzyme (glutathione-S-transferase), neurochemical enzyme (acetylcholinesterase) and energy metabolism biomolecules (total protein). Implications of obtained results for non-chemical control strategies are discussed.

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The house fly, *Musca domestica* L. (Diptera: Muscidae), is a cosmopolitan insect pest found in both urban and rural settings and capable of spreading widely, travelling as far as 20 km within 24 h in search of food. The insect pest is strongly believed to transmit at least 65 diseases of man and animals some of which are capable of causing very high mortality soon after infection (Blackburn *et al*., 2015). Disease transmission is aided by the natural habit of adult house flies to defecate and regurgitate on food items with the resulting ill-health taking a great toll on man, animal and global economy.

Synthetic chemical application in form of aerosol or bait is the main strategy for controlling house fly but overreliance on these pesticides or misuse of same has often been accompanied by adverse effects on man and his environment. House fly has also developed single- and multi-pesticide resistance (Acevedo *et al*., 2009) thus prompting some users to indulge in using higher-than-recommended dose rates. This act worsens pest situations in the ecosystem often leading to man and animal poisoning, and environmental pollution. The desire for a safe environment has increased interest in sourcing for cheap and friendly alternative control measures against house fly. Biological control strategies using pathogens and plant-based products provide more user- and environment-friendly alternatives to conventional chemical insecticides (Al-Olayan, 2013).

Plant oils vary in their modes of action but they generally alter functions of proteins in insect body. They can alter activities of digestive enzymes, transmission enzyme (acetylcholinesterase) and detoxifying proteins such as glutathione-S-transferases. Plant oils can also act by interfering with insect growth and development, behaviour and fitness (Moawad and Sadek, 2018). These changes have been attributed to the presence of alkaloids, steroids, anthraquinones, flavonoides, tannins, polyphenyls and terpenes in essential oils (EOs). The present study was, therefore, carried out to assess the bio-efficacy of five plant oils against house fly and identify a suitable extract that could be formulated into an effective botanical insecticide.

**Corresponding Author Email: jlekan2001@yahoo.co.uk; soyelu@oauife.edu.ng*

MATERIALS AND METHODS

Preparation of plant samples and oil extraction: Fresh samples of neem (leaf), siam weed (leaf and root) and vetiver (leaf and root) were collected within Obafemi Awolowo University (OAU), Ile-Ife and air-dried on a shaded platform to prevent oil degradation by direct sunlight. Thereafter, 800 g of each plant material was milled mechanically into fine powder and volatile oil was extracted by distillation using n-hexane in a Clevenger apparatus. Water in extracted oils was removed using Na_2SO_4 and the five extracts were kept in a fridge until they were needed for experiments. Oil extraction was carried out at the Drug Research and Production Unit, Faculty of Pharmacy, OAU, Ile-Ife.

Establishment of house fly colony: Adult house flies were collected from dumpsites within OAU, Ile-Ife and reared in the laboratory as described by Abulude *et al*. (2019). Newly emerged (≤ 3 d) 4th generation adult flies were used for experiments to ensure a stock free from influences of the wild.

Mortality test: Serial dilutions 20%, 10%, 5% and 2.5% were made from each stock solution of extracted plant oils and 2 ml of each dilution was added to 2 g granulated sugar in triplicate. The treated diet (in Petri dishes) was air-dried for the solvent (n-hexane) to evaporate completely before they were put inside separate 30 cm × 30 cm × 30 cm experimental cages. Ten adult house flies were introduced into each cage and provision of water was delayed for about 18 h to create a *no choice* situation and ensure that flies fed on treated diet. Each cage was monitored and mortality was recorded on daily basis for seven days. Control experiment (i.e. diet treated with n-hexane) was also set up for comparison of results.

Repellency test: Granulated sugar was mixed in separate Petri dishes with 20% of each oil extract as described for mortality test and placed in separate cages; a dish without plant extract served as control in each cage. Thirty adult house flies that had been starved for at least 6 h were introduced into each cage and the number of flies found on each Petri dish 3 min after introduction was recorded. Each test was replicated ten times and percent repellency for a plant oil was determined as:

$$\% \text{ Repel} = \frac{\text{NF}}{\text{TNF}} \times 100$$

Where: % Repel = Percentage repellency; NF = number of house flies on control Petri dish; TNF =Total number of house flies tested per trial

Anti-oviposition and offspring fitness tests: A 70 g paste of the larval diet was mixed with 2ml 20% of each plant oil in separate Petri dishes. After air-drying, the dishes were placed in separate cages to serve as oviposition substrates. Each cage had artificial diet with similar treatment in three Petri dishes and untreated diet was provided in a similar manner in separate cages. Each treatment cage was replicated thrice. Twenty naïve, but mated, female flies were introduced into each cage and allowed to oviposit for 48 h after which they were removed carefully. In order not to destroy the delicate eggs, emerged larvae in each Petri dish were counted as a measure of number of eggs laid per treatment. The contents of the Petri dishes were monitored on daily basis, pupae were picked and kept in labelled dishes and adult emergence per oviposition substrate was determined as:

$$\% \text{ AE} = \frac{\text{NAT}}{\text{TNL}} \times 100$$

Where % AE = adult emergence; NAT = number of eclosed adult per treatment; TNL = Total number of larvae per treatment

Body weight (mg), size (mm) and sex ratio of resulting progeny were determined as indices of offspring fitness. Body weight of each adult house fly was determined using a sensitive balance while the length of forewing was taken as index of body size (Fartyal *et al*., 2017).

Preparation of tissue extract: A mixture of granulated sugar and 20% plant oil was placed in five cages; a cage for one of five tested oils. The control cages had only granulated sugar without plant oils. Eighty adult flies were introduced into each cage and they were provided with water 20 h after exposure for just 1 h. This was to ensure that the flies did not choose water as an option for survival. The experiment was terminated after 36 h by lowering each cage into a deep-freezer; the flies were collected, weighed and homogenized in phosphate buffer pH 7.2. The supernatant for each treatment was collected after centrifuging at 4,000 rpm for 30 min at 4°C.

Biochemical assays: The total protein in each extract was determined according to the method of Bradford (1976) while α- and β-amylases as well as lipase were assayed as described by Oyebanji *et al*. (2014). Glutathione-S-transferase activity was determined following Vontas *et al*. (2000) and acetylcholinesterase activity was quantified as outlined by Ellman *et al*. (1961).

Statistical analysis: Percent mortality and repellency data were square root-transformed while number of emerging larvae in anti-oviposition trials were natural log-transformed before being subjected to analysis of variance (ANOVA) using SAS v. 9.0. Fitness data for body size and weight of offspring, and enzyme concentrations were also subjected to ANOVA. Mean values were separated among treatments using the Tukey's HSD test at $\alpha = 0.05$. Contribution of a source of variation to outcome of a particular event in adult house flies was calculated as:

$$\text{contribution to an event} = \frac{SS_{SV}}{SS_T} \times 100\%$$

Where SS_{SV} and SS_T are sum of square values for a given experimental factor and the whole model, respectively. The median lethal concentration (LC_{50}) and lethal time (LT_{50}) were determined from mortality data using the predictive function of Microsoft® Office Excel.

RESULTS AND DISCUSSION

Mortality test: Analysis of variance (Table 1) showed that house fly mortality varied significantly ($P < 0.0001$) with source of plant oil, concentration applied and time post-application. However, source of oil was the most important factor, contributing 31% of the variation in mortality followed by concentration of oil (24%) and time after application (3%). Generally, house fly mortality was concentration- and exposure time-dependent. Percent house fly mortality caused by essential oils of vetiver (leaf and root) and siam root was comparable but significantly higher than that from leaves of neem and siam weed (Table 2). However, the significantly lower LC_{50} and LT_{50} values showed that vetiver had the most potent essential oils. Comparison of results from earlier studies e.g., Ayvaz *et al*. (2010) showed that among other factors, insecticidal efficacy of EOs depends on the plant or part of plant from which the EO was extracted. Although vetiver grows in Nigeria, it is not as common as the other plants used in this study and little is known about its use as botanical insecticide in this locality.

Table 1: Analysis of variance showing effect of five plant oils, their concentrations and time post-exposure on survivability of adult house flies

Source of variation	*df*	Sum of squares	Mean square	F value	Percent contribution to house fly mortality
Source of EO	5	303799.52	60759.90***	92.16	31.05
Conc. of EO	4	236003.81	59000.95***	89.49	24.12
No. of days	6	33718.73	5619.79***	8.52	3.45
Replication	2	1293.78	646.89	4.49	0.13
Error	612	403528.44	659.36		41.25
Total	629	978344.29			

****: P < 0.001; EO: Essential oil*

Table 2: Mortality of house flies and lethal indices of different plant oils provided in insect diet over a period of seven days

Source of essential oil	Percent house fly mortality (minimum-maximum)	Median lethal values	
		LC_{50} (%)	LT_{50} (days)
Neem leaf	58.57b (20.00-96.19)	7.42 ± 0.18a	4.10 ± 0.59a
Siam leaf	59.88b (5.71-97.14)	7.24 ± 0.11a	3.97 ± 0.98a
Vetiver leaf	73.45a (19.52-99.52)	6.01 ± 0.03b	1.37 ± 0.27c
Siam root	74.29a (70.48-78.57)	6.54 ± 0.96a	2.35 ± 0.12b
Vetiver root	74.05a (56.67-96.67)	6.02 ± 0.64b	1.14 ± 0.13c
Untreated	0.48c (0.00-0.95)		

Mean values within each column with different letters are significantly different at 0.05 level of probability. Median lethal values are expressed as mean ± SE

Repellency, anti-oviposition and fitness tests: The five plant oils repelled adult flies effectively with efficacy of 93-100% (Table 3); an indication of strong repellents in the oils. Nootkatone, zizanol, bicyclovetivenol, tricyclic sesquiterpenoids, zizanal, epizizanal, α-vetivone, β-vetivone, khusimone and (C)-(1S,10R)-1,10-dimethylbicyclo [4,4,0]-dec-6-en-3-one were reported as repellents in vetiver oil (Babprasert and Karintayakit, 1996) while azadirachtin and khusimone were reported in neem (Khanam *et al*., 2017) and siam (Joshi, 2013) oils, respectively. The repelling ability of these EOs would offer significant protection against house fly if applied in an endemic area such as the abattoir. The plant oils also affected offspring fitness adversely as evident in significantly lower number of hatched larvae, percent adult emergence, size and weight of newly eclosed adults. A lower number of hatched larvae on oil-containing diet was an indication that the oils either showed anti-oviposition effect or they acted as ovicides. Neem oil was reported to have both ovicidal and oviposition deterrent activities against insects

(Kala *et al*., 2019). Similarly, a reduced adult emergence signified reduced survivability (Habiba *et al*., 2019) while inability of house fly to utilize treated diet optimally resulted in reduced size and weight of new adults (Mikami and Ventura, 2008). Nevertheless, essential oils did not have any significant effect on sex ratio of emerging offspring; agreeing with the 1♀:1♂ stable ratio reported by Meisel *et al*. (2016) in samplings done 32 years apart.

Table 3: The repelling efficacy of tested plant oils against adult house flies and performance of offspring on different plant oils

Source of essential oil	Percent adult repelled	Offspring performance				
		Average larvae per treatment	Percent adult emergence	Percent female offspring	Body size (mm)	Body weight (mg)
Neem leaf	93.33a	75.02c	68.47b	62.18a	6.65b	6.69b
Siam leaf	100.00a	114.26b	73.95b	58.86a	6.63b	6.67bc
Vetiver leaf	95.56a	38.14d	33.78c	48.69a	6.42cd	6.65c
Siam root	95.56a	107.32b	72.65b	40.04a	6.57bc	6.67bc
Vetiver root	98.89a	69.11c	39.26c	50.07a	6.50c	6.66bc
Untreated	16.67b	208.78a	96.27a	49.17a	7.33a	10.00a

Mean values within each column with different letters are significantly different at 0.05 level of probability.

Enzyme activity: With the exception of vetiver leaf oil, activity of the two amylases was stimulated by EOs (Table 4) and this was attributed to ability of the oils to increase enzyme affinity for the substrates (Macedo *et al*., 2007). The EOs from siam and vetiver leaves reduced activity of the fly lipase but it was more severe in the latter. A significant enzyme inhibition or stimulation above the control would result in metabolic imbalance, growth impairment and house fly mortality (Babu and Subrahmanyam, 2010). The EOs from vetiver and siam root showed potential as nerve agents because they reduced activity of acetylcholinesterase significantly. The oils would interfere with activity of the enzyme at synaptic joints, hindering it from breaking down acetylcholine effectively and, thus, permitting perpetual transmission of impulses. This condition may eventually lead to death of exposed insects. The neem oil did not have any significant effect on acetylcholinesterase and this is in agreement with earlier reports e.g., Campos *et al*. (2016) which concluded that the oil acts basically as a growth regulator by inhibiting the ecdysone. All the EOs reduced efficacy of glutathione-S-transferase which is one of the defense proteins in house fly. This is an indication that the EOs may be a potential threat to the defense mechanism of house fly. The elevated total protein reported in flies treated with siam and vetiver leaf oils could be attributed to induced physiological stress which necessitated enhanced protein production to replenish depleted protein and ensure insect survival (Chou *et al*., 2012).

Table 4: Biochemical activity in adult house flies fed plant oil-containing artificial diet

Source of essential oil	α-amylase (mg/ml/min)	β-amylase (mg/ml/min)	Lipase ($\times 10^{-5}$) (µmol/min)	AChE (µmol/min)	GST (µmol/min)	Total protein (mg/ml)
Neem leaf	4170.4a	4.1700ab	7.649a	65,813.00b	1916.730c	2.9000bc
Siam leaf	4326.0a	4.3267a	3.654d	67,181.25a	568.856e	4.8670a
Vetiver leaf	3125.9b	3.4567bc	2.800e	45,731.25e	565.474e	3.5887ab
Siam root	4170.4a	4.1700ab	7.500a	57,225.00c	2874.504b	2.9333bc
Vetiver root	4000.0a	4.0000ab	6.920b	50,981.25d	1352.38d	2.0553c
Untreated	2970.4b	2.9700c	4.980c	65,206.25b	4130.49a	2.2330c

Mean values within each column with different letters are significantly different at 0.05 level of probability. AChE: Acetylcholinesterase; GST: Glutathione-S-transferase

Conclusion: All the EOs tested showed potential as insecticidal agents against house fly with an appreciable level of adult mortality and excellent repellency. The reproductive success of the pest was significantly reduced by applied oils and the process of digestion was compromised. Similarly, the plant oils constitute potential threat to nervous and defense systems by inhibiting acetylcholinesterase and glutathione-S-transferase, respectively. Botanical insecticides are viable alternatives to synthetic chemicals and they create a safer environment.

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