Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com/journal/sajb/home

OPEN ACCESS

Chemistry

Evaluation of Adulticidal, Larvicidal and Repellent Activity of Selected Medicinal Plant Extracts and Essential Oil against *Musca domestica* L

Pedro César Machanguia, François Munyemana*

Department of Chemistry, Faculty of Science, Eduardo Mondlane University, Av. Julius Nyerere Nr 3453, P O Box 257, Maputo, Mozambique

DOI: 10.36347/sajb.2019.v07i12.008 | **Received:** 06.12.2019 | **Accepted:** 14.12.2019 | **Published:** 25.12.2019

***Corresponding author:** François Munyemana

Abstract **Original Research Article**

The use of plant extracts and essential oils has recently been referred to as an eco-friendly alternative in insect vector control. The present study evaluated the larvicidal, adulticidal and repellent potential of extracts and essential oils of *Zanthoxylum delagoense*, *Bridelia cathartica* Bertol and *Ocimum americanum* against the housefly *Musca domestica* L. The ethanolic and aqueous extracts were obtained by Soxhlet extraction and essential oil was obtained by hydrodistillation with modified Clevenger apparatus. Phytochemical screening has been conducted on aqueous and ethanolic extracts using standard methods. Essential oils have been analyzed qualitatively by GC-MS. For biological assays, concentrations of 5000, 3000 and 1000 ppm were prepared for the extracts and 2000, 1000 and 500 ppm for the essential oils. *Zanthoxylum delagoense* and *Ocimum americanum* essential oil mixtures were made to evaluate the synergistic effect. Phytochemical screening revealed the presence of alkaloids, anthraquinones, flavonoids, steroids, triterpenoids and condensed tannins in ethanolic and aqueous extracts. 7 compounds were detected in *Bridelia cathartica* Bertol leaves essential oil, Phytol was the major constituent; 5 compounds were identified in *Ocimum americanum* essential oil, Fenchone, D-limonene and Humulene were the major compounds; 11 compounds were identified in *Zanthoxylum delagoense* leaves essential oil, 1-methylene-4-(1-methylethenyl) Cyclohexane, 4-methylene-1-(1-methylethyl) Bicyclo [3.1.0] hexane and β-Myrcene were the major compounds. Essential oils showed higher larvicidal, repellent and adulticidal activity than extracts. *Zanthoxylum delagoense* essential oil and binary oil mixtures were found to have the highest insecticidal activity against *Musca domestica* with 100% mortality at 24 hours exposure. Extracts and essential oils of the three plants have been found to have potential larvicidal, adulticidal and repellent activity against *Musca domestica* L.

Keywords: Musca domestica, Insecticidal, Repellent, Zanthoxylum delagoense, Ocimum americanum, Bridelia cathartica.

INTRODUCTION

Mozambique is a country that has been affected by epidemics of diarrhea, cholera and malaria, diseases that have caused grief and sadness in families and one of the main causes of hospitalizations. These diseases are transmitted by insects like the housefly *Musca domestica* and Mosquito *Anopheles*. The housefly *M. domestica* is one of the major public health pests responsible for the transmission of more than 100 pathogens of humans, poultry, and livestock [1]. Housefly habits – such as walking and feeding on trash and excrement- make them superlative agents for transferring disease-causing pathogens to human and animal population [2]. Transmission of these diseases occurs when the fly comes into contact with the person and food [3]. Diarrheal diseases and avian influenza transmitted by the housefly can cause death while high density of flies can reduce the aesthetic value of livestock products and usually brings economic losses [1].

The main method for houseflies control worldwide is a chemical control using insecticides [4]. The development of resistance to the most of insecticides used and problems caused by organosynthetic insecticides on the environment and non-target organisms have stimulated the use of natural products as an alternative pest control strategy [3, 4]. The use of plant extracts and essential oils in housefly control is the most safe and environmentally-friendly major insecticide alternative because botanical insecticides are biodegradable, non-toxic to human organisms, animals and the environment [6]. Several studies have been conducted on the biological activities of natural products such as essential oils of various plant genera [7], and combinations with synthetic insecticides [8]. Some of

these studies have shown that secondary metabolites of plant extracts and essential oils are toxic and / or repellent to a variety of insect species [9]. Studies on the toxicity of 34 essential oils against *M. domestica* proved the potential insecticidal activity of some of them [10]. LC50 of medicinal plant essential oils of Central Argentina against the housefly have been determined by [11]. Essential oils from *Menta piperita*, *Cymbopogon citratus*, *Citrus sinensis* and *Eucalyptus globulus* and their combinations showed a strong repellent activity against the housefly *M. domestica* [12]. Several plants have been reported to have insecticidal activity and can be prepared in powders, extracts and oils for insect control. Its use has great advantages from the lower cost, ease of use without requiring qualified personnel and the lower toxicity. They have no negative effects on non-target organisms and have varied novel modes of action [13, 14].

Rutaceae family belongs to plant families known as excellent sources of essential oils with insecticidal properties. In that family, the genus Zanthoxylum provides a variety of secondary metabolites including alkaloids, aromatic and aliphatic amides, lignans and coumarins with important phytochemical and biological activities [14, 15]. Several researchers conducted studies on chemical analyses and biological activity of extracts and constituents of different plant parts of various species in the *Zanthoxylum* genus. Biological activity has been found against a wide range of organisms, including insects such as mosquitoes, beetles, cockroaches and houseflies . Alkaloids, amides and terpenoids appear to be the substances most often implicated in anti-insect effects [16]. *Zanthoxylum delagoense* is a species of plant in the Rutaceae family which is endemic in Mozambique. It is a small tree found along the coastline from Maputo to Inhambane Provinces in southern Mozambique (extent of occurrence = 55,062 km2), and occurs in some protected areas (Maputo Special Elephant Reserve, Pomene National Reserve) [17]. There is no information in the literature about insecticidal activity like other Zanthoxylum species and particularly no study has been found about bioactivity of its leaf extracts and essential oils against the housefly *M. domestica*.

Extracts and essential oils obtained from *Ocimum* species have been found to possess effective repellent, larvicidal and adulticidal activities against some insects. Essential oils of *Ocimum americanum* showed insecticidal activity against the hairy caterpillar, *Euproctis fraterna*l and the black cutworm, *Agrotis ípsilon* [18], and larvicidal activity against *Aedes aegypti* mosquito causing 100% mortality at concentration of 100 ppm [19]. Some Ocimum species like *Ocimum gratissum*, *Ocimum basilicum*, *Ocimum kilimandscharicum*, *Ocimum Sanctum* showed larvicidal, repellent and insecticidal activities against *M. domestica* [20].

Bridelia cathartica is an important medicinal plant throughout its distributional range in Sub-Saharian Africa. It is a shrub to small tree belonging to the family Phyllanthaceae, previously included in the Euphorbiaceae family [21]. *B. Cathatica* is a medicinal plant used in southern Mozambique to treat various diseases such as diarrhea, malaria, anemia, diabetes, infectious diseases, headaches [22]. Some studies showed that some species of this genus have insecticidal and repellent activity. *Bridelia ferruginea* extracts have been shown to possess insecticidal and repellent activity against *Dinoderus porcellus* in dry infested yam [23]. Another study showed that aqueous extracts of *Bridelia micrantha* have insecticidal activity in controlling *Podagria uniforma* and *Nisotra dilecta* [24]. However no studies have been reported about *B. cathartica* Bertol insecticidal activity.

It is within this context that in the present study was evaluated the repellent, larvicidal and adulticidal activities of leaf extracts and essential oils of medicinal plants *Z. delagoense*, *O. americanum* and *B. cathartica* Bertol against the housefly *M. domestica*.

MATERIALS AND METHODS

Plant Material Collection and Identification

Leaves of *B. cathartica* Bertol and *O. americanum* were collected in the district of Marracuene, province of Maputo and Leaves of *Z. delagoense* were collected in the district of Matutuine, Maputo province, Mozambique. The species were authenticated at the Herbarium unit of the Institute of Agricultural Research of Mozambique (IIAM) by comparison with existing specimen with Voucher No. 3990 (*Z. delagoense*), No. 4345 (*B. cathartica* Bertol) and No. 7366 (*O. americanum*). The leaves of the three species were dried at room temperature for a period of 30 days in the Laboratory of Natural Products of the Department of Chemistry- Eduardo Mondlane University. The dried leaves were ground into fine powder using an electrical grinder and stored in closed plastic containers at room temperature prior to extraction.

Preparation of Plant Extracts

The extracts were obtained by Soxhlet extraction using ethanol 96% and distilled water to obtain the ethanolic and aqueous extracts respectively. The ethanolic extracts were concentrated in the rotary evaporator at 40^{o} C and the aqueous extracts at 55^{o} C. The obtained crude extracts were kept in a refrigerator at 4^{o} C until use.

Extraction of Essential Oils

Essential oils were obtained by hydrodistillation using a modified Clevenger apparatus. The obtained oils were dried over anhydrous magnesium

sulphate, weighed and stored in a refrigerator at 4^{o} C before analysis.

Mixture of Essential Oils

Two mixtures of the obtained essential oils were prepared, 50/50 (0.5 mL for each oil) and 25/75 (0.25mL *Z. delagoense* and 0.75mL *O. americanum*). The essential oil of *B. cathartica* Bertol was not involved in the mixture because of the low yield obtained during the extraction.

Preliminary Phytochemical Analysis

Phytochemical tests were carried out according to standard procedures [25] with some modifications. The metabolites screened in the ethanolic and aqueous extracts were alkaloids, tannins, flavonoids, saponins, coumarins, anthraquinones, steroids, triterpenoids, resins and cyanogenic glycosides.

Analysis of essential oils by Gas Chromatography–mass spectrometry (GC–MS)

The qualitative analysis for identification of the chemical components present in the essential oils of the leaves of *Z. delagoense*, *B. cathartica* and *O. americanum* was performed on a GC-MS system comprising an Agilent Gas Chromatographer (Agilent GC 106 System 7820A) equipped with Mass Spectrometer (Agilent Mass Spectrometer detector 107 5977B). The column was a HP-5MS (30 m x 0.25 mm ID x 0.25 μm film thickness). The carrier gas used was Helium at a flow rate of 1mL/min. The injection temperature was 250^{o} C. The initial temperature was set to 50^{o} C in the first 5 minutes and then increased to 220^{o} C at 4^{o} C /min. The injection was of 1μL with a split ratio of 10:1. Electron ionization energy of 70 eV was used for GC-MS detection. The identification of the chemical compounds was performed by comparison with the library's database (NIST 14 Library). The relative percentages of the constituents were expressed as percentage by peak area.

Rearing of larvae and adults of Musca domestica L

The larvae were raised in a plastic tray, where a dead fish was placed as a host for egg exposure. After 24 h, larvae were hatched from the host and then placed in Petri dishes for further testing and others transferred to a cage of 40 cm x 30 cm x 30 cm containing an opening of about 7 cm in diameter on one side to facilitate the removal of flies for testing. The larvae were kept at laboratory temperature and fed corn bran until they reached the 3rd instar stage, where some larvae were tested and others kept in the cage until pupae were formed and subsequent fly emergence. After emergence, the flies were fed with moist milk powder until tested.

Preparation of different concentrations of extracts and essential oils for Bioactivity

Stock concentrations of 100000 ppm were prepared for extracts and 10000 ppm for essential oils and their binary mixtures. These stock concentrations were stored in the refrigerator protected from light at a temperature of 4^{o} C until the preparation of other smaller concentrations of 5000, 3000, 1000 ppm for ethanolic and aqueous extracts and 2000, 1000, 500 ppm for essential oils. Ethanol was used as the solvent for preparing ethanolic extracts and essential oils solutions.

Larvicidal Activity Test

The bioassay for larvicidal activity was carried out using the immersion method [26]. From the crude extract, concentrations of 5000 ppm, 3000 ppm and 1000 ppm were prepared and for essential oil, concentrations of 2000 ppm, 1000 ppm and 500 ppm were prepared. All tests were performed with 3rd instar larvae reared under the conditions described above. These bioassays followed the same pattern for all six concentrations prepared in the three plant species, differing only in the concentrations used. 10 third instar larvae were removed from the Petri dish, separated and soaked in 10 ml of each test solution over a 30 second period and then transferred to a filter paper in another Petri dish. Larval mortality was recorded after 24, 48 and 72 hours of exposure. The same experiment was performed for negative control (distilled water and ethanol) and positive control (cypermethrin). The experiment was conducted until adult emergence to verify changes in their life cycle. Pupae hatching and adult emergence were evaluated over 14 days.

Adulticidal Activity Test

The bioassay was performed using the WHO susceptibility test [27] with some changes. *Whatman* No. 1 filter paper was cut in a rectangular shape and impregnated with extracts and essential oil at different concentrations. It was then allowed to dry at room temperature for 30 minutes and was then placed in a plastic jar with small holes in the base. 10 adult houseflies were placed on each jar for one hour and then transferred to another jar. Cotton pads soaked in powdered milk solution were placed in the resting jar during the 24 hour waiting period. The mortality record was done at 1, 6, 12 and 24 h after the beginning of the test. Each test was replicated three times.

Repellent Activity Test on Larvae

The bioassay for repellent activity of essential oils on housefly larvae was performed according to the method described by [28] with some modifications. Filter paper discs and test concentrations were prepared. The filter papers were placed in Petri dishes (9 cm in diameter) and divided into two parts by a marker. Each concentration of the essential oil was applied to a half of the filter paper (treated fraction) and another half was

applied ethanol as a control (untreated fraction). The halves were allowed to dry out in the air for 10 minutes. 10 housefly larvae were placed in the middle of each filter paper circle. Each concentration was replicated three times. The number of larvae that were in each half of the filter paper disc was counted 1, 2, 3 and 24h after exposure.

To analyze the repellent activity data, the repellency index (RI) formula was used [29] :

$$RI = \frac{2G}{(G+P)}$$

Where: RI = repellency index
G = Number of larvae in the test extract.
P = Number of larvae in the control
RI values range from zero to two, indicating;
RI = 1, neutral extract; RI > 1, attractive extract; RI <1, repellent extract.

Attractant / Repellent Bioassay on adult *Musca domestica*

This test was performed according to the double choice method [30]. Twenty freshly emerged adults were released into a cage containing two conical flasks. 1mL of pure essential oil and their binary mixtures were dissolved in 1mL of ethanol. In the treated flask, the essential oil was dissolved in 5mL of milk while in another flask used as control, the ethanol was dissolved in 5mL of milk. The flasks were fitted with a funnel where in the apex was left a hole able to pass an adult fly. This assay was replicated three times. The number of flies attracted to the conical flask treated with pure essential oils and oil mixtures and the control conical flask were counted after 24h to calculate the repellency percentage. The results were expressed in terms of percentage of attraction / repulsion (% R) that was calculated using the formula [31].

% R = 100 (C - T) / C

C = number of flies trapped in the control flask
T = number of flies trapped in the treated flask

STATISTICAL ANALYSIS

Data obtained in the larvicidal, insecticidal and repellent activity tests were subjected to analysis of variance (ANOVA). In the case of significant treatment effects, the averages were compared by Tukey test at 5% significance level with the aid of the SPSS version 15.0 statistical program and Microsoft Excel 2013 was used to determine LC_{50} of the extracts, essential oils and their mixtures.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

The results of the phytochemical screening of extracts of the three plant species are shown in Table 1. The phytochemical screening revealed the presence of alkaloids, anthraquinones, condensed tannins, flavonoids, Steroids, Triterpenoids in all six extracts analyzed, while saponins were only detected in aqueous extracts. Cyanogenic glycosides were detected in aqueous and ethanolic extracs from *Z. delagoense* and *O. americanum*. Coumarins were detected in the three ethanolic extracts and in aqueous extract from *O. americanum*.

The secondary metabolites identified in ethanolic and aqueous extracts of *Z. delagoense* are similar to results reported in the literature on different species of *Zanthoxylum*. For many *Zanthoxylum* species, the qualitative analysis showed the presence of alkaloids, coumarins, glycosides, Flavonoids, phenolic compounds, tannins, terpenoids and quinones in crude ethanolic extracts [32].

Most of the phytochemical compounds identified from the *B. cathartica* aqueous and ethanolic leaves extracts in the present study (alkaloids, anthraquinones, condensed tannins, saponins, flavonoids, steroids and triterpenoids) corroborate with the results reported by other authors. Tannins, flavonoids, anthracene glycosides, fatty acids, steroids, emodins, triterpenoids, volatile oils and anthocyanins have been previously identified from leaves of *B. cathartica* [33]. Several authors reported the presence of these secondary metabolites in different parts of the *B. cathartica* including the leaves [21].

The results obtained in the present study on phytochemical screening of aqueous and ethanolic extracts of *O. americanum* leaves are in agreement with the results of the previous studies [34, 35]. Alkaloids, phenolic compounds, tannins, lignin, starch, saponins, flavonoids, terpenoids and anthraquinones have been identified in leaves of *O. americanum* [34] and similarly carbohydrates, tannins and flavonoids have been identified in aqueous and alcoholic extracts of *O. americanum* [35].

The variation in the chemical composition of the extracts verified in the results obtained should be due to the geographical origin, the genetic variations, method of extraction, age or maturity, harvest time, among other factors [36].

GC – MS analysis of essential oils

The essential oils obtained by hydrodistillation yielded 1.8% for *O. americanum*, 0.6% for *Z. delagoense* and 0.0028% for *B. cathartica* Bertol.

The low yield obtained for *B. cathartica* essential oil did not allow the realization of the mixtures and biological activities.

Qualitative analysis of essential oils by GC - MS led to the identification of 7 components in *B. cathartica* Bertol leaves essential oil: Phytol (83.2 %), (Z) 6, (Z) 9-Pentadecadien-1-ol (6.4 %), 6,10,14-trimethylpentadecan-2-one (4.0 %), Tridecanal (3.4%), 1-methyl-4-(2-methyloxiranil)-7-oxabiciclo [4.1.0] heptane (1.3 %), (Z) 6, 10-dimethylundeca-5,9-dien-2-one (0.7%) and Tetradec-13-en-11-yn-1-ol (0.6 %); 5 compounds in *O. americanum* essential oil: Fenchone (32%), D-limonene (21.6%), Humulene (15.3%), 2-(4a,8-dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphtalen-2-yl) propan-2-ol (3.5%) and Nerolidol (2.9%); 11 components in *Z. delagoense* leaves essential oil: 1-methylene-4-(1-methylethenyl) Cyclohexane (29%), 4-methylene-1-(1-methylethyl) Bicyclo [3.1.0] hexane (21%), β-Myrcene (13%), 4-methyl-1-(1-methylethyl) Cyclohex-3-en-1-ol (9.3 %), α-Phellandrene (6.5%), Citral (6.1 %), (1R)-2, 6, 6-Trimethylbicyclo[3.1.1] hept-2-ene (4.9 %), 3, 7-dimethyl- (Z) 2, 6-Octadienal (4.7 %), γ-Terpinene (2.8%), Linalool (2.1%).

Researches on chemical composition of most of the essential oils from *Ocimum* species and *Zanthoxylum* species have been undertaken by various researchers using GC, GC-MS and other techniques [37] . The data given in the literature about the chemical composition of essential oils from different *Zanthoxylum* and *Ocimum* species showed a considerable difference depending to geographical regions [38].

Among the components identified in *Z. delagoense* essential oil, β-Myrcene is the most reported in other *Zanthoxylum* species like *Z. readelianum* [39], *Z. schinifolium* and *Z. piperitum* [40]. In the current study, GC-MS analysis revealed other compounds that are not reported in other studies on species of the genus *Zanthoxylum* which means that the components of their oils vary according to the origin (36). In the list of essential oil components of *O. americanum*, *D-Limonene* is the most found in other *Ocimum* species [18, 41, 42]. In literature there is little information about essential oil components of the genus *Bridelia*. The most prominent compound found in the leaf essential oil of *B. retusa* [43] was phytol that was also identified in *B. cathartica* Bertol as the major component in the current study. Most of the components identified in the current study (D - Limonene, Nerolidol, β-Myrcene, Phytol, Tridecanal, α - Phellandrene, Fenchone) are described in the literature that have insecticidal and repellent activity [44]. These and others identified in the current study, alone or in combination may be responsible for insecticidal and repellent activity.

Larvicidal activity

The results of larvicidal activity of ethanolic extracts, aqueous extracts and essential oils from *Z. delagoense*, *O. americanum* and *B. cathartica* Bertol against the 3rd instar larvae of *M. domestica* are presented in Table 2 and Table 3. In the same tables were also shown the development of treated larvae in pupae and adult houseflies.

The percentage of larval mortality increased with the time of exposure and was dose-dependent reaching the range 73.33 ± 5.77 – 76.67 ± 5.77% for ethanolic extracts and 53.33 ± 5.77 – 90.00 ± 10.00 % for aqueous extracts at the highest concentration (5000 ppm) after 72 h of exposure.

The percentage of larval mortality reached 100% in the case of *Z. delagoense* essential oil and binary oil mixtures *Z. delagoense*/*O. americanum* (50/50 and 25/75) at the concentration of 2000 ppm. At the same concentration and time of exposure, the percentage of larval mortality for *O. americanum* was 80 ± 10%. The essential oil from *B. cathartica* Bertol was not tested because of the low yield obtained from hydrodistillation (0.0028%). Essential oils presented higher larval mortality in comparison with the extracts.

At the concentration of 1000 ppm, neither extracts, nor oils, nor binary oil mixtures reached 100% larval mortality as it was the case with the insecticide Cypermethrin used as a positive control. No larval mortality was observed in the negative control. As shown in Tables 2 and 3, larvae treated with plant extracts could develop in pupae and even emerge in adult houseflies but in case of treatment with essential oils no larvae developed in adult housefly.

The results obtained in the larvicidal bioassays of the extracts, essential oils and the binary oil mixtures against the 3rd instar larvae of *M. domestica* are similar to those obtained by [45] and [46]. The larvicidal activity of extracts and oils varied according to plant species and larval mortality increased with the increase of the concentration, the higher the concentration the higher the mortality. Similar results were obtained by [47] who investigated the Insecticidal, biological and biochemical response of *M. domestica* to some indigenous weed plant extracts. They found that mortality of 2nd instar larvae of *M. domestica* was significantly increased with the increase in dose rate and exposure time.

The results revealed also that there was higher mortality in the first 24 h, which corroborates with [48] who evaluated the larvicidal activity of *Piper capitarianum* extracts and essential oils on *Aedes aegypti* and *Anopheles* at 24 h, 48 h and 72 h and observed that the highest mortality occurred at 24 h in all extracts tested.

In Tables 2 and 3 was also illustrated the developmental reduction results of larvae treated with extracts, pure essential oils and oil mixtures in pupae and adult houseflies. There was observed a greater development in the concentration of 1000ppm reaching 86.67%. However, the developed larvae showed difficulties in locomotion and several anomalies. Those results are in agreement with those obtained by [49] that observed the reduction in the emergence of adult *M. domestica* in larvae treated with *Eucalyptus* essential oils. The degree of insect deformation depends on concentrations, the higher the concentration the greater the deformations [50]. Various morphological abnormalities of larvae, pupae and adults of *M.domestica* have been observed by other authors [51]. In another study it was confirmed that essential oils and extracts affect larval development in pupae or adult flies depending on the concentration applied [5].

Plant extracts greatly affect the life cycle parameters of houseflies and can be used in vector control programs to control housefly-borne diseases in place of conventional insecticides [52].

The results obtained in the current study showed that in the essential oil mixtures there was no larvae development in pupae and adult flies, showing that the essential oil mixtures may be a correct alternative for the control of the *M. domestica* pest. Many plants have been reported about their potential insecticidal actions on different stages of *M. domestica* via crude extracts or extracted active compounds. Some results also showed their effects on metamorphosis or emergence or fecundity or life span of houseflies [53]. The literature offers much information about development effects of vast number of plant extracts and their phytochemical constituents against housefly [53]. Larvicidal bioassays against the 2nd instar larvae of *M. domestica* using whole-plant boiled extracts of 3 indigenous plants have also been reported [54].

Adulticidal activity

The results of adulticidal activity of aqueous extracts, ethanolic extracts and essential oils from *Z. delagoense*, *O. americanum* and *B. cathartica* Bertol against the adult *M. domestica* are shown in Table 4 and 5.

The results showed that after 24h of exposure at the highest concentration (5000 ppm), all extracts tested exhibited more than 50% of insecticidal mortality but none of them reached 100% of mortality. In case of essential oils, in the first hour of exposure, the two essential oils tested exhibited more than 50% of mortality at 2000 ppm while the binary oil mixtures exhibited 100% insecticidal mortality. After 24h of exposure at 2000 ppm, the essential oil from *Z. delagoense* and binary oil mixtures exhibited 100% insecticidal mortality while the essential oil from *O. americanum* exhibited 83.33% insecticidal mortality.

As noted in larvicidal activity, the results of adulticidal activity also showed that the increase of concentration also increased the % mortality of *M. domestica*. These results are similar to those found by [47] who reported that the insecticidal activity of the weed plants against *M. domestica* was time and dose dependent.

Extracts of leaves and stems of *Cupressus* species produced 90% mortality rate in adults of *M. domestica* within 24 h at a concentration of 10,000 mg / l [55] . In the study of the bioactivity of extracts of some medicinal plants against *M. domestica* was found that the percentage of mortality increased with higher concentrations [6] . Five percent of *Bridelia ferrugenia* propanolic extract caused 88.9% mortality of *Dinoderus porcellus* after 24 h exposure [23] .

Essential oils and their mixtures showed a higher percentage of mortality compared to extracts, especially in the case of essential oil mixtures that exhibited 100% mortality at 2000 ppm and in the first hours of exposure. In pure essential oils, *Z. delagoense* essential oil was more active with 100% mortality in the highest concentration. Those results are in agreement with those of [14] who reported that *Z. limonella* essential oil caused 100% mortality in *Aedes aegypti* and *Culex quinquefasciatus.* In the study on the insecticidal activity of *O. gratissimum* extracts and essential oil against pests and vector insects including *M. domestica* was found that the essential oil was more active than the extracts [56]. Other studies have shown that essential oils have strong insecticidal activity against insects such as whitefly and housefly and other arthropods [57-59]. The insecticidal activity of monoterpenoids and their derivatives against *M. domestica* adults have been previously reported [11, 60, 61]. The terpenic hydrocarbons (myrcene, γ-terpinene, limonene, α-phellandrene), acyclic monoterpene alcohol (linalool), aliphatic aldehyde (citral) and bicyclic monoterpenic ketone (Fenchone) identified in essential oils of *Z. delagoense* and *O. americanum* in the current study, have been shown to have adulticidal activity against *M. domestica*.

LC_{50} values of Extracts and Essential oils

In Table 6 were presented LC_{50} values of extracts, essential oils and binary oil mixtures after 24 h exposure against adult houseflies and 72 h exposure against 3rd instar larvae of *M. domestica*.

Among the extracts, the ethanolic extract of *Zanthoxylum delagoense* showed the lowest LC_{50} value (2384.5 ppm) after 72 h exposure against 3rd instar larvae of M. domestica and the lowest LC_{50} value (2505.6 ppm) after 24 h exposure against adult *M.domestica*.

Among the essential oils, *Z. delagoense* essential oil showed the lowest LC_{50} value against 3rd instar larvae (328.0 ppm after 72 h exposure) and *O. americanum* essential oil showed the lowest LC_{50}value against adult *M. domestica* (560.0 ppm after 24 h exposure).

The binary mixture of *Z. delagoense* and *O. americanum* showed the lowest LC_{50} value against 3rd instar larvae (61.4 ppm for 50/50 mixture) and lowest LC_{50} value for adult *M. domestica* (0.2 ppm for 25/75 mixture).

These results are close to those found when evaluating the insecticidal effect of *O. basilicum* essential oil against larvae and adult insect pests *Attagenus fasciatus* and *Lasioderma serricorne* [60]. LC_{50} of essential oils from medicinal plants native to Central Argentina [11] and from fruits and leaves of *Zanthoxylum schinifolium* [62] have been determined, obtaining values ranging from the most potent 0.5 mg / dm^3 to the least potent 46.9 mg / dm^3, values that resemble to those obtained in the current study.

Repellent Activity Test against *M. domestica Larvae*

The results of the repellent activity against 3rd instar larvae are shown in Table 7. The results of this bioassay showed that the essential oils of *Z. delagoense*, *O. americanum* and their binary mixtures have repellent activity against the 3rd instar housefly larvae at the concentrations of 2000 ppm and 1000 ppm. At the concentration of 500 ppm only the binary oil mixtures have repellent activity. *Z. delagoense* and *O. americanum* were found attractive at lower concentration.

Those results are similar to those obtained by [47] who reported that weed plant extracts showed significant repellency and mortality against *M. domestica* larvae. They found that the repellency potential of extracts was increased as the test concentration was increased. Attraction responses were observed in mosquitoes to essential oils [63]. Citronellal, linalool, citral and geraniol were attractive at lower concentrations and repellent at higher concentrations to *Aedes albopictus*. The dose-dependent behavioral response was also found for the honey bee (*Apis florae*) to the nerol [64]. Various authors reported the repellent effect of essential oils of plants against *M. domestica* larvae [65, 66] . The repellent activity of essential oils may be related to their active constituents.

Attractant /Repellent Bioassay against adult *M. domestica*

The results of attractant/repellent bioassay against adult *M. domestica* were summarized in Table 8. The percentage repellency calculated after 24 h was in the range 75.0 – 94.7% at 2000 ppm.The repellent activity increased with the increase of the test concentration. *Z. delagoense* essential oil showed higher repellent activity than *O. americanum*. Binary oil mixtures exhibited the highest percentage repellency.

These results fit with those found in the literature. The essential oil of *O. gratissimum* and *T. Serpyllum* showed 100% repellency against *M. domestica* [67]. Essential oils of *Tymus vulgaris* and *Eugenia coryphyllus* showed 90.2% and 80.68% of repellency against *M. domestica* [30]. The repellency to stable fly, *Stomoxys calcitrans* (L.), of *Zanthoxylum piperitum* (L.) DC pericarp steam distillate (ZP-SD), *Zanthoxylum armatum* DC seed oil (ZA-SO) and their constituents alone or in combination with *Calophyllum inophyllum* L. nut oil (CI-NO, using an exposed human hand bioassay have been reported [66].

Other studies reported repellent activity against *M. domestica* of plants such as *Artemisia niligirica*, *Blumula ariantha*, *Hyptis suaveoleus* and *Lavendula bipounata* [6].

Eucalyptus saligna showed strong repellent activity against *Sitophilus zeamais*, *Tribolium confusum* and induced some repellent effect of adult female *Culex pipiens* [68].

Ocimum and *Zanthoxylum* species belong to the group of plants with repellent and insecticidal activity. Essential oils from *O. basilicum* L, *O. gratissimum* L, *O. sanctum* L, *O. kilimandscharicum*, *Z. xanthoxyloides* showed repellency to adult Housefly [67, 69].

The repellent activity of essential oils has been linked mainly to the presence of monoterpenes and sesquiterpenes. The chemical compounds Limonene identified in essential oil of *O. americanum* ; Citral , Linalool and γ-terpinene identified in essential oil of *Z. delagoense* in the current study, have been reported to show significant repellency to adult Housefly [37, 70]. Although repellent activity of essential oils is generally attributed to some particular compounds, a synergistic phenomenon among these metabolites may result in a higher bioactivity compared to the isolated components [70].

Table-1: Results of preliminary phytochemical tests

Extracts		Alk	HT	CT	Sap	Flav	Cou	Ant	ST	Tr	Res	CG
Z. delagoense	Ethanolic	+	-	+	-	+	+	+	+	+	+	+
	Aqueous	+	-	+	+	+	-	+	+	+	+	+
B. cathartica	Ethanolic	+	-	+	-	+	+	+	+	+	+	-
	Aqueous	+	-	+	+	+	-	+	+	+	+	-
O. americanum	Ethanolic	+	-	+	-	+	+	+	+	+	+	+
	Aqueous	+	-	+	+	+	+	+	+	+	-	+

Legend: +: detected; - : not detected; Alk: alkaloids; HT: Hydrolysable tannins; CT: Condensed tannins; Sap: Saponins; Flav: Flavonoids; Cou : Coumarins; Ant: Anthraquinones; ST: Steroids; Tr: Triterpenoids; Res: Resins ; CG: Cyanogenic glycosides.

Table-2: Larval Mortality at different concentrations of ethanolic and aqueous extracts after 24 h, 48 h and 72 h of exposure and its development after treatment.

Extracts		Conc. (ppm)	Mortality Mean ± SD (%)			Mean no. (%) of developed Larvae after treatment in	
			24h	48h	72h	Pupae	Adult
Aqueous	*Z.delagoense*	5000	66.67±5.77c	76.67±5.77g	90.00±10.00b	10.00±10.00	10.00±10.00
		3000	33.33±5.77d	43.33±5.77d	56.67±5.77d	43.33±5.77	43.33±5.77
		1000	16.67±5.77ef	23.33±5.77e	26.67±5.77e	76.67±5.77	76.67±5.77
	B. cathartica	5000	33.33±5.77d	46.67±5.77d	76.67±5.77bc	23.33±5.77	23.33±5.77
		3000	30.00±10.00d	40.00±10.00d	56.67±5.77d	43.33±5.77	43.33±5.77
		1000	13.33±5.77ef	20.00±10.00e	23.33±5.77fg	76.67±5.77	76.67±5.77
	O.americanum	5000	23.33±5.77e	33.33±5.77d	53.33±5.77d	46.67±5.77	46.67±5.77
		3000	13.33±5.77ef	16.67±5.77e	23.33±5.77fg	76.67±5.77	76.67±5.77
		1000	6.67±5.77f	6.67±5.77f	13.33±5.77f	86.67±5.77	86.67±5.77
Ethanolic	*Z. delagoense*	5000	63.33±5.77c	66.67±5.77cg	73.33±5.77cd	26.67±5.77	26.67±5.77
		3000	43.33±5.77d	46.67±5.77d	56.67±5.77d	43.33±5.77	43.33±5.77
		1000	30.0±10.00d	33.33±5.77e	36.67±5.77e	63.33±5.77	63.33±5.77
	B. cathartica	5000	56.67±5.77c	66.67±5.77c	73.33±5.77cd	26.67±5.77	26.67±5.77
		3000	50.0±10.00c	56.67±5.77c	63.33±5.77d	36.67±5.77	36.67±5.77
		1000	20.0±10.00e	23.33±5.77eh	30.00±10.00eg	70.00±10.00	70.00±10.00
	O.americanum	5000	56.67±5.77c	63.33±5.77c	76.67±5.77bc	23.33±5.77	23.33±5.77
		3000	43.33±5.77d	53.33±5.77d	56.67±5.77d	43.33±5.77	43.33±5.77
		1000	13.33±5.77ef	16.67±5.77h	23.33±5.77fg	76.67±5.77	76.67±5.77
Control	Water	-----	0.00±0.00a	0.00±0.00a	0.00±0.00a	100.00±0.00	100.00±0.00
	Ethanol	-----	0.00±0.00a	0.00±0.00a	0.00±0.00a	100.00±0.00	100.00±0.00
	Cypermethrin	1000	100.00±0.00b	100.00±0.00b	100.00±0.00b	0.00±0.00	0.00±0.00

The mean followed by different letters in the columns are significantly different at $p < 0.05$

Table-3: Larval mortality at different concentrations of essential oils after 24 h, 48 h and 72 h of exposure and its development after treatment

Extracts		Conc. (ppm)	Mortality Mean ± SD (%)			Mean no. (%) of developed Larvae after treatment in	
			24h	48h	72h	Pupae	Adult
Essential oils	*Z. delagoense*	2000	80.00±10.00c	86.67±5.77c	100±0.00b	0.00±0.00	0.0±0.0
		1000	56.67±5.77df	66.67±5.77e	73.33±5.77c	26.67±5.77	0.0±0.0
		500	43.33±5.77e	50.0±10.00f	53.33±5.77de	46.67±5.77	0.0±0.0
	O. americanum	2000	73.33±5.77c	76.67±5.77d	80.0±10.00c	20 ±10.00	0.0±0.0
		1000	53.33±5.77df	56.67±5.77ef	66.67±5.77d	36.67±5.77	0.0±0.0
		500	30.00±10.0e	33.33±5.77g	46.67±5.77e	56.67±5.77	0.0±0.0
	Mixture 50/50	2000	100±0.00b	100±0.00b	100±0.00b	0.00±0.00	0.0±0.0
		1000	63.33±5.77d	73.33±5.77d	83.33±5.77c	0.00±0.00	0.0±0.0
		500	46.67±5.77ef	56.67±5.77ef	56.67±5.77d	0.00±0.00	0.0±0.0
	Mixture 25/75	2000	100±0.00b	100±0.00b	100±0.00b	0.00±0.00	0.0±0.0
		1000	66.67±5.77d	73.33±5.77d	73.33±5.77c	0.00±0.00	0.0±0.0
		500	46.67±5.77ef	53.33±5.77f	53.33±5.77de	0.00±0.00	0.0±0.0
Control	Ethanol	------	0.00±0.00a	0.00±0.00a	0.00±0.00a	100±0.00	100±00
	Cypermethrin	1000	100±0.00b	100±0.00b	100±0.00b	0.00±0.00	0.0±0.0

The mean followed by different letters in the columns are significantly different at $p < 0.05$

Table-4: Mortality of adult *M. domestica* at different concentrations of aqueous and ethanolic extracts after 1 h, 6 h, 12 h and 24 h of exposure

Extracts		Conc. (ppm)	Mortality Mean ± SD (%)			
			1 h	6 h	12 h	24 h
Aqueous	*Z. delagoense*	5000	13.33±5.77cd	20.0±10.00c	46.67±5.77c	73.33±5.77c
		3000	6.67±5.77d	13.33±5.77cd	26.67±5.77de	53.33±5.77c
		1000	6.67±5.77d	13.33±5.77cd	23.33±5.77de	36.67±5.77cd
	B. cathartica	5000	13.33±5.77cd	23.33±5.77c	46.67±5.77cd	53.33±5.77c
		3000	6.67±5.77d	13.33±5.77cd	26.67±5.77de	36.67±5.77cd
		1000	6.67±5.77d	6.67±5.77d	13.33±5.77e	20.0±10.00d
	O. americanum	5000	23.33±5.77c	33.33±5.77c	53.33±5.77c	56.67±5.77c
		3000	6.67±5.77d	16.67±5.77c	23.33±5.77de	43.33±5.77cd
		1000	6.67±5.77d	6.67±5.77d	13.33±5.77e	16.67±5.77d
Ethanolic	*Z. delagoense*	5000	13.33±5.77cd	33.33±5.77c	53.33±5.77c	76.67±5.77c
		3000	6.67±5.77d	13.33±5.77cd	23.33±5.77de	43.33±5.77cd
		1000	6.67±5.77d	6.67±5.77d	13.33±5.77e	16.67±5.77d
	B. cathartica	5000	20.00±10.00c	33.33±5.77c	43.33±5.77cd	56.67±5.77c
		3000	6.67±5.77d	13.33±5.77cd	30.0±10.0cde	40.00±10.00cd
		1000	6.67±5.77d	6.67±5.77d	13.33±5.77e	13.33±5.77d
	O. americanum	5000	23.33±5.77c	33.33±5.77c	56.67±5.77c	70.00±10.00c
		3000	6.67±5.77d	16.67±5.77c	23.33±5.77e	40.0±10.00cd
		1000	6.67±5.77d	6.67±5.77d	16.67±5.77e	23.33±5.77d
Control	Water	-----	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Ethanol	-----	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Cypermethrin	1000	100.00±0.00b	100.00±0.00b	100.00±0.00b	100.00±0.00b

The mean followed by different letters in the columns are significantly different at $p < 0.05$.

Table-5: Mortality of adult *M. domestica* at different concentrations of essential oils and their binary mixtures after 1 h, 6 h, 12 h and 24 h of exposure

Extracts		Conc. (ppm)	Mortality Mean ± SD (%)			
			1 h	6 h	12 h	24 h
Essential oils	*Z. delagoense*	2000	53.33±5.77c	63.33±5.77c	76.67±5.77c	100.00±0.00b
		1000	33.33±5.77d	46.67±5.77d	53.33±5.77d	63.33±5.77d
		500	20.00±10.00d	33.33±5.77e	36.67±5.77e	46.67±5.77e
	O. americanum	2000	63.33±5.77c	66.67±5.77c	73.33±5.77c	83.33±5.77c
		1000	30.00±10.00d	33.33±5.77e	46.67±5.77de	63.33±5.77d
		500	23.33±5.77d	26.67±5.77e	33.33±5.77e	46.67±5.77e
	Mixture 50/50	2000	100.00±0.00b	100.00±0.00b	100.00±0.00b	100.00±0.00b
		1000	63.33±5.77c	66.67±5.77c	73.33±5.77c	76.67±5.77c
		500	46.67±5.77d	53.33±5.77c	53.33±5.77d	60.00±10.00cde
	Mixture 25/75	2000	100.00±0.00b	100±0.00b	100±0.00b	100.00±0.00b
		1000	63.33±5.77c	66.67±5.77c	70.0±10.00cd	73.33±5.77cd
		500	46.67±5.77d	46.67±5.77d	56.67±5.77d	63.33±5.77d
Control	Ethanol	------	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
	Cypermethrin	1000	100.00±0.00b	100.00±0.00b	100.00±0.00b	100.00±0.00b

The mean followed by different letters in the columns are significantly different at $p < 0.05$

Table-6: LC_{50} of extracts, essential oils and binary oil mixtures after 24 h and 72 h exposure against adults and 3rd instar larvae of *Musca domestica* L respectively

Plant material	Extract	LC_{50} (ppm)		
		Larvae		Adult
		24h	72h	24h
Z. delagoense	Aqueous	3888.8	2513.7	3296.2
	Ethanolic	3547.2	2384.5	2505.6
	Essential oil	754.5	328.0	606.8
O. americanum	Aqueous	NE	NE	4111.0
	Ethanolic	4141.5	4141.5	4618.1
	Essential oil	1086.9	475.9	560.0
B. cathartica	Aqueous	NE	2843.1	4618.1
	Ethanolic	3834.6	2494.8	4244.2
Mixtures of essential oils	50/50	606.8	61.4	63.5
	25/75	568.0	328.0	0.2

NE – Not estimated

Table-7: Repellency of essential oils and their mixtures against *M. domestica* larvae

Extracts	Conc. (ppm)	Mean in treatment	Mean in control	Index of Repellency	Classification
Z. delagoense	2000	1.75±0.50	8.75±0.50	0.35	Repellent
	1000	3.42±1.34	6.58±1.34	0.68	Repellent
	500	7.33±0.72	2.67±0.72	1.46	Attractive
O. americanum	2000	2.00±0.90	8.00±0.90	0.40	Repellent
	1000	3.83±1.13	6.17±1.13	0.76	Repellent
	500	6.08±1.10	3.91±1.10	1.36	Attractive
Mixture 50/50	2000	1.70±0.64	8.30±0.64	0.34	Repellent
	1000	2.58±0.83	1.72±0.83	0.52	Repellent
	500	3.75±0.99	6.25±0.99	0.75	Repellent
Mixture 25/75	2000	1.90±1.16	8.10±1.16	0.38	Repellent
	1000	2.42±1.42	7.58±1.42	0.48	Repellent
	500	2.75±0.99	7.25±0.99	0.55	Repellent

Table-8: Attraction / repellency test of the essential oils and their mixtures in 24h exposure against adult *M. domestica* (N=20)

Extracts	Conc. (ppm)	Mean No. of flies trapped in treated zone	Mean No. of flies trapped in control	Percentage repellency
Z.delagoense	2000	1.33±0.57	18.67±0.57	92,9
	1000	3.33±0.57	16.67±0.57	80.0
	500	5.00±1.00	15.00±1.00	66.7
O. americanum	2000	4.00±1.00	16.00±1.00	75.0
	1000	6.33±0.57	13.67±0.57	53.7
	500	8.33±0.57	11.67±0.57	28.6
Mixture 50/50	2000	1.00±1.00	19.00±1.00	94.0
	1000	3.33±0.57	16.67±0.57	80.0
	500	5.00±1.00	15.00±1.00	66.7
Mixture 25/75	2000	1.00±1.00	19.00±1.00	94.7
	1000	3.00±1.00	17.00±1.00	92.4
	500	5.33±0.57	14.67±0.57	63.7

CONCLUSION

The results of the current study indicated that extracts and essential oils of *Z. delagoense*, *O. americanum* and *B. cathartica* Bertol, have potential insecticidal and repellent activity against *Musca domestica* L and would be effective against the housefly *M. domestica* at different stages of its life cycle.

The results indicated also that the essential oils of the leaves of *Z. delagoense*, *O. americanum* and their mixtures have higher insecticidal activity compared to their extracts and highlighted the synergistic effect of essential oils combination.

The essential oil of *Z. delagoense* and *O. americanum* and their binary mixtures have a good potential to be developed into effective natural larvicide, repellent and adulticide agents for controlling the housefly *M. domestica* L. population.

ACKNOWLEDGEMENTS

To the District Government of Memba for the authorization given to Mr. Pedro César Machanguia to continue his studies and to the Department of Chemistry, Faculty of Science, Eduardo Mondlane University for laboratory facilities and support.

REFERENCES

1. Wang JN, Hou J, Wu YY, Guo S, Liu QM, Li TQ, Gong ZY. Resistance of House Fly, Musca domestica L.(Diptera: Muscidae), to Five Insecticides in Zhejiang Province, China: The Situation in 2017. Canadian Journal of Infectious Diseases and Medical Microbiology. 2019;2019.
2. Farooq M, Freed S. Infectivity of housefly, Musca domestica (Diptera: Muscidae) to different entomopathogenic fungi. brazilian journal of microbiology. 2016 Dec;47(4):807-16.
3. Malik A, Singh N, Satya S. House fly (Musca domestica): A review of control strategies for a challenging pest. J Environ Sci Heal - Part B Pestic Food Contam Agric Wastes. 2007;42(4):453–69.
4. Levchenko MA, Silivanova EA, Balabanova GF, Bikinyaeva RH. Insecticide susceptibility of house flies (Musca domestica) from a livestock farm in Tyumen region, Russia. Bulg J Vet Med. 2019;22(2):213–9.
5. Sharma PP, Pardeshi AB, Vijigiri D. Bioactivity of some medicinal plant extracts against Musca domestica L. J Ecobiotechnology. 2011;3(9):14–6.
6. Prieto JA, Patiño OJ, Delgado WA, Moreno JP, Cuca LE. Chemical composition, insecticidal, and antifungal activities of fruit essential oils of three colombian Zanthoxylum species. Chil J Agric Res. 2011;71(1):73–82.
7. Suwannayod S, Sukontason KL, Pitasawat B, Junkum A, Limsopatham K, Jones MK, Somboon P, Leksomboon R, Chareonviriyaphap T, Tawatsin A, Thavara U, Sukontason K. Synergistic Toxicity of Plant Essential Oils Combined with Pyrethroid Insecticides against Blow Flies and the House Fly. Insects. 2019;10(6):178.
8. Tripathi AK, Prajapati V, Khanuja SPS, Kumar S. Effect of d-Limonene on Three Stored-Product Beetles. J Econ Entomol. 2009;96(3):990–5.
9. Pavela R. Insecticidal Properties of Several Essential Oils on the House Fly (Musca domestica L.). Phyther Res. 2008;1208(May):1200–8.
10. Palacios SM, Bertoni A, Rossi Y, Santander R, Urzúa A. Insecticidal activity of essential oils from native medicinal plants of Central Argentina against the house fly, Musca domestica (L.). Parasitol Res. 2009;106(1):207–12.
11. Chauhan N, Malik A, Sharma S. Repellency potential of essential oils against housefly, Musca domestica L. Environ Sci Pollut Res. 2018;25(5):4707–14.
12. De Souza AP, Marques M, Mahmoud T, Bolzani V, Caputo B, Canhete G, Leite CB, De Lima DP. Insecticidal Effect of Extracts from Native Plants to Mato Grosso do Sul, Brazil, on Sitophilus zeamais

Mots. (Coleoptera: Curculionidae). BioAssay. 2010;5(0):0–5.
13. Soonwera M, Phasomkusolsil S. Adulticidal, larvicidal, pupicidal and oviposition deterrent activities of essential oil from Zanthoxylum limonella Alston (Rutaceae) against Aedes aegypti (L.) and Culex quinquefasciatus (Say). Asian Pac J Trop Biomed [Internet]. 2017;7(11):967–78.
14. Overgaard HJ, Sirisopa P, Mikolo B, Malterud KE, Wangensteen H, Zou YF, Paulsen BS, Massamba D, Duchon S, Corbel V, Chandre F . Insecticidal activities of bark, leaf and seed extracts of Zanthoxylum heitzii against the African Malaria Vector Anopheles gambiae. Molecules. 2014;19(12):21276–90.
15. Matimele A Raimondo D, Bandeira S, Burrows JE, Darbyshire I, Massingue AO, Timberlake, J. Zanthoxylum delagoense. 2016;8235. The IUCN Red List of Threatened Species 2016: e.T38743A85955359. http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T38743A85955359.en.
16. Shadia E, El-Aziz A, Omer EA, Sabra AS. Chemical Composition of Ocimum americanum Essential Oil and Its Biological Effects Against, Agrotis ipsilon, (Lepidoptera: Noctuidae). Res J Agric Biol Sci [Internet]. 2007;3(6):740–7. Available from: http://www.academia.edu/21092547/Chemical_Composition_of_Ocimum_americanum_Essential_Oil_and_Its_Biological_Effects_Against_Agrotis_ipsilon_Lepidoptera_Noctuidae
17. Cavalcanti ESB, Morais SM de, Lima MAA, Santana EWP. Larvicidal activity of essential oils from Brazilian plants against Aedes aegypti L. Mem Inst Oswaldo Cruz. 2004;99(5):541–4.
18. Chere JMC, Dar MA, Pandit RS. Evaluation of Some Essential Oils against the Larvae of House Fly , Musca domestica by Using Residual Film Method. Adv. Biotech. Microb. 2018;9(1):1–8.
19. Maroyi A, Maroyi A. Bridelia cathartica Bertol. f. (Phyllanthaceae): a review of its pharmacological properties and medicinal potential. 2018;6(3):424–33.
20. Cumbane P, Munyemana F. Antioxidant and antibacterial activity of leaves and stem extracts of Bridelia cathartica Bertol. Sky Journal of Microbiology Research. 2017;5(2):18–26.
21. Loko LY, Alagbe O, Dannon EA, Datinon B, Orobiyi A, Thomas-Odjo A, Densi A, Tamo M . Repellent Effect and Insecticidal Activities of Bridelia ferruginea, Blighia sapida, and Khaya senegalensis Leaves Powders and Extracts against Dinoderus porcellus in Infested Dried Yam Chips. Psyche (New York). 2017;2017.
22. Adesina JM, Ileke KD, Yallappa R, Ofuya TI. Insecticidal evaluation of bridelia micrantha and dalbergia lactea aqueous extracts for the control of podagrica uniforma (Jacoby) and nisotra dilecta (Jacoby) (Coleoptera: Chysomelidae) infestation on okra. Agrivita. 2016;38(3):269–74.
23. Sivanandham V. Phytochemical techniques - a review. World J Sci Res. 2015;1(2):80–91.
24. Soonwera M, Sinthusiri J. Thai Essential Oils as Botanical Insecticide Against House Fly (Musca domestica L.) Int. Conf of Agricultural, Ecological and Medicinal Sciences. 2014;66(0):67–9.
25. WHO/ GMP. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes: Second edition. World Heal Organ Tech Rep Ser [Internet]. 2016;22. Available from: https://www.who.int/malaria/publications/atoz/9789241511575/en/
26. Hamouda A Ben, Mechi A, Zarred K, Chaieb I, Régional C. Insecticidal Activities of Fruit Peel Extracts of Pomegranate (Punica granatum) against the red flour beetle Tribolium castaneum. Tunisian Journal of Plant protection. 2014;9(1):91–100.
27. Girão Filho JE, Alcântara Neto F, Pádua LEM, Pessoa EF. Repelência e atividade inseticida de pós vegetais sobre Zabrotes subfasciatus Boheman em feijão-fava armazenado. Rev Bras Plantas Med. 2014;16(3):499–504.
28. Chintalchere JM, Lakare S, Pandit RS. Bioefficacy of Essential Oils of Thymus Vulgaris and Eugenia Caryophyllus Against Housefly , Musca. Int Q J Life Sci. 2013;8(3):1029–34.
29. Campbell MM. A test for repellency to non-biting flies and a comparison of repellents using Musca domestica L. Pestic Sci. 1983;14(2):199–212.
30. Ayangla N, Singh N, Kumar A. Phytochemical Analysis of Plant Species of genus Zanthoxylum. Internatinal J Med and Pharmaucetical Sci. 2016;6(1):1–8.
31. Chhabra SC, Uiso FC, Mshiu EN. Phytochemical screening of tanzanian medicinal plants. I. J Ethnopharmacol. 1984;11(2):157–79.
32. Dhale D, Birari A, Dhulgande S. Preliminary Screening of Antibacterial and Phytochemical Studies of Ocimum americanum Linn. J Ecobiotechnology. 2010;2:11–3.
33. Sarma SDK, Babu AVS. Pharmacognostic and phytochemical studies of Ocimum americanum. J Chem Pharm Res. 2011;3(3):337–47.
34. Munyemana F, Alberto AL. Evaluation of larvicidal activity of selected plant extracts against Plutella xylostella (Lepidoptera: Plutellidae) larvae on cabbage. Adv Med Plant Res. 2017;5(1):11–20.
35. Pandey AK, Singh P, Tripathi NN. Chemistry and bioactivities of essential oils of some Ocimum species: An overview. Asian Pac J Trop Biomed. 2014;4(9):682–94.
36. Miller SL, Haber WA, Setzer WN. Chemical composition of the leaf essential oil of an undescribed species of Zanthoxylum from Monteverde, Costa Rica. Am J Essent Oils Nat Prod AJEONP. 2013;1(12):38–40.

37. Guy I, Charles B, Guinaudeau H, Ferreira ME, Rojas de Arias A, Fournet A. Essential oils from Zanthoxylum chiloperone and Z. riedelianum growing in Paraguay. Pharm Biol. 2001;39(2):152–4.
38. Donald GR, Fernandes PD, Boylan F. Antinociceptive Activity of Zanthoxylum piperitum DC. Essential Oil. Evidence-based Complement Altern Med. 2016;2016.
39. Özcan, M.; Chalchat JC. Essential Oil Composition of Ocimum basilicum L . and Ocimum minimum L . in Turkey. Czechoslov J Food Sci. 2002;20(6):223–8.
40. Souza Filho APS, Bayma JC, Guilhon GMSP, Zoghbi MGB. Atividade potencialmente alelopática do óleo essencial de ocimum americanum. Planta Daninha. 2009;27(3):499–505.
41. Sanseera D, Niwatananun W, Liawruangrath S, Baramee A, Khantawa B, Pyne SG, Liawruangrath B. Comparison of Chemical constituents and antibacterial activities and antioxidant activities of the essential oil from leaves and fruits of Bridelia retusa (L.) A. Juss. J Sci Ind Res (India). 2012;71(11):733–9.
42. Aparecida L, Morais S De. Química Dos Óleos Essenciais. 2009;2:4050–63.
43. Chantawee A, Soonwera M. Larvicidal, pupicidal and oviposition deterrent activities of essential oils from Umbelliferae plants against house fly Musca domestica. Asian Pacific Journal of Tropical Medicine. 2018;11(10):621–9.
44. Ahmad F, Iqbal N, Zaka SM, Qureshi MK, Saeed Q, Khan KA, Ghramh HA, Mohammad JA, Jaleel W, Muhammad A, Awar MB . Comparative insecticidal activity of different plant materials from six common plant species against Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Saudi J Biol Sci [Internet]. 2018;0–4. Available from: https://doi.org/10.1016/j.sjbs.2018.02.018
45. Attaullah, Zahoor MK, Zahoor MA, Mubarik MS, Rizvi H, Majeed HN, Zulhussnain M, Ranian K, Sultana K, Imran M, Qamer S. Insecticidal, biological and biochemical response of Musca domestica (Diptera: Muscidae) to some indigenous weed plant extracts. Saudi J Biol Sci [Internet]. 2019;(xxxx). Available from: https://doi.org/10.1016/j.sjbs.2019.05.009
46. Maria C, Dos B. Universidade federal do amazonas programa multi-institucional de pós-graduação em biotecnologia doutorado em biotecnologia. 2006;1966:0–171.
47. Sukontason KL, Boonchu N, Sukontason K, Choochote W. Effects of eucalyptol on house fly (diptera: muscidae) and blow fly (diptera: calliphoridae). Rev Inst Med Trop Sao Paulo. 2004;46(2):97–101.
48. Adamski Z, Niewadzi M, Ziemnicki K. Inheritance of chorionic malformations and insecticide resistance by Spodoptera exigua. J Appl Entomol. 2005;129(9–10):526–33.
49. Pandey SK, Tandon S, Ahmad A, Singh AK, Tripathi AK. Structure-activity relationships of monoterpenes and acetyl derivatives against Aedes aegypti (Diptera: Culicidae) larvae. Pest Manag Sci. 2013;69(11):1235–8.
50. Kumar P, Mishra S, Malik A, Satya S. Insecticidal evaluation of essential oils of Citrus sinensis L. (Myrtales: Myrtaceae) against housefly, Musca domestica L. (Diptera: Muscidae). Parasitol Res. 2012;110(5):1929–36.
51. Ahmed S, Khan AS, Nisar MS. Effect of Aqueous Extracts of Different Plants on Life Cycle and Population Build up Parameters of House Fly , Musca domestica L(Diptera: Muscidae). Research &Reviews: Journal of Agriculture Science and Technology. 2015; 4(1): 1- 6.
52. Mansour SA, Bakr RFA, Mohamed RI, Hasaneen NM. Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, Musca Domestica L. Open Toxinology J. 2011;4:1–13.
53. Aktar MJ, Islam MS. An eco-friendly approach to reduce reproductive attributes in the housefly, Musca domestica L. using crude plant extracts. Scholars Academic Journal of Biosciences. 2015;3(5):443–51.
54. HUANG S qing, ZHANG Z xiang, LI Y zhi, LI Y xia, XU H hong. Anti-Insect Activity of the Methanol Extracts of Fern and Gymnosperm. Agric Sci China [Internet]. 2010;9(2):249–56. Available from: http://dx.doi.org/10.1016/S1671-2927(09)60090-0
55. Benelli G, Pavela R, Maggi F, Nkuimi Wandjou JG, Yvette Fofie NGB, Koné-Bamba D, Sagratini G, Vittori S, Caprioli G . Insecticidal activity of the essential oil and polar extracts from Ocimum gratissimum grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. Ind Crops Prod. 2019;132(February):377–85.
56. Ahmed KM. The effect of aqueous plant extracts of tobacco on Third larvae of house fly (Musca dmestica L., (Diptera: Muscide). J Entomol Zool Stud. 2018;6(3):403–8.
57. El-sherbini GT, Osman N. Original Research Article Insecticidal effects of Fortunella crassifolia essential oil used against house fly (Musca domestica). Int. Journal Curr. Microbiol. App. Sciences 2014;3(6):1–9.
58. Mwangi EN, Hassanali A, Essuman S, Myandat E, Moreka L, Kimondo M. Repellent and acaricidal properties of Ocimum suave against Rhipicephalus appendiculatus ticks. Exp Appl Acarol. 1995;19(1):11–8.
59. Lee S, Tsao R, Peterson C, Coats JR. Insecticidal Activity of Monoterpenoids to Western Corn. J Econ Entomol. 1997;90(4):883–92.

60. Reda FAB, Abdel Fattah H, Salim N, Atiya N. Insecticidal activity of Four Volatile Oils on Two Museum Insects Pests. Egypt Acad J Biol Sci F Toxicol Pest Control. 2010;2(2):57–66.
61. Wang CF, Yang K, Zhang HM, Cao J, Fang R, Liu ZL, Du ZL, Wang YY, Deng ZW, Zhou L . Components and insecticidal activity against the maize weevils of Zanthoxylum schinifolium fruits and leaves. Molecules. 2011;16(4):3077–88.
62. Hao H, Sun J, Dai J. Dose-Dependent Behavioral Response of the Mosquito Aedes albopictus to Floral Odorous Compounds . J Insect Sci. 2013;13(127):1–8.
63. Naik DG, Vaidya HS, Namjoshi TP. Essential oil of indian propolis: Chemical composition and repellency against the honeybee apis florea. Chem Biodivers. 2013;10(4):649–57.
64. Elbermawy S, Elkattan N, Ahmed K, Abdel-Gawad R. Screening for Bioactive Plant Materials against House Fly, Musca domestica L.(Diptera: Muscidae). Funct Plant Sci Biotechnol. 2011;5(1):45–51.
65. Hieu TT, Kim S Il, Kwon HW, Ahn YJ. Enhanced repellency of binary mixtures of Zanthoxylum piperitum pericarp steam distillate or Zanthoxylum armatum seed oil constituents and Calophyllum inophyllum nut oil and their aerosols to Stomoxys calcitrans. Pest Manag Sci. 2010;66(11):1191–8.
66. Singh D, Singh AK. Repellent and insecticidal properties of essential oils against housefly, Musca domestica L. Int J Trop Insect Sci. 1991;12(04):487–91.
67. Tapondjou AL, Adler C, Fontem DA, Bouda H, Reichmuth C. Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against Sitophilus zeamais Motschulsky and Tribolium confusum du Val. J Stored Prod Res. 2005;41(1):91–102.
68. Bisseleua HBD, Gbewonyo SWK, Obeng-Ofori D. Toxicity, growth regulatory and repellent activities of medicinal plant extracts on Musca domestica L. (Diptera: Muscidea). African J Biotechnol. 2008;7(24):4635–42.
69. Tian Y. Toxicity and repellency of essential oils to the house fly (Musca domestica). 2017;
70. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: A review. Bioresour Technol [Internet]. 2010;101(1):372–8. Available from: http://dx.doi.org/10.1016/j.biortech.2009.07.048.