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KNOCK DOWN EFFECT OF *Syzygium aromaticum* AND *Cinnamonam cassia* PLANT VOLATILE AGAINST THE *Aedes aegypti* L. (DIPTERA: CULICIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author JS conducted the data. Author KE designed and prepared the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

In the present study, knock down activity of *Syzygium. aromaticum* and *Cinnamomum cassia* were tested against the freshly emerged adults of *Ae. aegypti* in a condutive laboratory. The plant volatile oils were individually tested with different concentrations of 50, 100, 150 and 200 mg/mL concentrations concentrations as stated in the experiments larval mortality against the freshly moulted fourth in star larvae of *Ae. Aegypt* with the selected concentration of the plant volatile oil. After 24, 48, 72 hrs of exposure the mosquitoes dead and counted the percent mortality was calculated by using standard formula. In the present study, the knockdown activity of *C. cassia* indicated that the significant mortalities were observed at 200mg/mL concentration. In 50 mL *S. aromaticum* induced, 26.2, 34.2 and 38.4%, 100 mg/mL concentration 2.8, 46.8 and 52.8% in the 150 mg/mL concentration 62.2, 72.2 and 76.4% and in 200 mg/mL concentration of the essential oil, *S. aromaticum* induced significant knockdown effect of 82.2, 84.6 and 90.2% at 24, 48 and 72 hrs exposure period respectively on *Ae. aegypti* .In the same way *C. cassia* was tested with different concentrations larval mortality against the on *Ae. aegypti* 50, 100, 150 and 200 mg/mL. In 50 mg/mL concentration of the essential oil, *C. cassia* induced 24.8, 38.6 and 40.2%, 100 mg/mL concentration 36.2, 45.2 and 52.4%, in the same way the 150 mg/mL 64.4, 74.6 and 78.2%, in the 200 mg/mL concentration of the essential oil *C. cassia* induced significant knockdown effect of 80.4, 86.8 and 92.2% at 24, 48 and 72 hrs exposure period. It is inferred that the plant oils persuaded remarkable mortality on the test mosquitoes. Thus, the phytochemicals are eco-friendly, safer to non-target organisms.

Keywords: Plant volatile oil *Syzygium aromaticum*; *Cinnamonam cassia*; knockdown activity.

1. INTRODUCTION

Many measures have undertaken some successfully, to prevent or minimize the number of infections spread by *Aedes* mosquitoes [1,2]. Successful vaccination campaigns and legal requirements of countries for international travelers have contained mainly the global spread of yellow fever. Viral infections spread by *Aedes* spp [3,4,5,6]. cause significant morbidity and mortality globally. Despite having an effective vaccine for yellow fever, the annual estimated death of yellow fever still exceeds 30,000 people [WHO, 2016]. The knockdown effect accounts for the efficacy of insecticide to control the

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mosquito populations. Therefore, good should have the ability to bring 90% of the reduced insect population [7]. Knockdown is the initial effect such as unhealthy or unusual behavior due to the alteration of a specific physiological process or processes that taken placed upon contact with the toxicant [8-9]. The repellency of the essential oil of *C. sativum* was evaluated using the human-bait technique, to simulate the condition of human skin on which repellents will be applied [10-11]. *Aedes aegypti* are responsible for the transmission of several critical viral infections, including yellow fever, dengue, chikungunya, and Zika virus. Yellow fever is currently endemic in 47 countries and is responsible for between 29,000 to 60,000 deaths annually [12]. Dengue-related deaths are far fewer in number, but the estimated number of clear cases globally was 58.4 million in 2013, is a sharp increase from the estimate in 1990 of 8.3 million. The total number of disability-adjusted life years [DALYs] lost due to dengue was 1.14 million in 2015 alone [13]. Unlike the globally prevalent diseases mentioned above, both chikungunya and Zika virus infections usually occur in localized outbreaks. However, with increased international travel, they also can cause epidemics across borders [14-15]. The full spectrum of disease manifestations in people who infected with Zika virus is not yet known, and its association with microcephaly has recently raised concerns [16]. Chikungunya, though rarely fatal, causes significant long-term morbidity in the form of debilitating arthralgia [17,18]. In addition to the well-known diseases mentioned above, these *Aedes* mosquito species are also the vectors of several other endemic viral infections such as West Nile fever, Mayaro virus infection, and Eastern equine encephalitis virus infection [10,11,19]. In another research, the petroleum ether extract from Korean coriander showed toxic effects against *Ae. aegypti* larvae [20]. The composition and the bioactivity of the essential Moil extracted from *C. sativum* were able to wield outstanding repellency against *A. albopictus* [21]. Neem seed oil (NSO) of *Azadirachta indica* contains more than one hundred determined biologically active compounds, and many formulations deriving from them showed toxicity, antifeedant and repellence against several arthropod pests [22-24]. The two formulated products have excellent repellency and knockdown effect due to synergism among the plants' extracts [19]. The combination of *Ocimum* and *Eucalyptus* oil led to a more superior product with a knockdown effect [15]. The increased concentration of essential oil showed maximum repellent activity with them increase in the concentration of the essential oil leads to increase in the protection time leading to prolonged repellent activity against *A. aegypti* mosquitoes [15]. Recently, reported [7] that the larvicidal activity of *Origanum marjorana* oil and ovicidal activity of *O. marjorana* against *Ae. aegypti, An. stephensi* and *C. quinquefasciatus* [26-30]. In Ayurveda, a lot of herbal formulations are in use widely [31-33,34,35]. However, it is not easy to analyze their pharmacological activity unless we analyze their phytochemical constituents. *Artemisia nilagirica*, a herb is reported for its mosquito larvicidal and insect-repellent activities [36]. Many measures have undertaken some successfully, to prevent or minimize the number of infections spread by *Aedes* mosquitoes. Successful vaccination campaigns and legal requirements of countries for international travelers have contained mainly the global spread of yellow fever [37-39,20,40]. Viral infections spread by *Aedes* spp. cause significant morbidity and mortality globally. Despite having an effective vaccine for yellow fever, the annual estimated death of yellow fever still exceeds 30,000 people [10]. The widespread mosquitoes are controlled by using essential oils like *S. aromaticum* and *C. cassia.* researchers to find worthwhile alternatives that are more environmentally friendly [41,42-45]. In this context, based on botanical extracts is attracting substantial interest in recent years. Among botanical extracts used as pesticides, plant volatile oils are a hopeful alternative because of their availability worldwide and low cost effectiveness.

2. METHODOLOGY

Knock down activity: The knockdown activity of *S. aromaticum* and *C. cassia* oils have studied by following the modified method of (Jaswanth et al. 2002). The experiments have conducted in a closed chamber. Fifty adult female mosquitoes (3-5 hrs. old) have released into the chamber. The plant essential oils have individually soaked with cotton and kept inside the chamber. The different concentrations i.e., 50, 100, 150 and 200mg/ml of *S. aromaticum* and *C. cassia* oils have tested individually with the exposure period of 24, 48 and 72 hrs. The Knockdown of selected plant volatile oils were then recorded from the average of five replicates.

$$\text{Knockdown (\%)} = \frac{\text{No. of adult knocked down}}{\text{No. of adults released}} \text{ X } 100$$

Determination of lethal concentration: Lethal concentration (LC_{50} and LC_{90}) represents the concentration of the test material that caused 100% mortality of the test organisms within the specified period of exposure (Krishnappa et al., 2012a & 2012b). It was determined by exposing various development stages of the (*Ae. aegypti)* to a different a concentration of the oil. Based on the mortality of the test organisms a recorded a in the bioassays, LC_{50}

and LC_{90} were calculated along with their fiducial a limits at 95% confidence level by probit analysis using SPSS (version 20.0).

Analysis of volatile compounds: The volatile constituents of *S. aromaticum and C. cassia* oils have established by Gasa Chromatography-Mass Spectrometry/Quadropolea detector analyses using a Shimadzu QP 5050asystem fitted with a Free Fatty Acida Phase (50 m × 0.32 mm (i.e.) film thickness: 0.25 µm) capillary column. 230°C was the detector and injector temperature and program for the column was from 120°C (1 min) to 230°C at a rate of 6°C/mina and then held at 200°C for 35 min. Helium gas was used as a carrier gas at a flow of 14 psi (Split 1:10), and the injection volume of each sample was one µl. The identification of the components was based on comparison of their mass spectra with those of a Wiley and a NIST Tutorea Libraries. The ionization energy was set 70 eV.

3. RESULTS

3.1 Knock down Activity of *Syzygium aromaticum* Tested against *Ae. Aegypti*

The Knock down activity of *S. aromaticum* was tested with different concentrations as stated in the previous experiments (*i.e.,* 50, 100, 150 and 200 mg/mL concentrations). It is pertinent to note that the 50 mg/mL concentration of the essential oil, *S. aromaticum* induced 26.2, 34.2 and 38.4% knockdown effect against the freshly emerged adults of *Ae. aegypti*. It is pertinent to note that the 100 mg/mL concentration of the essential oil, *S. aromaticum* induced 32.8, 46.8 and 52.8% knockdown effect on *Ae. aegypti*. It is also pertinent to note that the 150 mg/mL concentration of the essential oil, *S. aromaticum* induced 62.2, 72.2 and 76.4% knockdown effect against the *Ae. aegypti* adults. Besides, the 200 mg/mL concentration of the essential oil, *S. aromaticum* induced significant knockdown effect of 82.2, 84.6 and 90.2% at 24, 48 and 72 hrs exposure period respectively on *Ae. aegypti* [Fig. 1]. The lethal concentration values the above data are represented in Table 1.

3.2 Knock down Activity of *Cinnamonam cassia* Tested against *Ae. Aegypti*

The knockdown activity of *C. cassia* was tested with different concentrations as stated in the previous experiments (*i.e.,* 50, 100, 150 and 200 mg/mL concentrations). It is pertinent to note that the 50 mg/mL concentration of the essential oil, *C. cassia* induced 24.8, 38.6 and 40.2% knockdown effect against the freshly emerged adults of *Ae. aegypti*. It is pertinent to note that the 100 mg/mL concentration of the essential oil, *C. cassia* induced 36.2, 45.2 and 52.4% larval mortality against the on *Ae. aegypti*. In the same way the 150 mg/mL concentration of the essential oil, *C. cassia* induced 64.4, 74.6 and 78.2% knockdown effect. Besides, the 200 mg/mL concentration of the essential oil *C. cassia* induced significant knockdown effect of 80.4, 86.8 and 90.4% at 24, 48 and 72 hrs exposure period respectively [Fig. 2]. The lethal concentration values the above data are represented in Table 2.

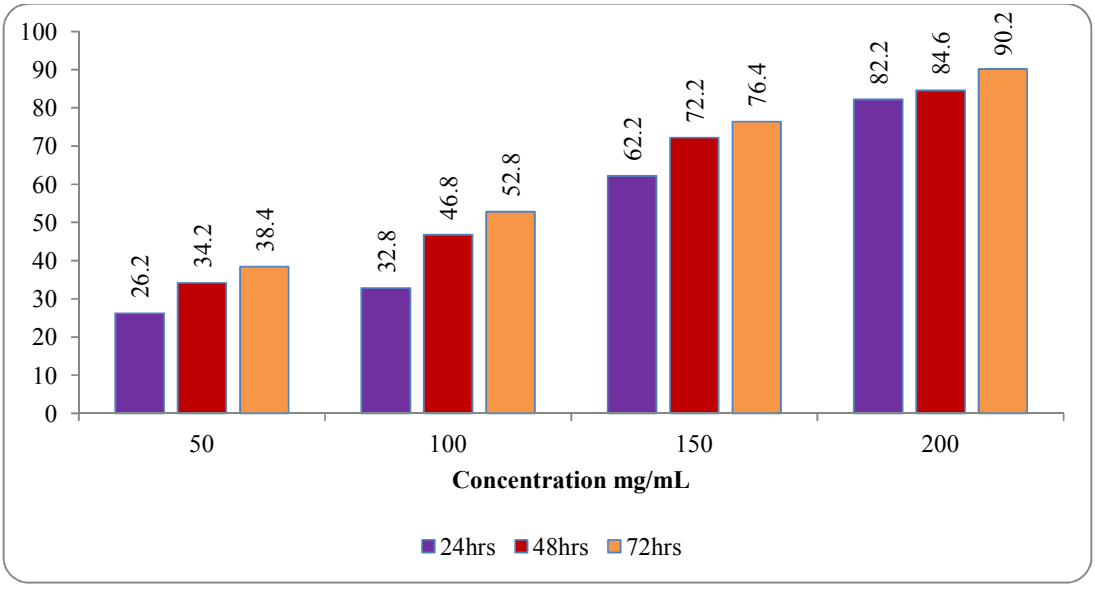

Fig. 1. Knock down activity of *Syzygium aromaticum* tested against *Ae. aegypti*

Table 1. Determined lethal concentration values of *S. aromaticum* tested against *Ae. Aegypti*

Exposure periods (hrs)	LC_{50}	95 % fiducial limit		LC_{90}	95% fiducial limit		χ^2
		LCL	UCL		LCL	UCL	
24	122.30	109.84	134.61	240.16	216.10	276.67	3.952
48	96.97	80.73	110.38	226.32	201.93	264.65	1.203
72	83.85	66.90	97.10	203.67	183.06	235.02	1.043

[LCL stands for lower control limit and UCL stands for upper control limit]

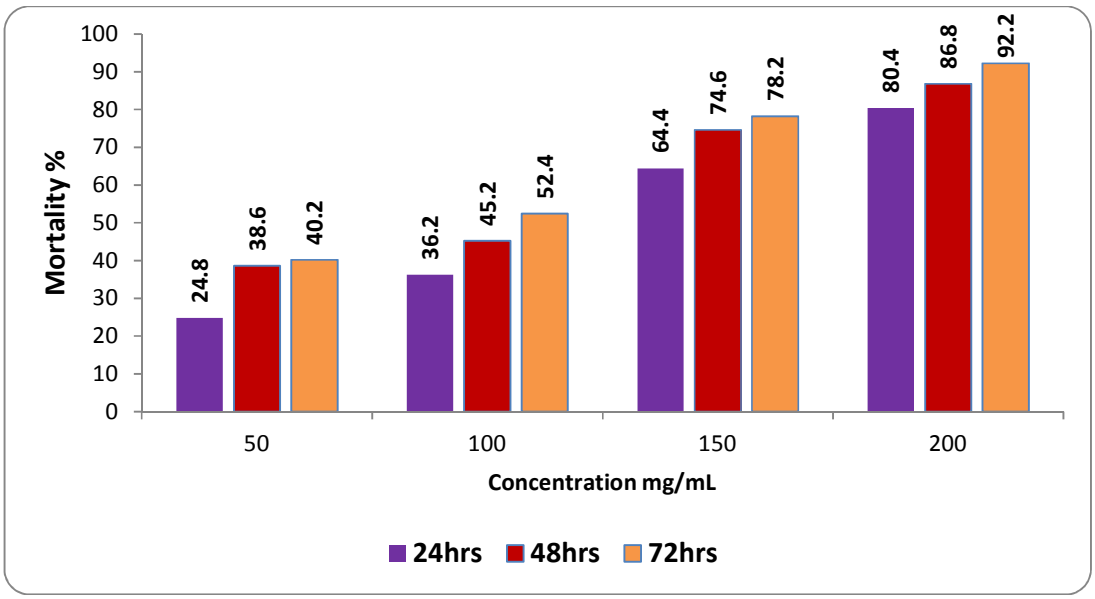

Fig. 2. Knock down activity of *Cinnamonam cassia* tested against *Ae. Aegypti*

Table 2. Determined lethal concentration values of *Cinnamonam cassia* tested against *Ae. Aegypti*

Exposure periods (hrs)	LC_{50}	95 % fiducial limit		LC_{90}	95% fiducial limit		χ^2
		LCL	UCL		LCL	UCL	
24	120.84	108.12	133.24	240.38	216.06	274.47	1.549
48	91.27	74.21	104.90	220.34	196.59	257.62	4.028
72	81.13	63.58	94.64	201.63	181.10	232.43	1.860

[LCL stands for lower control limit and UCL stands for upper control limit]

4. DISCUSSION

Mosquitoes spread serious human diseases, causing millions of deaths every year and the development of resistance to synthetic insecticides, resulting in bounce back vectorial capacity. The mosquito has approximately 3500 species and present in tropical and subtropical regions of the world [46,47,25,48,7, 10,11]. The effect of the plant and plant-based products have well documented by several researchers, this attributed due to the presence of a wide array of phyto chemical groups and novel compounds present in the plants [49]. The development of resistance to chemical mosquitoes results in rebounding vectorial capacity. Worldwide synthetic repellents are used for protection against mosquito-borne diseases and it adversely affects the environment by contaminating water, soil and air. There is an urgent necessity to find substitutes to synthetic insecticides. Plants are amusing source of alternative agents for control of mosquitoes and its vectors. Plant oils and their compounds from different plant families have evaluated for their promising mosquito repellent activities. Literature has documented that essential oils have traditionally used as effective repellents. In the present investigation, many compounds from the four selected oils predominantly belong to the terpenes and phenolics. Essential oils are composed of volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has linked to the presence of monoterpenes and sesquiterpenes. The present

study focused on the potential and mosquito repellent activity of different volatile oils of medicinal plants. From an economic point of view, the synthetic chemical is still more frequently used as repellents than essential oils, these essential oils have the potential to provide efficient and can be used as a cheap, eco-friendly, safer for humans and the environment and also an efficient alternative to the chemical larvicides. Mosquitoes spread serious human diseases, causing millions of deaths every year and the development of resistance to synthetic insecticides, resulting in bounce back vectorial capacity [50] published the efficacy of repellency of *Hyptis suaveolens* and *Ocimum gratissimum* against the adults of *Ae. Aegypti.* Mosquito coil made from the above said plants offered complete protection from *Ae. aegypti* bite. The combined use of plant increased the percent protection than with individual plant extracts. [51] described the knock activity of *Thymus vulgaris, Salviaa officinalis, Lippia origanoides, Eucalyptus globulus, Cymbopogon nardus, Cymbopogon martinii, Lippia alba, Pelargonium graveolens, Turnera diffusa, and Swinglea glutinosa* has larvicidal action against *Ae. aegypti.* The main compounds, thymol, and p-cymene of the oils have possessed the highest activity. Mosquitoes spread serious human diseases, causing millions of deaths every year and the development of resistance to synthetic insecticides, resulting in bounce back vectorial capacity. The mosquito has approximately 3500 species and present in tropical and subtropical regions of the world [52-60]. The larvicidal effect of the plant and plant-based products have well documented by several researchers, this attributed due to the presence of a wide array of phyto groups and novel compounds present in the plants [57]. The secondary metabolites like the monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellol, citronellal, camphor and thymol are the universal constituents in several essential oils presenting mosquitocidal activity [61-66]. In the present study also a remarkable knock down activity was noted with *C. aurantium* oil against the fourth instar larvae of *Ae. aegypti*. In this corroborating with the earlier works of several authors [58]. Evaluated knock down efficacy of the most promising oils such as *Mentha piperita*, *Myrtus caryophyllus* and *Acorus calamus.* [67] reported that, *Ocimum* the most active essential oils against third instar larvae of *A. aegypti*. *Mentha longifolia* and *Lavandula dentata* were evaluated for their insecticidal activity against adult females of *Culex pipiens* [68]. In reported the presence of 24, 17, 20, 21, and 12 compounds from the oils of *Citrus hystrix*, *Citrus reticulata*, *Zingiber zerumbet*, *Kaempferia galanga*, and *Syzygium aromaticum*, respectively. [60] Documented 90% larval mortality with *M. longifolia* and *M. suaveolens*, which were containing a majority of piperitenone oxide, and also had the maximum activity. Kiran and Devi [2007] stated that the essential oil and the sesquiterpenes isolated from the leaves of *C. swietenia* were screened for mosquitocidal activity by fumigant toxicity against three mosquito species, *Anopheles gambiae*, *C. quinquefasciatus,* and *Ae. aegypti.*

4. CONCLUSION

In past years, mosquito repellents play a substantial role in preventing mosquito contact and there by diminish the chance of impurities and its hostile effects. There is a crucial necessity to find alternates to synthetic insecticides. The plants based products are amusing source of another agents for control of mosquitoes and its vectors. Plant oils and their complexes from changed plant families have evaluated for their capable mosquito repellent activities. The present study has documented that essential oils have traditionally used as current repellents. In the present investigation, compounds from the four selected oils predominantly belong to the terpenes and phenolics. Essential oils are composed of volatile mixtures of hydrocarbons with a diversity of functional groups, and their repellent activity has linked to the presence of monoterpenes and sesquiterpenes. From an economical view, the synthetic chemical is still more normally used as repellents than essential oils these essential oils have the feasible to provide efficient and can be used as a cheap, biodegradable and harmless for humans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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