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Effect Of Thymus Vulgaris, Mentha Piperita And Pelargonium Citrosum Leaf, Syzygium Aromaticum Buds And Citrus Limonoids Peels Extracts As Mosquito Larvicidal And Pupicidal Agent

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Abstract – Mosquito control depends on application of synthetic insecticides. This has been limited due to its harmful effect on human health. This increased needs for botanical origin alternatives as a sustainable and more safe method of mosquito control. The current study aimed to evaluate larvicidal and pupicidal effect of thyme (Thymus vulgaris), peppermint (Mentha piperita) and citronella leaf (Pelargonium citrosum), clove buds (Syzygium aromaticum) and lemon peels (Citrus limonoids) methanolic extracts. Tested plants were extracted and three concentrations of these extracts were prepared (25, 50 and 100 mg/ml) then their larvicidal and pupicidal activities were evaluated against 20 larvae and 20 pupas for each concentration. Mortality rate were calculated after 24 hrs. Adult mosquitoes were identified to genus level. Clove showed the highest larvae mortality rate (100%) for the three tested concentrations, followed by thyme, lemon and peppermint. Citronella had no effect as larvicidal agent. On the other hand, pupicidal activity test of thyme showed the highest results followed by clove. While lemon didn't show any activity. Mosquito was identified as Culex. The result showed that buds extract of S. aromaticum and leaf extracts of T. vulgaris and peels extract of C. limonoids could serve as potential larvicidal extracts against the Culex mosquito.

Keywords – Mosquito, Larvicidal effect, Pupicidal effect.

I. INTRODUCTION

Among all groups of arthropods, mosquitoes considered the most disease transmitters, which affect millions of people around the world. World Health Organization (WHO) has declared the mosquitoes as "public enemy number one" (WHO reports). They act as a vector of many life threatening diseases such as malaria, yellow fever, dengue fever, etc. [1]. Mosquito control is essential to prevent spreading of mosquito borne diseases as well as improving quality of environment and public health. Applying synthetic chemical insecticides is the major tool in mosquito control operation, some of these chemicals are organochlorine and organophosphate compounds. In last years, many of synthetic insecticides in mosquito control program has been limited due to human health, economic and ecological issues [2, 3]. This has resulted in an increasing need to investigate environment friendly, cost-effective and biodegradable substitutions. The application of eco-friendly alternatives such as biological control of vectors has become the central focus of the control program instead of the chemical insecticides [1]. One of the most vital steps of biological control program is to explore the floral biodiversity and using more safe insecticides of a botanical origin as a simple and sustainable way of mosquito control. In addition, unlike synthetic traditional insecticides which consist of a single active ingredient, plant

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derived insecticides involve botanical mixture of many chemical compounds which act conjointly on both behavioural and physiological processes. Thus, there is very little chance of insects to develop resistance to such substances [1]. Plant secondary metabolites like limonoids contain larvicidal and antifeedant activity, therefore, the citrus oils that contain limonoids have more potential than citrus varieties without limonoid contents [4] as they detain the metabolic activities of the larvae [5]. It was reported that larvicidal potential of aqueous extracts from *Thymus vulgaris* L. leaves was tested on different larval instars of *Culex pipiens* and *Culiseta longiareolata*. The obtained results showed that this plant material had significant activity and can be considered as a powerful natural larvicidal agent against *Cx pipiens* [6]. Citronella oil was studied for its chemical composition and it was found that the crude essential oil consists of active ingredients that remarkably suppressed the growth of several mosquito species. Citronellal and linalool were the most active compounds among the 16 volatiles examined in Citronella oil, consisting of 6 main constituents of the essential oil and 10 other related monoterpenes [7]. Some essential oil and its components were found to have repellent and larvicidal properties [8]. Some essential oils and their compounds exhibited both repellent and larvicidal effects, volatile oils involving camphor, cineole, methyl eugenol, limonene, myrcene and thymol powerfully repelled adult mosquitos and showed larvicidal activity at the same time [9]. It was also found that mortality effect of clove and cinnamon essential oils against wild and laboratory-reared larvae had variations in their LC50 and LC95 values. The mortality of wild and laboratory-reared larvae at different concentrations of cinnamon and clove post exposure were dose-dependent and were higher for cinnamon compared to clove essential oils [10]. Another study concluded that the essential oil of *M. piperita* possessed excellent larvicidal efficacy against dengue vector [11]. This study aimed to evaluate the larvicidal and Pupicidal efficacy of lemon peels, citronella, thyme, peppermint and clove methanolic extract.

II. MATERIALS AND METHODS

2.1 Apparatus

Beaker (50 and 100 mL), adjustable pipettes (1-10, 10-100, and 100-1000 µL) sensitive balance, spatulas and white plastic trays (250 ml) were obtained from the Faculty of Pharmacy, University of Tripoli. Dimethyl sulfoxide (DMSO), solvent (methanol) were obtained from Merck, Germany.

2.2 Plant Sample Collection

Thyme was freshly collected from the west mountain (Libya), lemon fruits were collected from local markets. Citronella and peppermint were freshly collected from local garden. Clove buds were purchased from local markets. The voucher specimens were confirmed in Botany department, Faculty of Science, University of Tripoli. Plant samples were washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (thyme, peppermint and citronella leaves. Lemon peels and clove buds).

2.3 Extraction

Powder, plant materials were extracted with methanol 72hrs x 3 (maceration). Extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. Crude plant extracts were evaporated to dryness in a rotary vacuum evaporator.

2.4 Plant Dosage Preparation

One hundred mg of the plant residue was dissolved in 1 mL of DMSO (stock solution) and considered as 100 % stock solution. From this stock solution, different concentrations were prepared (50 mg/ml and 25 mg/ml).

2.5 Larvae Collection and Rearing

Wild larvae were collected from local farm in Tripoli, Libya. Larvae were sampled from irrigated fields using a standard dipper (350 ml) (WHO standardized procedure 1975) and transported to the insectary. Larvae were reared on dog's biscuit as a food source in Research Unit in pharmacognosy department, faculty of pharmacy, University of Tripoli. Libya. Colonies were maintained at 27 ± 2 °C, with a 12 h light: 12-h dark cycle. Insectary reared third and fourth instars larvae were tested against plant samples crude extracts at different concentrations.

2.5.1 Mosquito Larvicidal test

Larvicidal tests were carried out based on the WHO standardized procedures and guidelines for larvicidal test method (WHO. Guidelines 2005). Initial screening was conducted using the selected plant extracts with different dosages (25, 50 and 100mg/ml). Each plant extract was dissolved in DMSO to prepare (25, 50 and 100mg/ml) concentration. Batches of 25 3rd and 4th instar larvae of mosquito were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (25, 50, 100 mg/ml). A control group consisted of 1 mL of DMSO and 199 mL of distilled water. After treatment, treated larvae were observed and recorded immediately at time intervals and no food was offered to the larvae. At the end of 24 h, the larvae were considered dead, when they showed no sign of swimming movements even after gentle touching with a glass rod. The percentage of mortality was calculated with Abbott's formula [12].

2.5.2 Mosquito Pupicidal test

The same plant concentration (25, 50 and 100mg/ml) were used. Batches of 25 pupas of mosquito were transferred to 250 mL enamel bowl containing 199 mL of distilled water and 1 mL of plant extracts (25, 50, 100 mg/ml). A control group consisted of 1 mL of DMSO and 199 mL of distilled water. After treatment, treated larvae were observed and recorded immediately at time intervals and no food was offered to the pupas. At the end of 24 h, pupas were considered dead when they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the WHO's technical report series. The percentage of mortality was calculated with Abbott's formula [12].

$$Mortality\ \% = \frac{Survival\ in\ the\ control\ larvae - Survival\ in\ the\ treated\ larvae}{Survival\ in\ the\ control\ larvae} x100$$

2.5.3 Mosquito Identification

Samples of adult mosquitoes and larvae were tested in Department of Entomology, Faculty of Science, University of Tripoli, Libya to be identified.

III. RESULTS AND DISCUSSION

3.1. Percentage of Yield of Plant Extracts

The percentage yields of extracts ranged from 56% to 5% and are represented in Table 3.1. It revealed that, the Thyme (56%) showed maximum yield and followed by clove and peppermint (13%) (Table 1).

Table 1: Percentage extraction of Methanolic extracts of tested plant species

Plant name	Weight of plant part (g)	Yield of extract	
		Weight (g)	Percent (%)
Thyme	50	28	**56**
Clove	30	3.8	13
Peppermint	7	0.9	13
Lemon	50	3.8	8
Citronella	50	2.3	5

3.2. Mosquito Larvicidal Tests Result

The results were obtained in the preliminary assays of five extracts from thyme (*Thymus vulgaris)*, citronella (*Pelargonium citrosum*), lemon (*Citrus limonoids*), clove (*Syzygium aromaticum*) and peppermint (*Menthe piperita*) against third and fourth instar mosquito larvae showed that four of them were active. The larvicidal activities of the extracts against the mosquito larvae under

laboratories conditions are given in (Figure 1). The results of the present study indicate that the mortality rates at a 100mg/ml concentration were highest amongst all plant extracts tested for larval mortality. As shown in figure 1, clove had the highest mortality percentage (100%) among larvae in the three tested concentrations (25, 50 and 100mg/ml), followed by thyme, lemon peels and peppermint leaf extract respectively. While citronella leaf extract gave no result (mortality % = 0) in the three tested concentrations.

In Figure 2 it can be noticed that clove had 100% mortality percentage among the five tested plant species. This result was compatible with another study conducted by Susheela et al. 2016 which reported that the highest mortality (100%) was observed in the extract of cloves and alcohol among some studied plant extracts, which showed moderate larvicidal efficacy [13]. This work demonstrates the potency of clove extract in the control mosquito larvae. Clove extract appeared as the most lethal among the various extracts tested. The high mortality recorded for clove extract might be attributed to the deficiency of dissolved oxygen in the water. Eugenol which is the main constituent of clove buds extract was reported to have larvicidal activity [14].

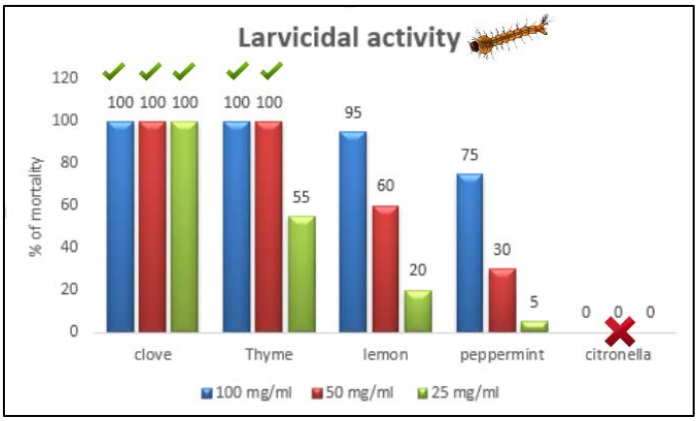

Figure 1 – Larvicidal activity of clove, thyme, lemon, peppermint and citronella (100mg/ml, 50 mg/ml, and 25 mg/ml).

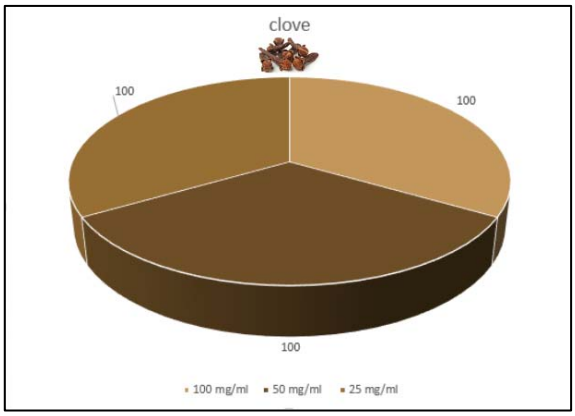

Figure 2 – Larvicidal activity of clove bus extract (100mg/ml, 50 mg/ml, and 25 mg/ml).

Thyme leaf methanolic extract (*Thymus vulgaris*) exhibited significant toxicity (100% mortality% for 50 and 100 mg/ml and 55% mortality% for 25 mg/ml) in the three concentrations (Figure 3). This bioactivity reflects a study by Massebo et al. (2009), who studied EO extracted from leaves and seeds of plants from Ethiopia [15]. Also, *T. vulgaris* extracts from plants grown in the Czech Republic with *Culex quinquefasciatus* confirmed this result [16]. Thymol and p-cymene were the major compounds identified in this plant, and the toxicity against mosquitoes was consistent with other reports on these metabolites [17]. Thymol was found to have a significant toxic effect against early third-stage larvae of *Culex tritaeniorhynchus*, *Aedes albopictus*, and *A. subpictus* [18]. This effect was followed by lemon peel extract, which showed 95% mortality% at 100mg/ml, 60% mortality% at 50 mg/ml and 20% at 25 mg/ml concentration. (Figure 4). Limonene which is the main component of lemon peels has natural resistance to insects and also can stop the activities of larvae [19]. This might explain the high effect of the extract against mosquito larvae. Govindarajan et al. 2013 showed that limonene appeared to be most effective among tested terpenes against mosquito larvae. Peppermint leaf extract showed 75% mortality% at 100mg/ml, 30% mortality% at 50 mg/ml and 5% at 25 mg/ml concentration [18]. (Figure 5).

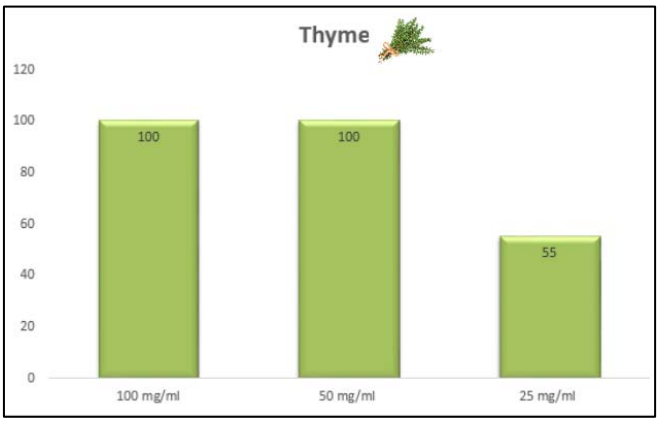

Figure 3 – Larvicidal activity of thyme leaf extract (100mg/ml, 50 mg/ml, and 25 mg/ml).

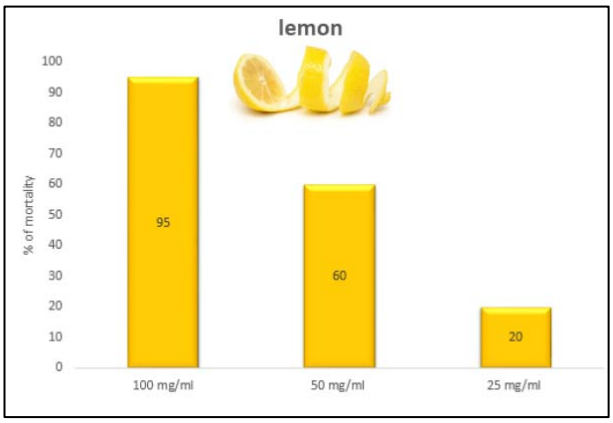

Figure 4 – Larvicidal activity of lemon peels (100mg/ml, 50 mg/ml, and 25 mg/ml).

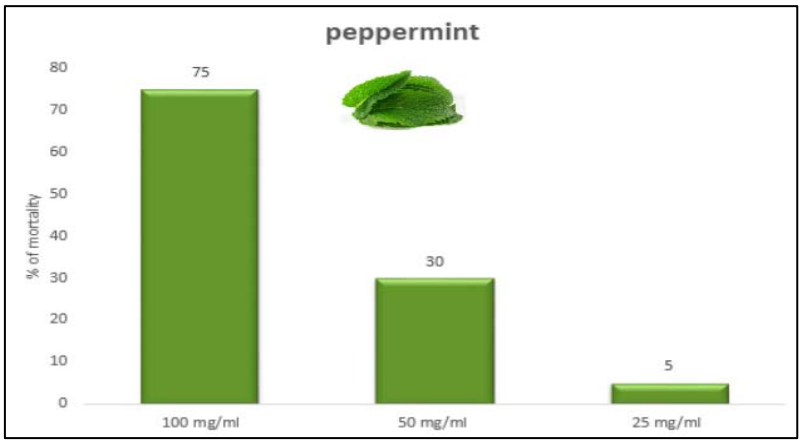

Figure 5 – Larvicidal activity of peppermint leaf extract (100mg/ml, 50mg/ml, and 25mg/ml).

The result was compatible with other study by [20], where *Mentha piperita* L (Peppermint oil), was evaluated for larvicidal activity against different species of mosquito. The oil showed strong larvicidal activity and repellent action against adult mosquitoes when applied on human skin. Other study [11] confirmed that essential oil extracted from *M. piperita* possessed excellent larvicidal efficiency against dengue vector. The toxicity of the oil increased 11.8% when the larvae were exposed to the oil for 48 h. The remarkable repellent properties of *M. piperita* essential oil were established against adults *Ae. aegypti*. The application of oil resulted in 100% protection for 150 min. After the next 30 min, only 1-2 bites were recorded as compared with 8-9 bites on the control arm. Citronella leaf extract showed no effect against 3rd and 4th instar larvae in the three tested concentrations (25, 50 and 100 mg/ml). This was compatible with other research published in 1996 in The Journal of the Mosquito Control Association that claimed no significant difference between citrosa-treated and nontreated subjects [21]. In another study in the same journal, scientists assessed the citrosa "mosquito plant" as a wide area repellent against adult, host seeking female mosquitoes. This involve the two species *Aedes albopictus* and *Culex quinquefasciatus*. Researchers noted no significant differences in the number of mosquitoes landing on the forearms of human subjects in locations where plants were applied compared with areas without any plant application [22]. On other hand, a study that tested Micro-encapsulated Citronellas oil, showed that the capsules were potential to be larvicides of *Aedes aegypti* larvae. This might be explained as the extraction technique in this study was not designed to extract essential and volatile oil which were responsible for such effect.

3.3. Mosquito Pupicidal Tests Result

As the study continued on pupicidal activity, three plant extracts were chosen according to the highest mortality percentage on tested larvae. These plant extracts were clove buds, thyme leaves, and lemon peels extracts. As shown in figure 6, thyme resulted in the highest mortality% among tested extracts (100% mortality% for 100mg/ml, 40% mortality% for 50 and 25mg/ml), followed by clove (80% mortality% for 100mg/ml, 40% mortality% for 50 and 25mg/ml), while lemon peels extract had no effect as pupicidal agent among the three chosen extracts. This might be explained due to the active secondary metabolites in clove and thyme such as linalool, thymol and eugenol which is well known for their insecticide efficacy. Lemon did not affect pupa, this might be explained as the pupa is stronger than the larvae and has thicker skin that protects it against the environment.

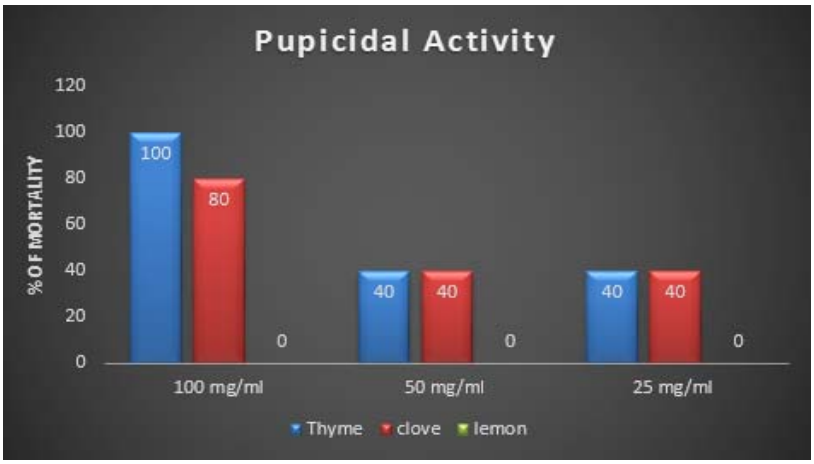

Figure 6 – Pupicidal activity of thyme leaves, clove buds and lemon peels extracts (100mg/ml, 50 mg/ml, and 25 mg/ml).

3.4. Mosquito Identification Result

Samples of adult mosquitoes and larvae were identified as Culex (genus level) in the Department of Entomology, Faculty of Science, University of Tripoli, Libya. This genus is common in Libya in addition to others. Identification to species level is under progress (Figure7, a and b).

Figure 7 – Culex mosquito adult and larvae standard (a) and tested adult and larvae samples (b)

IV. CONCLUSION

This result clearly displays those buds extract of *S. aromaticum* and leaf extracts of *T. vulgaris* and peels extract of *C. limonoids* could be potential larvicidal agents against the Culex mosquito. This approach could reduce the possibilities of physiological resistance development in the mosquito population. The mode of action and larvicidal efficiency of these plant spp. extracts should be investigated and determined. Besides, further investigation regarding the effect on the non-target organism is extremely important and imperative in the near future.

REFERENCES

[1] Ghosh A., Chowdhury N. and Chandra G., 2012. Plant extracts as potential mosquito larvicides. Indian J Med Res. 135(5):581-598.

[2] Brown AW.,1986. Insecticide resistance in mosquitoes: a pragmatic review. J Am Mosq Control Assoc. 2(2):123-40.

[3] Russell TL., Kay BH. and Skilleter GA., 2009. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. Aquat Biol. 6:77-90.

[4] Roy A. and Saraf S. Limonoids: 2006. overview of significant bioactive triterpenes distributed in plants kingdom. Bio and Pharma Bulletin. 29(2):191–201.

[5] Senthilkumar N., Varma P. and Gurusubramanian G., 2009. Larvicidal and adulticidal activities of some medicinal plants against the Malarial Vector, Anopheles stephensi (Liston). Parasitol Res. 104(2):237–244.

[6] Dahchar Z., Bendali-Saoudi F. and Soltani N., 2016. Larvicidal activity of some plant extracts against two mosquito species *Culex pipiens* and *Culiseta longiareolata*. Journal of Entomology and Zoology Studies. 4(4):346-350.

[7] Nakahara K., Alzoreky N. and Yoshihashi T., 2003. Chemical composition and antifungal activity of essential oil from *Cymbopogon nardus* (citronella grass). JARQ. 37(4):249–252.

[8] Krishnappa K., Elumalai K., Dhanasekaran S. and Gokulakrishnan J., 2012. Larvicidal and repellent properties of *Adansonia digitata* against medically important human malarial vector mosquito *Anopheles stephensi* (Diptera: Culicidae). *J. Vector Borne Dis*. 49(2): 86–90.

[9] Wahyuni D., 2012. Larvicidal Activity of Essential Oils of Piper betle from the Indonesian Plants against *Aedes Aegypti* L. J. Appl. *Environ. Biol. Sci*. 2(6):249–254.

[10] Thomas A., Humphrey D., Manjurano A., Morona D. and Eliningaya J., 2017. Evaluation of active ingredients and larvicidal activity of clove and cinnamon essential oils against *Anopheles gambiae* (sensu lato). Parasites & Vectors. 10(1):411.

[11] Kumar S., Wahab N. and Warikoo R., 2011. Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti* L. Asian Pac J Trop Biomed. 1(2):85-88.

[12] Abbott WS., 1987. A method of computing the effectiveness of an insecticide. *J Am Mosq Control Assoc*. 3(2):302-303.

[13] Susheela PR. and Padmapriyanga S., 2016. Evaluation of larvicidal action of natural extracts on mosquito larvae of *Aedes aegypti* (Diptera: Culicidae). International Journal of Mosquito Research. 3(6):26-30.

[14] Simas Nk., Lima ED., Conceição SD., Kuster RM., Oliveira AM. and Lage CLS., 2004. Natural products for dengue transmission control - Larvicidal activity of Myroxylon balsamum (red oil) and of terpencids and phenylpropanoids. Quim Nova. 27(1):46–49.

[15] Massebo F., Tadesse M., Bekele T., Balkew M. and Gebre-michael T., 2009. Evaluation on larvicidal effects of essential oils of some local plants against *Anopheles arabiensis* Patton and *Aedes aegypti* Linnaeus (Diptera, Culicidae) in Ethiopia. African J. Biotechnol. 8(17):4183-4188.

[16] Pavela R., 2008. Larvicidal effects of various Euro-Asiatic plants against Culex quinquefasciatus Say larvae (Diptera: Culicidae). Parasitol. Res. 102(3):555-559.

[17] Dias CN. and Moraes DFC., 2014. Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides. review Parasitol. Res. 113(2):565-592.

[18] Govindarajan M., Sivakumar R., Rajeswary M. and Veerakumar K ., 2013. Mosquito larvicidal activity of thymol from essential oil of *Coleus aromaticus* Benth. against *Culex tritaeniorhynchus*, *Aedes albopictus*, and *Anopheles subpictus* (Diptera: Culicidae). Send to Parasitol Res. 112(11):3713-21

[19] Wahyuni D., 2012. Larvicidal Activity of Essential Oils of Piper betle from the Indonesian Plants against *Aedes Aegypti* L. J. Appl. Environ. Biol. Sci. 2(6):249–254.

[20] Ansaria MA., Vasudev P., Tandonb M. and Razdana R., 2000. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Bioresource Technology. 71(3):267-271.

[21] Matsuda B., Surgeoner G., Heal J., Tucker A. and Maciarello M., 1996. Essential oil analysis and field evaluation of the citrosa plant "*Pelargonium citrosum*" as a repellent against populations of Aedes mosquitoes. J Am Mosq Control Assoc. 12(1):69-74.

[22] Cilek J. and Schreiber E., 1994., Failure of the "mosquito plant", Pelargonium x citrosum 'van Leenii', to repel adult *Aedes albopictus* and *Culex quinquefasciatus* in Florida. J Am Mosq Control Assoc. 10(4):473-6.