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Evaluation of Larvicidal and Ovicidal Effects of Mundulea Sericea in the Control of Culex Quinquefasciatus)

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ABSTRACT

Crude extract of M. sericea from Coastal region and Western Kenya were applied in 100 mls cups with thirty 3rd instar larvae in three replicates in the laboratory and compared with alpha cypermethrine and untreated. Samples with higher efficacy were further tested in five semi field and field conditions. There was high significant larval and pupal mortality in semi fields (F (290,314) =2.58, p = 0.0378) and field (F (285, 309) =8.20, p=0.0000). There was also statistical significance in mortality among various instars of larvae and pupa (F (290,314) =2.77, p = 0.0276). However in semi-field, there was no significant mortality on larval instars and pupa (F (290,314) =1.37, p = 0.1561). No eggs hatched at 0.126g concentration of alpha cypermethrine. Coast samples had a higher efficacy of 0.28g/100mls of water compared to 0.5g of the sample from Western Kenya. In both Semi field and field habitats, double LC_{90} was more effective than any other M. sericea concentrations. At 0.3g of crude extract from Coast sample completely hindered hatchability of C. quinquefasciatus eggs both in the laboratory and semi field treatment (t (26) = 3.0987, p=0.0046). Coast and Western samples of M. sericea crude extracts can be used as a biological larvicides and ovicides to control C. quinquefasciatus.

Keywords: *Mundulea service, Culex quinquefasciatus, Alpha cypermethrin, ovicidal, larvicidal.*

INTRODUCTION

The principal vector of lymphatic filariasis (LF), mainly in Africa is *Culex quinquefasciatus.* The Carter Center [1] estimated that 120 million persons (40 million of them with symptoms) in more than 80 countries of Asia, the Pacific, Africa and the Americas are infected and at least one billion people are at risk of this disease. Found mainly in tropical and sub-tropical climates, the parasitic filarial worms which live in the lymphatic system cause extreme swelling of the extremities and genitals. In Kenya, LF has been reported since 1910 [2] and the disease remains endemic at the coastal region of Kenya where about 2.5 million people are at risk of infection [3]. Whereas the disease remains the leading cause of permanent and long term disabilities worldwide, control programs of the disease remain inadequate. Among the strategies in the management of the disease, reinforcement in the control of *C. quinquefasciatus* is very fundamental. Larval treatment is much more effective for managing this notorious insect because of their localization at this stage [4]. Synthetic pesticides in use for the control of most biting Diptera are not environmentally friendly. They are also not readily available besides being too costly particularly to the slum and rural communities where the mosquitoes are most common. *Mundulea sericea* has been reported to contain insecticidal rotenoids which are which are rotenone [5] deguelin and munetone [6].

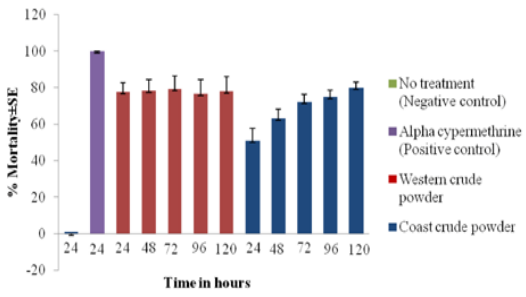


Figure1

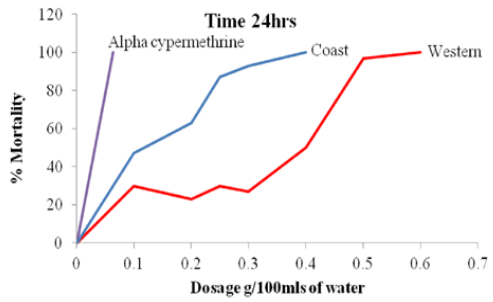


Figure2

Cheaper and effective alternatives have to be sought. Botanical pesticides could therefore safely replace the synthetic forms, particularly in the tropics where higher plant diversity offers even a wider variety of resources. In addition medicinal plant extracts do not induce pesticide resistance in mosquitoes [7]. Previous laboratory studies have shown that crude root-bark, stem-bark and leaf extracts from *M. sericea* are toxic to various stages of *Phlebotomus duboscqi*, *Anopheles gambiae* and *C. quinquefasciatus* [8]. In addition, *M. sericea* is locally available, not an endangered species and is well adapted to tropical climate. Though many plants have been shown to possess larvicidal and growth inhibition activity against mosquitoes, most of these reports are based on laboratory observations only [9]. There was therefore need to test the insecticidal effect of *M. sericea* in the natural habitats of *C. quinquefasciatus*.

MATERIALS AND METHODS

Study Design, Material Collection and Processing

A randomized block design technique was used in this study. The technique enabled the area to be divided into three relatively homogeneous blocks. According to Trochim [10], obtaining experimental data from within each block makes estimates of the treatment effect to be more efficient than if the estimates were done across the entire sample. Mainly mature *M. sericea* leaves were collected from Majaoni Kilifi County (coast) and from Luanda, (Vihiga county), western Kenya. The leaves were temporarily dried in darkness before packaging in sisal gunny bags for transportation to the laboratory kwhere they were spread on the floor covered with flattened carton boxes for further drying. About 6.5kg leaves from Majaoni and about 2.5kg leaves from Western were then separately crushed and ground repeatedly into fine powder by means of a commercial electrical stainless steel blender [11]. Alpha cypermethrine a synthetic larvicide was purchased from a government agro-vet for use as the positive control.

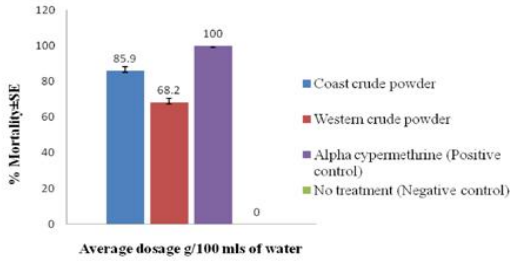


Figure.3

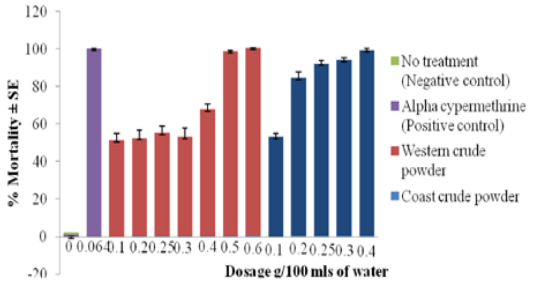


Figure.4

Field Surveillance and Site Identification

Larval/pupa surveillance was carried out in the study area to confirm the presence of *C. quinquefasciatus* larvae whose identification was done by an entomologist. Three natural breeding habitat blocks 1, 2 and 3 of about 40m2 each, were identified. The area provided a suitable environment for the study due to its gentle slope with considerable vegetation cover and minimal human interference. According to O'Malley [12], mosquito larvae are usually found where surface vegetation or debris is present. About 90% of all Kibera waste waters drain and stagnate in this area, popularly known as 'Silanga' and which includes the adjacent Nairobi dam. Five experimental (replicate) sites comprising mostly the abandoned cess pits were identified in every block and marked A, B, C, D and E according to WHO [13] guidelines. Each site was numbered and named by systematic randomization with reference to the block occupied. The volume of water in each site was estimated from measurements taken by means of a meter rule and their Global Positioning System (GPS) and altitude were established. Too open sites and those that appeared relatively too deep were avoided following O'Malley [12]

(1989) recommendation that, usually larvae are not found in open and or deep water.

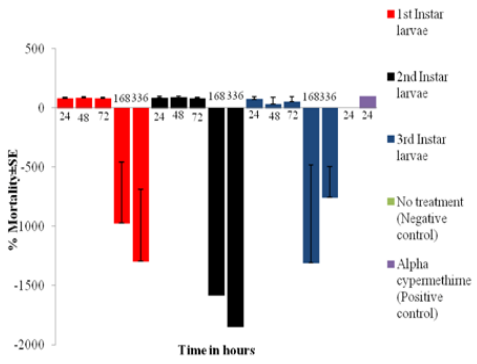


Figure5

Sampling Techniques

The mosquito larvae and pupae present were sampled by standard dipping procedure as described by Service [14]. According to Leisnham *et al.* [15] dipping is the most common method used to sample immature mosquitoes. Approach to each site was done to avoid disturbing the larvae/pupae. A delay of 3 minutes following arrival at each sampling site and prior to dipping was observed to allow mosquito larvae to return to the water surface [16]. Sampling was carried out randomly within the site targeting areas around floating debris, aquatic and emergent vegetation, logs in the water and grasses around the margins [12]. Ten dips were taken from each replicate site at subsequent 3 minutes interval using a standard 50 mls dipper every dip sample was reserved in mini-trays and returned to the site only after the count to minimize density interference. Approach on every occasion was done facing the sun to prevent shadows which could easily disturb the larvae and cause them to dive [12]. Counting of the immature mosquitoes was carefully done by means of a dropper 13]. Samples were obtained from waste water habitats ranging from partially clear to very dark appearance posing a great counting challenge. The mini-trays were used where necessary to transfer the samples in bits hence facilitating the counting process. Part of the samples was carefully carried in whirl packs to the laboratory for raising a laboratory colony. The process was done for 3 consecutive days and repeated after 1 week for 3 weeks. The final sampling was done a day just before treatment and this enabled the larval and pupal densities/dip to be determined for both experimental and control sites.

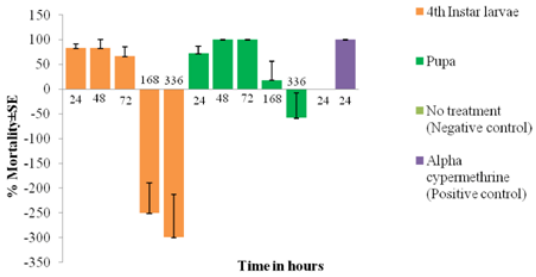


Figure6

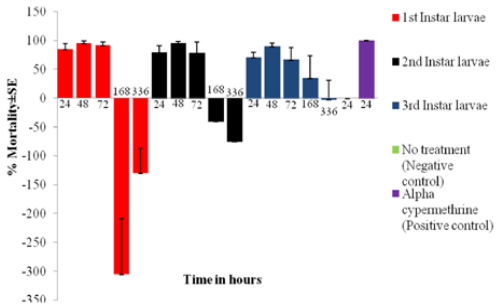


Figure7

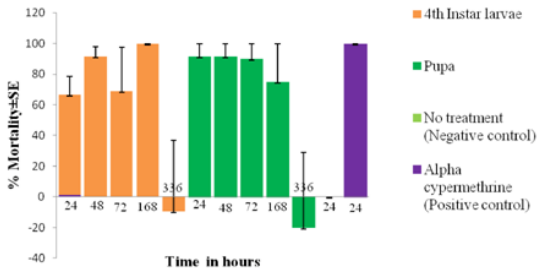
Rearing of the Mosquito Colony

All *C. quinquefasciatus* larval instars collected (1st, 2nd, 3rd and 4th) from the field were transferred into labeled rearing pans with clean dechlorinated water. The largest tray available 40 cm x 32 cm x 7 cm contained over 500 larvae. The rest of the larvae were reared in 33 cm x 22 cm x 8 cm tray. Five trays were maintained throughout the period and the larvae were fed with about 0.01g sera mikropan juvenile fish food. Larval identification by its siphon, colour and inclination angle was done to establish the species obtained after transferring the immature stock to the rearing trays.

Field Treatment

Surveillance in the field realized more larvae in the cess pits with dark waste waters, rich in decomposed vegetation. Multiples of LC_{90} from the laboratory bioassay were also used in the field as in the semi-field with similar replicate sites various dosage quantities as in the semi field treatment, the sites were sampled at 24 hours interval after every treatment for 72 hours to check for the larval viability, the larval stage and to determine the post-treatment density of the larvae and pupa. Similarly, 10 scoops were taken and the mean number of larvae and pupae collected per dip for each treatment and control sites was calculated and recorded. Larval mortality was determined by considering the difference between density before treatment and the density after treatment. The efficacy of the

larvicide was determined from the post-treatment counts of live larvae and pupae in the treated and control sites (and the floating dead).



Ovicidal Effect of M. *Sericea* Test

Various concentrations of the Coast sample *M. sericea* crude extract were prepared and placed inside the rearing cage with adult *Culex* mosquitoes in the laboratory. The set up was monitored to check for any possible oviposition after a blood meal with hamster. Hatchability was monitored after 24 hours for five days. Untreated dechlorinated water was also placed within the rearing cage for oviposition. A bioassay was also prepared to determine the hatchability of the eggs in the semi field. Five rafts were collected by means of a small wooden rod and placed in a 100 mls cup, replicated three times for every *M. sericea* crude extract and bestox cypermethrine (synthetic) concentrations used In each cup about 0.01g of sera mikropan food was applied to feed larvae in case of hatching. A control replicate set up was also prepared with no treatment. After 120 hours, the eggs which never hatched in both the laboratory and semi field bioassay, were transferred to clean fresh water and observed for 48 hours.

DATA ANALYSIS

The laboratory results were analyzed using probit analysis [17], in SPSS programme to determine the LC_{90} for both Coast and Western samples. Student t-test in STATA software was employed to compare the mortality means of the two *M. sericea* samples. One-way ANOVA was used to compare the mean mortality variation with time for both Coast and Western crude extract samples. Mortality per crude extract dosage applied from the two samples was similarly compared. Factorial ANOVA was used to compare mortality means as a result of semi-field and field treatments. Comparison of the means was done following excel and STATA software procedures. Percentage hatchability of *C. quinquefasciatus* rafts between control set up and the treated ones was compared using student t-test.

RESULTS

Efficacy of M. service crude extract on C. quenquefasciatus in the laboratory

The number of dead larvae for every dosage of crude extract was determined for all the three replicates. The effects of the two samples (Coast and Western) at various concentrations were compared 24 hours after application up to 120 hours**.** The average number of dead larvae for the three replicates in every concentration obtained was recorded as percentage mortality (Table 1). Crude extract sample from Coast showed LC_{90} at 0.3g dosage with 93 % mortality in 24 hours while within the same duration of time at equal dosage, the sample from Western region had only 27% mortality (Figure 1). For crude extract from Coast 0.4g was required to kill 100% of the larvae within 24 hours while up to 0.6g was required to achieve the same effect for the crude extract from Western Kenya (Table 1). The percentage mortality was clearly dose and time dependent and overall the sample from Coast performed better than the sample from the Western region. Mean mortality of larvae due to *M. sericea* crude extract application from Coast was significantly higher than that from Western ($t(178) = 5.36$, $p=0.000$, $a=0.05$). Average mortality of various crude powder concentrations by both samples from Coast and Western indicated a gradual increase in mortality with time. Mortality variations with time for both Western and Coast samples including variations within controls was statistically significant ($F(177, 181) = 6.11$, $p=0.0001$, $a=0.05$). Alpha cypermethrine showed 100% mortality within 24 hours while on average no larvae died in a set-up with no treatment (Figure 2).

Mortality comparison and lethal concentrations

Both samples showed an increase in mortality with increase in the concentration of crude extract applied with alpha cypermethrine showing higher efficacy. The difference in mortality due to various dosages of *M. sericea* crude extract was statistically significant ($F(173,181) = 14.84$, $P=0.0000$, $a=0.05$,) for Coast and Western samples in relation to the controls. Alpha cypermethrine showed 100% mortality of *C. quinquefasciatus* compared to 85.9% of *M. sericea* crude extract from Coast and 68.2% from Western for five days post-treatment investigation. There was no larval mortality in

the set up with no treatment (negative control) (Figure 3).

Efficacy of M. Sericea Crude Extracts in the Semi Field Conditions

After about 3-4 days of organic matter decomposition, oviposition occurred in virtually all sites. There was more oviposition in the sheltered sites and those with more decomposed organic matter than those exposed and with less decomposed vegetation. Applied crude extract temporarily remained floating before settling at the bottom of the artificial sites with water colour changing to faint green. There was reduction in the number of larvae and pupa in most of the treated sites compared to the sites with no treatment. Lethal concentration (LC) Probit analysis indicated that on average 0.5464g of *M. sericea* crude extract from Western killed 90% of *Culex* larvae within 24 hours duration (Table 2) while it required 0.2898g of the sample from Coast to kill the same percentage of the larvae (Figure 4) (Table 3). A similar trend of larval mortality with time for both samples was noted with lethal concentrations that were able to kill 50% of the larvae. The number of dead larvae for every dosage of crude extract was determined for all the three replicates. The effects of the two samples (Coast and Western) at various concentrations were compared 24 hours after application up to 120 hours. The average number of dead larvae for the three replicates in every concentration obtained was recorded as percentage mortality. Crude extract sample from Coast showed LC90 at 0.3g dosage with 93 % mortality in 24 hours while within the same duration of time at equal dosage, the sample from Western region had only 27% mortality (Table 1). For crude extract from Coast 0.4g was required to kill 100% of the larvae within 24 hours while up to 0.6g was required to achieve the same effect for the crude extract from Western Kenya. The percentage mortality was clearly dose and time dependent and overall the sample from Coast performed better than the sample from the Western region. Mean mortality of larvae due to *M. sericea* crude extract application from Coast was significantly higher than that from Western ($t(178) = 5.36$, $p=0.000$, $a =0.05$) (Figures 5-8).

Ovicidal Effect of M. Sericea Crude Extract

In the rearing cage there was oviposition in both the treated and the untreated set up. The rafts subjected to 0.3g crude extract remained intact throughout the testing period. However no oviposition occurred in the alpha cypermethrine treated set up. There was 0% hatchability in 0.3g concentration for the 120 hours the rafts remained in the treatment set up. In the control set-up, 100% of the rafts hatched within 48 hours and on average the larvae lived throughout post-treatment time moulting to successive instars. A set-up with 0.1g and 0.2g crude extract, had rafts also hatching within 48 hours with some larvae surviving while others died with time. While there was 100% hatching within 24 hours in 0.064g and 0.128g of alpha cypermethrine treatments, no rafts hatched (0%) in the set up treated with 0.256g of the same synthetic insecticide. No larvae survived in the alpha cypermethrine treatment set up after hatching (Table 4).

DISCUSSION

The samples obtained from the designated areas showed that, although *C. quinquefasciatus* is the major breeding species in the organic waste waters, other species also exist. The study however confirmed that the more the organic wastes the breeding site has, the better the breeding environment for *C. quinquefasciatus*. Such a site has minimal or no breeding for the other species of mosquito, which probably oviposit in fresh clear waters. Under laboratory conditions both *M. sericea* crude extracts from Coast and Western showed toxicity to *C. quinquefasciatus* as demonstrated by larval and pupal mortality at different concentrations.

It has been shown that some medicinal plants containing natural toxins were effective against mosquito larvae [7]. A study by Satoto [18], found that *Annona squamosa* seed was one of the most effective larvicide against both *C. tritaeniorhynchus* and *Aedes aegypti*. Since crude extract formed a homogenous mixture with Sera mikropan put as larvae food, mortality could have resulted from consumption of the toxic mixture. Trudel and Bomblies [19] reported that Neem seed powder effectively targets mosquito larvae in part because it floats on the water surface where the *Anopheles* larvae feed; ingesting the crushed seeds whose active chemicals have growth-inhibiting property. There is therefore possibility that *C. quinquefasciatus* larval mortality resulted from consumption of *M. sericea* crude extract together with sera mikropan food leading to death. In each sample the percentage mortality increased with increase in the crude extract concentrations. In the study by Okumu *et al.* [20] Neem oil was observed to be highly larvicidal at high

concentrations (32 ppm), but declined progressively as the dose decreased. It has also been reported that percent larval mortality due to oil larvicide increased with increasing concentrations such that, a concentration of 400 ppm of the oil was 100% larvicidal whereas 100 ppm was only about 45% of third and fourth instars [21] (Cetin *et al*., 2009) . Similarly Chaudhary *et al*. [22] noted that plants and plant derived samples efficiently killed the larval stages of *Plutella xylostella* (diamondback moth) and the activity increased with increasing concentrations with concentration up to 10,000 µg/ml showing 100% mortality. Crude extract from Coast attained 100% mortality of third instar larvae at 0.4g concentration within 24 hours while 0.2g was the lowest dosage that realized a significant control (63% after 24 hours) of *C. quinquefasciatus*. Senthilnathan [23] observed that higher larvicidal effect of *Eucalyptus tereticornis* oil (leaf extract) were obtained with increased doses on *A. stephensi*. Similarly, in this study higher larvicidal effects were realized with increased crude extract concentrations of *M. sericea*. Difference in secondary metabolites in plants varies with environmental factors such as altitude, rainfall and soil composition among others. Morison and Lawler [24] reported that local geoclimate, seasonal changes, external conditions such as light, temperature, humidity affect composition of secondary metabolites. Gupta *et al*. [25] reported that linalool (a chemical derived from flower and spice plants used to control fleas and cockroaches) in female plant leaves was found to be highest at low altitude while it decreased at high altitude. It is most probable that Coast sample had a higher concentration of larvicidal compound than the Western sample.

Under semi field conditions the study showed that *C. quinquefasciatus* female would majorly oviposit in a highly decomposed organic matter habitat than otherwise. Like in the laboratory bioassay, sites in the semi field subjected to higher crude extract concentrations showed relatively higher mortality. Double LC90 (0.56g/100 mls of water) was the most effective crude extract concentration against *Culex* larvae and pupa. There were relatively similar mortality trends among various instar larvae and pupa with time. This therefore slightly differs with Senthilnathan [23] who observed that first and second instar larvae of *A. stephensi* were most susceptible to treatments with *E. tereticornis* oil (leaf extract). Generally there was increase in mortality with time for the 72 hours the larvae were exposed to the crude extracts of *M. sericea*. However, the extracts showed efficacy reduction after some time resulting into high density of larvae 7 days after treatment. A negative reduction was an indication that there was no mortality caused by the crude extracts and instead larvae increased in number. Choochote *et al*. [26] noted that chemicals from medicinal plants have high degree of biodegradation. This suggests that the active ingredient in *M. sericea* crude extract had a low residual effect. Apart from rotenone, which is found in seeds, stem and leaves that have insecticidal activities [27], the tephrosin, deguelin, munduserone including other bioactive chemicals[28] are found in most parts of the plant [29, 30, 31]. It is possible that the insecticidal effects displayed by the plant extract/powder could be caused by all these compounds.

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REFERENCES

[1] The Carter Centre. Summary of the Third Meeting of the International Task Force for Disease Eradication. www.cartercenter.org/health/lf/. 2009.

[2] Wamae, C.N., Mwandawiro, C., Wambayi, E., Njenga, S., Kiliku, F. (2001). Lymphatic filariasis in Kenya since 1910, and the prospcets for its elimination: a review. East African Medical Journal, 78: 595-603.

[3] Central Bureau of Statistics Population and housing census. Volume II. Population distribution by administrative areas and urban centers. Ministry of Finance and Planning, Republic of Kenya. Government of Kenya, 2001.

[4] Rana, I.S., and Rana, A.S. (2012). Efficacy of essential oils of aromatic plants as larvicide for the management of filarial vector Culex quinquefasciatus (Say) (Diptera: Culicidae) with special reference to Foeniculum vulgare. Asian Pacific Journal of Tropical Disease, 184-189.

[5] Nagai, N. (2005). Researches on the poisonous principals of rhoten. Journal of the Tokyo Chemical Society, 23: 744-777.

[6] Lee, S.K., Luyengi, L., Gerhäuser, C., Mar, W., Lee, K., Mehta, R.G., Kinghorn, A.D and Pezzuto, J.M. (1999). Inhibitory effect of munetone, an

isoflavonoid, on 12-O-tetradecanoylphorbol 13-acetate-induced ornithine decarboxylase activity. Cancer Letters, 136 (1):59-65.

[7] Monzon, R.B., Alvior, J.P., Luczon, L.L., Morales, A.S and Mutuc, F.E. (1994). Larvicidal potential of five Philippine plants against Aedes aegypti (Linnaeus) and Culex quinquefasciatus (Say). Southeast Asian Journal of Tropical Medicine and Public Health, 25: 755-759.

[8] Kiania, N.M., Mueke, J., Rukunga, G., and Anjili, C. (2014). Larvicidal efficacy of Mundulea sericea (Leguminosae) plant extract against Anopheles gambiae (Giles) and Culex quinqefasciatus (Say) (Diptera: Culicidae). Open Science Repository Pharmaceutics, Online (open-access), 2014; e23050491. doi:10.7392/openaccess.2305049.

[9] Mittal, P.K and Subbarao, S.K. (2003). Prospects of using herbal products in the control of mosquito vectors. Indian Council Medical Research Bulletin, 33: 1.

[10] Trochim, W.M. (2006). The Research Methods Knowledge Base, @nd Edition. http://www.socialresearchmethods.net/kb/.

[11] Rahuman, A.A., Bagavan, A., Kamaraj, C., Saravanan, E., Zahir, A.A and Elango, G. (2009). Efficacy of larvicidal botanical extracts against Culex quinquefasciatus Say (Diptera: Culicidae). Parasitology Research, 104(6):1365-1372.

[12] Pangnakorn, U., Kanlaya, S., Kuntha, C. (2012).Effect of Wood Vinegar for Controlling on Housefly (Musca domestica L.) International Journal of Medical and Biological Sciences, 6(5).

[13] WHO. (2005). Guidelines for laboratory and field testing of mosquito larvicides. World Malaria Report. World Health Organization, Geneva Switzerland, pgs 20-24.

[14] Service, M.W. (1993). Mosquito Ecology. Field Sampling Methods. 2nd Edition, Elsevier Applied Science, London, pg 988.

[15] Leisnham, P.T., Slaney, D.P., Lester, P.J and Weinstein, P. (2005). Evaluation of two dipping methods for sampling immature Culex and Ochlerotatus mosquitoes (Diptera: Culicidae) from artificial containers. New Zealand journal of Marine and Fresh Water Research, 39: 1233-1241.

[16] Bogh, C., Clarke, S.E., Jawara, M., Thomas, C.J and Lindsay, S.W. (2003). Localized breeding of Anopheles gambiae complex (Diptera: Culicidae) along the river Gambia, West Africa. Bulletin of Entomological Research, 93: 279-287.

[17] Finney, D.J. (1971). Probit analysis. Cambridge University Press, London, 68-72.

[18] Satoto, T.B.T. (1993). A laboratory study of the biological effects of some medicinal plants on Culex tritaeniorhynchus. MSC thesis in Tropical Medicine, Faculty of Graduate Studies, Mahidol University, Bangkok, 119.

[19] Trudel, R.E and Bomblies, A. (2011). Larvicidal effects of Chinaberry (Melia azederach) powder on Anopheles arabiensis in Ethiopia. Parasites and Vectors, 4:72.

[20] Okumu, F.O., Knols, B.G and Fillinger, U. (2007). Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae. Malaria Journal, 6:63

[21] Cetin, H., Kurt, Y., Isik, K and Yanikoglu, A. (2009). Larvicidal effect of Cedrus libani seed oils on mosquito Culex pipiens. Pharmaceutical Biology, 47(8): 665–668.

[22] Chaudhary, A., Sharma, P., Nadda, G., Tewary, D.K and Singh, B. (2011). Chemical composition and larvicidal activities of the Himalayan cedar, Cedrus deodara essential oil and its fractions against the diamondback moth, Plutella xylostella. Journal of Insect Science, 11:157.

[23] Senthilnathan, S. (2007). The use of Eucalyptus tereticornis Sm. (Myrtaceae) oil (leaf extract) as a natural larvicidal agent against the malaria vector Anopheles stephensi Liston (Diptera: Culicidae). Biosource Technology, 98: 1856-1860.

[24] Morison, J.I and Lawlor, D.W. (1999). Interactions between increasing CO2 concentration and temperature on plant growth. Plant Cell Environment, 22:659-682.

[25] Gupta, S., Bhaskar, G and Andola, H.C. (2011). Altitudinal variation in essential oil content in leaves of Zanthoxylum alatum Roxb. A. high value aromatic tree from Uttrakhand. Research Journal of Medicinal Plants, 348-351.

[26] Choochote, W., Kanjanapothi, D., Panthong, A., Taesotikul, T., Jitpakdi, A., Chaithong, U and Pitasawat, B. (1999). Larvicidal, adulticidal and repellent effects of Kaempferia galanga. South-east Asian Journal of Tropical Medicine and Public Health, 30: 470-476.

[27] Ray, D.E. (1991). Pesticides derived from plants and other organisms. In Hayes Jr., Wayland, and Edward R. Laws, Jr. (eds.). Handbook of Pesticide Toxicology. Academic Press, Inc. New York, 1991, NY 2-3.

[28] Pezutto, J.M. (1995). Natural produtcs for cancer chemopreventive agents. Recent Advance in Phytochemistry, Vol 29, In Phytochemistry of medicinal plants (eds.) Arnason, J.T., Mata, R. and Romeo, J.T. 1995, pp 29. Springer Science + Business Media, LLC.

[29] Worsley, R.R., Le, G. (1993). The insecticidal properties of some East African Plants. 1. Annals of Applied Biology, 1934; DOI: 101111/j1744-7348.1934.tb07467.x.

[30] Nakatani, N. and Matsui, M. (1968). Synthetic Studies on tenoids. Agricultural and Biological Chemistry, 32:6, 769-772

[31] Khyade, M.S. (2017). Chemical Profile and Antioxidant Properties of Mundulea sericea. Pharmacognosy Journal, 9(20): 213-22.