PSM Microbiology ISSN(Online): 2518-3834

Research Article 2019 | Volume 4 | Issue 1 | 7-16

Article Info

Open Access

Citation: Ishtiaq, G., Sheikh, R., Siddiqui, M.A., Farid, I., 2019. Larvicidal Efficacy of Aqueous Extracts of *Citrus grandis* (Grapefruit) against *Culex* Larvae. PSM Microbiol., 4(1): 7-16.

Received: November 9, 2018

Accepted: February 27, 2019

Published: March 31, 2019

***Corresponding Author:**
Ghazal Ishtiaq

Email:
ghazalsiddiqui32@gmail.com

Copyright: ©2019 PSM. This work is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial 4.0 International License.

Scan QR code to see this publication on your mobile device.

Larvicidal Efficacy of Aqueous Extracts of *Citrus grandis* (Grapefruit) against *Culex* Larvae

Ghazal Ishtiaq[1]*, Rabia Sheikh[2], Arif Mehmood Siddiqui[3], Imtiaz Farid[4]

[1]Department of Zoology, Lahore College for Women University, Lahore, Pakistan.
[2]Department of Zoology, Govt. Post Graduate Islamia College for Women, Cooper road, Lahore, Pakistan.
[3]Social Welfare Officer, Government of the Punjab, Lahore, Pakistan.
[4]Department of Health Sciences, Government of the Punjab, Lahore, Pakistan.

Abstract:
Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to humans. Development of resistance to insecticides and wide spread environmental pollution necessitate a continued search for alternative pest control as well as vector control strategies. The present study was undertaken to evaluate the larvicidal activity of various extracts of boiled and unboiled leaves of *Citrus grandis* against *Culex quinquefasciatus*. Various concentrations (20 mg/200ml, 40 mg/200ml and 60 mg/200ml) of the *Citrus grandis* extracts were tested against fourth instar larvae of *Culex quinquefasciatus*. The larval mortality was observed after each 24 h of exposure. All the extracts showed significant larvicidal activity against *Culex* mosquito at 0.05 level of significance ($p<0.05$) however, the highest larval mortality was found in 30% boiled extract of leaves as it took least time (5 days) to give 97% mortality. The results indicated that the aqueous leaf extract of *Citrus grandis* is effective against *Culex quinquefasciatus* mosquito larvae and need to be explored for its possible use in the control of mosquito population.

Keywords: Larvicidal activity, resistance to insecticides, control of mosquito population.

INTRODUCTION

Vector borne diseases are a major source of illness and death worldwide. Mosquitoes are one of the most important vectors that alone transmit diseases to more than 700 million people per annum (Tripathi, 2003). They transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis; contribute significantly to poverty and social debility in tropical countries (Jang *et al.*, 2002). The mosquitoes have attained their place in the world by the evolution of highly specialized anatomical characters (Service, 1983).

Mosquitoes, in their role as vectors, are critical components in the transmission cycle of many disease causing pathogens that affect hundreds of millions of people world-wide (Reiter, 2001). Mosquitoes are regarded as public enemies because of their biting annoyance, noise nuisance, sleeplessness, allergic reactions and disease transmission due to their bites. Species of *Culex*, *Aedes*, and *Anopheles* mosquitoes are responsible for the transmission of many human pathogens that cause various diseases. The capacity of different mosquito species to transmit the pathogens varies greatly, and much of this variation can be attributed to the mosquito immune system's ability to recognize and eliminate the pathogen (Barr, 1957).

Culex quinquefasciatus (the southern house mosquito) is an important mosquito vector of viruses such as West Nile virus and St. Louis encephalitis virus that cause lymphatic filariasis. The ability of *C. quinquefasciatus* to take blood meals from birds, livestock, and humans contributes to its ability to vector pathogens between species. This nighttime-active, opportunistic blood feeder is a vector of many of pathogens, several of which affect humans (Barr, 1957). Although the *Culex* mosquito is not a primary vector for prevalent mosquito-borne diseases such as malaria, dengue and yellow fever, it can transmit a number of other illnesses that can present serious health problems to human beings. It is known to contribute to the spreading of the West Nile Virus, filariasis, and encephalitis (WHO, 2010).

Culex larvae feed on biotic material in the water and require between five to eight days completing their development at 30°C. The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and molt to the pupal stage. Following 36 hours at 27°C the adults emerge from the pupal stage (Gerberg *et al.*, 1994).

Control of larvae can be accomplished through use of contact poisons, growth regulators, surface films, stomach poisons (including bacterial agents), and biological agents such as fungi, nematodes, copepods, and fish (Walker *et al.,* 2002). Insecticides can be used to control larvae and adults. Insecticide application although highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity (Liu *et al.,* 1990). Furthermore, they are responsible for substantial hazards to a variety of non-target organisms and environment in the form of biomagnifications (Sarwar *et al.,* 2009; Yildirim *et al.*, 2012). Therefore, researchers have diverted their attention since few decades ago towards the plant world, which are eco-friendly and cost effective.

To alleviate these problems, major emphasis has been on the use of natural plant based products as larvicides which can provide an alternate to synthetic insecticides (Zhu *et al.*, 2006). Larvicides are applied to bodies of water near where the larvae are concentrated. This method reduces the greatest number of immature mosquitoes with the least amount of pesticide.

Larvicides of plant origin are currently receiving considerable attention because of their relatively harmless biodegradable properties. Since 1920s more than 2000 plants have been tested for insecticidal properties.

Recently, lot of research work is being carried out to search for alternative eco-friendly effective larvicides from botanical origin (Singh *et al*., 2010). Many plants have been found to contain chemicals which are helpful for the control of insects (Robert, 2001; Ullah *et al.,* 2018; Iqbal and Ashraf, 2018; Shahzad *et al.,* 2018) and are useful for field applications in mosquito control programme (Kalyanasundaram and Das, 1985).

The grapefruit is the fruit of a citrus tree (*Citrus Decumana* or *Citrus Grandis*) called *Pampelmusa*. Alcoholic extract of its leaves possess moderate antibacterial activities against *Bacillus subtilis*, *B. cereus*, *B. magaterium*, *Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi* and *Vibrio cholerae* (Khan, 2009).

The aim of this work was to find out an easiest, cheap and nontoxic way to control mosquito population at its larval stage. For this purpose, the larvicidal efficacy of *Citrus grandis* was assessed against the fourth instar larvae of *Culex quinquefasciatus* by making different concentrations of aqueous extract of leaves of Citrus grandis (grapefruit).

MATERIALS AND METHODS

Collection and identification of mosquito larvae

Mosquito larvae were collected from different localities of Lahore in the 1st week of April. They were brought to the Zoology research laboratory, Govt. Post Graduate Islamia College for Women Copper Road Lahore and the larvae were transferred into a beaker containing 500ml of water (Figure 1).

The larvae were identified as *Culex quinquefasciatus* with the help of the following morphological key features.

- Make an angle with water surface.
- Short and slender breathing tube.
- Gills are blunt.
- Five and more branches of hair on head.

They were brought to the Entomology Department of IPH where they were confirmed as 4th instar larvae of *Culex quinquefasciatus* and were placed in water filled beaker covered with fine net (Sattar *et al.,* 2016).

Fig. 1. *Culex* larvae collection.

Collection of plant material

The leaves of grapefruit were collected from Shah-kott town, near Faisalabad. They were identified and authenticated by the Botany Department of Govt. Post Graduate Islamia College for Women Cooper Road Lahore (Figure 2).

Fig. 2. Leaves of grapefruit (*C.grandis*).

Two parameters were selected for study:

1. Unboiled leaves of grapefruit
2. Boiled leaves of grapefruit

Fresh leaves of grapefruit were washed with tap water. 1200g of leaves were divided into two equal amounts, for two parameters of leaves, one for boiled and other one for unboiled.

Preparation of unboiled leaves extract

For making 10% concentration 20g of leaves were taken. Leaves were grinded in 200ml of distilled water. This extract was then filtered through muslin cloth and the filtrate thus obtained was stored in plastic bottle for experimental use as a stock solution. Similar procedure was adopted for making 20% and 30% by taking 20g and 40g leaves in 200ml of water respectively.

Preparation of *Boiled* leaves extract

For making 10% concentration, 20g of leaves were taken. Washed leaves were boiled in 200ml of distilled water for 30 minutes at moderate flame. This extract was cooled then filtered by muslin cloth. Similar procedure was adopted for making 20% and 30% by 20g and 40g leaves in 200ml of water respectively.

Three stock solutions were made for boiled leaves by this method (10%, 20% and 30%) in 200ml of water respectively. The filtrate thus obtained was stored in plastic bottle for experimental use as it will be used as stock solution.

Bioassay

Two sets of plastic glasses of 300ml capacity were taken, each set consisted of 6 glasses. These glasses were fitted into the thermo pore sheet by making holes into the sheet. Set one was labeled for boiled leaves extract and second for unboiled leaves extract. In each set two replicates were made for each extract. Replicates of each concentration were named as sample A, sample B and sample C. Glasses of one set had boiled leaves extract of different concentrations (10%, 20% and 30%) while the other set of glass have unboiled leaves extract of different concentration (10%, 20% and 30%). To determine the larvicidal bioassay ten 4th instar larvae of *Culex quinquefasciatus* were transferred in each glass with the help of dropper (Figure 3).

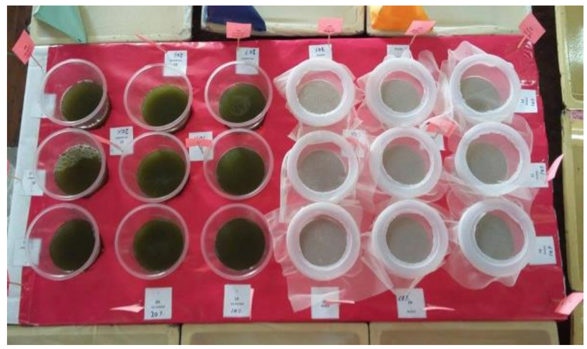

Fig. 3. Experimental setup, boiled and unboiled leaves extract of *C. grandis*.

Two dishes (4.5cm x 25cm x 18cm) were used as control and they just contained 1000 ml distilled water and ten larvae. One control was run for each parameter. A pinch of food which consisted of dog biscuit and yeast powder in 3:1 ratio was sprinkled on each glass and dish. All dishes and glasses were covered with fine net to avoid egg laying of any other insects (Figure 4). The glasses and dishes were held at a temperature of 30-33°C.

The mortality and survival rate of these larvae was checked after 24 hours. Motionless larvae were considered as dead ones for mortality calculation. Dead larvae can be recognized easily as they do not show any movement on disturbance and they were removed from dishes to avoid any confusion and degradation. The percentage morality was computed/calculated by the Abbot formula.

Percentage mortality =

Number of dead larvae /

Number of introduced larvae x 10

Fig. 4. Larvae in disposable glasses covered with fine net.

Statistical Analysis

The statistical tools that were used in this study are Abbot's formula for determining the % mortality, Analysis of variance (ANOVA) to determine the significant difference on the mortality of *Culex quinquefasciatus* between the control and experimental group. The SPSS version 18 was used to calculate the F and P values of the ANOVA. The mean value equal or less than 0.05 was considered significant.

RESULTS

The crude aqueous extract of *Citrus grandis* leaves (10%, 20% and 30% concentrations) showed the larvicidal activity when tested against *Culex quinquefasciatus* mosquito 4th instar larvae.

Larvicidal effect of boiled leaves extract

The 10% concentration of boiled leaves extract showed 80% mortality of larvae after 5 days of treatment, while 87% mortality of larvae after 6 days. The survival rate of larvae in 10% concentration was 13% after 6 days. Boiled leaves crude aqueous extract of 20% concentration showed 73% mortality of larvae on 6th day of experiment while overall mortality was 83% after 7 days. And the survival rate of larvae was 17% after 7 days. In 30% concentration 93% mortality was recorded on 5th day and 97% mortality was observed after 6 days of treatment. The survival rate of larvae was 30% after 6 days. In the control of boiled leaves extracts, all the larvae transformed into pupae in 2-3 days on average and then into adult at about 7th day, so the survival percentage was 100% and the mortality percentage were zero (Table 1).

A comparison of all the three concentrations on 5th day showed that 30% concentration gave 93% mortality, while 10% and 20% concentrations showed 80% and 73% mortality. The results shown in (Figure 5) demonstrated that 30% concentration was significantly more effective against *Culex* larvae as it showed highest mortality than lower concentrations (10% and 20%). Although 20% concentration has less effect than 10% and 30% but it can prolong their larval and pupal period. While in control group the larvae transformed into the pupae within 2-3 days (Table 1).

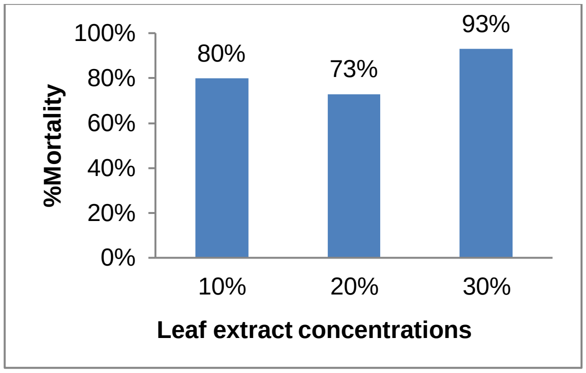

Fig. 5. Comparison of the % mortality of 4th instar larvae of *Culex quinquefasciatus* in different concentration of boiled leaves on 5th day.

Larvicidal effect of unboiled leaves extract

The 10% concentration of unboiled leaves extract showed 80% mortality of larvae after 5 days of treatment, while 87% mortality of larvae after 6 days. The survival rate of larvae in 10% concentration was 13% after 6 days. Unboiled leaves crude aqueous extract of 20% concentration showed 70% mortality of larvae on 6th day of experiment while 77% mortality after 7 days. The survival rate of larvae was 23% on 7th day. In 30% concentration 90% mortality was recorded after 5 days and 93% mortality was observed after 6 days of treatment. The overall survival rate was 7% in this concentration. While in control group, survival percentage was 100% and no mortality was observed. All the larvae transformed into pupae within 2-3 days and then into adults after 2-3 days (Table 1).

A comparison of all the three concentrations on 5th day showed that 30% concentration gave 90% mortality, while 10% and 20% concentration s showed 87% and 66% mortality. The results shown in (Figure 6) demonstrated that 30% concentration was significantly more effective against *Culex* larvae as it showed high mortality than lower concentrations (10% and 20%). Like boiled extract, although 20% concentration has less effect than 10% and 30% but it can prolong their larval and pupal period. And in control group the larvae transformed into the pupae within 2-3 days (Table 1).

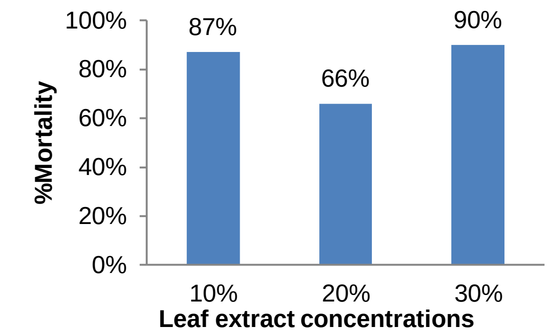

Fig. 6. Comparison of the % mortality of 4th instar larvae of *Culex quinquefasciatus* in various concentrations of unboiled leaves on 5th day.

Table 1. Comparison of mortality and survival of 4th instar larvae of *Culex quinquefasciatus* at different concentrations of leaf extracts.

Parameter	Concentration of leaf extracts	Time													
		1st		2nd		3rd		4th		5th		6th		7th	
		Mortality (%)	Survival (%)	Mortality (%)	Survival (%)	Mortality (%)	Survival (%)	Mortality (%)	Survival (%)	Mortality (%)	Survival (%)	Mortality (%)	Survival (%)	Mortality (%)	Survival (%)
Boiled water	10%	10	90	43	57	63	37	73	27	80	20	87	13	-	-
	20%	23	77	56	44	66	34	73	27	73	27	76	24	83	17
	30%	37	64	66	34	66	34	86	14	93	7	97	3		
	Control	0	100	0	100	0	100	50	50	-	0	-	0	-	0
Un-boiled water	10%	33	67	47	53	63	37	76	24	87	13	-	13	-	-
	20%	23	77	36	64	50	50	60	40	66	34	70	30	77	23
	30%	20	80	30	70	43	57	63	37	90	10	93	10	-	-
	Control	0	100	0	100	0	100	40	60	-	0	-	0	-	0

Comparison of the efficacy of boiled and unboiled leaves extracts

The results obtained were analyzed using One-way ANOVA, comparing experimental and control group, with a significance level established d at P<0.005. The results shown in table 2 indicate a highly significant difference between treatments and control. When a multiple comparison was made between 10%, 20% and 30% concentrations of boiled and unboiled leaves extracts at 5th day, it showed that all the extracts have significant potency against fourth instar larvae of *Culex quinquefasciatus*. However 30% concentration of boiled leaves extract was found to be most effective as it gave 97% mortality in 6 days.

Table 2. ANOVA results on the mortality of Culex quinquefasciatus 4th instar larvae treated on the various concentrations of the leaves extracts and the control groups.

Variable compared	F-computed	P-value	Implication
Control VS 10% boiled leaves	21.130	.001*	
Control VS 20% boiled leaves	45.613	.000*	At 0.05 level of significance.
Control VS 30% boiled leaves	55.858	.000*	
10% VS 20% boiled leaves	1.018	.359	
10% VS 30% boiled leaves	0.963	.350	
20% VS 30% boiled leaves	0.668	.431	
Control VS 10% unboiled leaves	44.873	.000*	*Implies that there is a significant difference
Control VS 20% unboiled leaves	36.859	.000*	
Control VS 30% unboiled leaves	16.303	.002*	
10% VS 20% unboiled leaves	1.750	.243	
10% VS 30% unboiled leaves	0.340	.573	
20% VS 30% unboiled leaves	0,019	.893	
10% boiled VS 10% unboiled leaves	0.175	.685	
20% boiled VS 20% unboiled leaves	0.847	.376	
30% boiled VS 30% unboiled leaves	1.255	.289	

DISCUSSION

In recent years much effort has been focused on plant extracts or phytochemicals as potential sources of mosquito control agents and as a viable component of Integrated Pest Management (Zhu *et al*., 2008). Plant products have revolutionized the fields of vector control as they possess different bioactive components and can be used as general toxicants against various larval stages of the mosquito (Sukumar *et al*., 1991; Mohan *et al*., 2005).

The present study evaluates the effect of *Citrus grandis* leaves (10%, 20% and 30% concentrations) against larvae of *Culex quinquefasciatus*. The larval mortality was observed after each 24 h of exposure. All the extracts showed significant larvicidal activity against *Culex* mosquito at 0.05 level of significance (p<0.05) however, the highest larval mortality was found in 30% boiled extract of leaves as it took least time (5 days) to give 97% mortality. These results indicate the presence of some toxic components in the leaves of *Citrus grandis* which contributed in the mortality of the fourth instar larvae of *Culex quinquefasciatus*. While in the control group all the larvae metamorphosed into pupae and then into adults so the mortality percentage of this group remained zero.

Our results are in accordance with the work of Sattar et al. (2012) who observed increasing larval mortality with increase in extract concentration and increase in exposure time in all treatments. Similar results were reported by Ubulom et al. (2012) who demonstrated larvicidal effect of *Blighia sapida* leaf extracts against mosquito larvae.

The plant *Citrus grandis* used in our study gave good results even on larval stage as it prolonged its larval duration and then caused their death. The phytochemicals present in the leaf extracts of *Citrus grandis* contain alkaloids, steroids, flavonoids and tannins. The phytochemicals of the plants serve as huge storage of compounds that have biological action (Howard *et al*., 2007). Plant barks are good source of valuable therapeutic agents as they have active components (Shahzad *et al.,* 2017). Alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties (Azmathullah *et al*., 2011). The high mortality rate might be due to the high amount of the components (alkaloids, saponins, and tannins) in the leaf of the *Citrus grandis*. Citrus plants include limonoids that work both as toxicant and feeding deterrent. The results of a previous study indicated that the extracts from rough lemon and lemon were more effective as larvicides (Akram *et al*., 2010). Various studies have demonstrated ethnopharmacological uses of plants (Iqbal *et al.,* 2015; Kalim *et al.,* 2016; Hussain *et al.,* 2016; Ali *et al.,* 2017; Iqbal and Ashraf, 2019; Shuaib *et al.,* 2019).

The control in current study produced no mortality at the various time intervals in the test samples. The larvicidal activity could have come from no other source but the presence of the phytochemicals in the extracts. This suggested that these compounds should be considered as alternative insecticide candidates for mosquito control in breeding sites, especially in cases where susceptibility is decreasing.

CONCLUSION

It is concluded that leaf of *Citrus grandis* could be a potential source of active larvicidal agents. The botanical derivatives used in mosquito control could diminish the cost and environmental pollution instead of synthetic insecticides. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

ACKNOWLEDGEMENT

The author thanks the Department of Zoology, Govt. Post Graduate Islamia College for Women, Cooper road, Lahore, Pakistan, for access to equipment and materials in this investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akram, W., Khan, H.A.A., Hafeez, F., Bilal, H., Kim, Y.K., Lee, J.J., 2010. Potential of Citrus Seed Extracts against Dengue Fever Mosquito, *Aedes alpobictus* (CULICIDAE: DIPTERA). Pak. J. Bot. 42(4): 3343-3348.

Ali, K., Shuaib, M., Ilyas, M., Hussain, F., Hussain, F., 2017. Medicinal Uses of Chemical Extracts from *Withania somnifera* and Its Antimicrobial Activity: A Mini-Review. PSM. Microbiol., 2(1): 20-23.

Azmathullah, N., Sheriff, A.M., Mohideen, S.A.K., 2011. Phytochemical screening of *Calotropis procera* flower extracts and their bio-control potential on *Culex* sp. mosquito larvae and pupae. Int. J. Pharm. Biol. Arch., 2(6): 1718-1721.

Barr, A.R., 1957. The distribution of *Culex quinquefasciatus* and *Culex pipiens* in North America. Am. J. Tropical Med. Hyg., 6(1): 153-165.

Gerberg, E.J., Barnard, D.R., Ward, R.A., 1994. Manual for Mosquito Rearing and Experimental Techniques. American

Mosquito Control Association Bulletin NO. 5, 61-62.

Howard, A.F., Zhou, G., Omlin, F.X., 2007. Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. BMC Public Health., 7: 199.

Hussain, F., Kalim, M., Ali, H., Ali, T., Khan, M., Xiao, S., Iqbal, M.N., Ashraf, A., 2016. Antibacterial Activities of Methanolic Extracts of *Datura inoxia*. PSM Microbiol., 01(1): 33-35.

Iqbal, M.N, Ashraf, A., 2018. Recombinant Protein Production in Plants: Biofactories for Therapeutics. Int. J. Mol. Microbiol., 1(1): 38-39.

Iqbal, M.N, Ashraf, A., 2019. *Withania somnifera*: Can it be a Therapeutic Alternative for Microbial Diseases in an Era of Progressive Antibiotic Resist- ance? Int. J. Nanotechnol. Allied Sci., 3(1): 16-18.

Iqbal, M.N., Anjum, A.A., Ali, M.A., Hussain, F., Ali, S., Muhammad, A., Irfan, M., Ahmad, A., Irfan, M., Shabbir, A., 2015. Assessment of microbial load of un-pasteurized fruit juices and *in vitro* antibacterial potential of honey against bacterial isolates. Open Microbiol. J., 9: 26-32.

Jang, Y.S., Kim, M.K., Ahn, Y.J., Lee, H.S., 2002. Larvicidal activity of Brazilian plants against *Aedes Aegypti* and *Culex pipens* pallens (Diptera:Culicidae) Agric. Chem. Biotechnol., 45(3): 131-134.

Kalim, M., Hussain, F., Ali, H., Iqbal, M.N., 2016. Antifungal activities of Methanolic Extracts of *Datura inoxia*. PSM Biol. Res., 01(2): 70-73.

Kalyanasundaram, M., Das, P.K., 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Ind. J. Med. Res., 82: 19-23.

Khan, Y.S., 2009. Response of *Culex quinquefasciatus* to deltamethrin in Lahore District, J. Parasitol. Vector Biol., 1(3): 19-24.

Liu, Y., Liu, D.C., Wu, B., Sun, Z.H., 1990. Genetic diversity of pummeloo (*Citrus grandis Osbeck*) and its relatives based on simple sequencerepeat markers. Chinese J. Agric. Biotechnol., 3(2): 119-126.

Mohan, L., Sharma, P., Srivastava, C.N., 2005. Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. J. Environ. Biol., 26(2): 399-401.

Reiter, P., 2001. Vecrot-born diseases and global warming. Environ. Health Perspect., 109 (Suppl 1): 141-61.

Robert, S.H., 2001. Discover a native plant extract that repels and kill termites. J. Econ. Entomol., 9: 1200-1208.

Sattar, M., Iqbal, M.N., Ashraf, A., Ali, S., Shahzad, M.I., Alam, S., Ali, T., Sheikh, R., 2016. Larvicidal Efficacy of *Citrus sinensis* Extracts against *Culex quinquefasciatus*. PSM Microbiol., 01(2): 56-61.

Sarwar, M., Ahmad, N., Toufiq, M., 2009. Host plant resistance relationshiphs in chickpea (*Cicer arietinum* L.) against gram pod borer (Helicoverpa armigeraHubner). Pak. J. Bot., 41(6): 3047-3052.

Service, M.W., 1983. Management of vectors. In: Pest and vectors management in Tropics, (Eds.): A. Youdeowei and M.W. Service. 5: 265-280.

Shahzad, M.I., Ashraf, H., Iqbal, M.N., Khanum, A., 2017. Medicinal Evaluation of Common Plants against Mouth Microflora. PSM Microbiol., 2(2): 34-40.

Shuaib, M., Ali, S., Ali, K., Hussain, F., Ilyas, M., Arif, M., Hussain, F., 2019. Validation of the Ethnopharmacological Uses of *Withania somnifera*. Int. J. Nanotechnol. Allied Sci., 3(1): 1-6.

Singh, N., Hoette, Y., Miller, R., 2010. Tulsi 'The Mother Medicine of. Nature' 2nd Edition. International Institute of Herbal Medicine, Lucknow. 2, 28-47.

Sukumar, K., Perich, M.J., Boobar, L.R., 1991. Botanical derivatives in mosquito control: A review J. Am. Mosquito Assoc., 7(2): 210-237.

Tripathi, A.K., Prajapati, V., Khanuja, S.P.S., 2003. Herbal synergistic formulation effective against mosquito larvae and use thereof in controlling malaria vector. J. Sci. Technol., 63: 67-74.

Ubulom, P.M. E., Imandeh, G.N., Ettebong, E. O. Udobi, C.E., 2012. Potential larvicidal properties of *Blighia sapida* leaf extracts against larvae of *An. gambiae*, *Cu. quinquefasciatus* and *Ae. aegypti*. Br. J. Pharm. Res., 2(4): 259-268.

Ullah, M., Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Farooq, T.H., Zaffar, M.H., Ullah, R., Khan, S. U., Hussain, W., Ullah, I., Shaheen, S., Ali, M., 2018. Plants as Antidiabetic Agents: Traditional Knowledge to Pharmacological Validation. PSM Biol. Res., 3(3): 111-119.

Walker, K.R., Ricciardone, M.D., Jensen, J., 2002. Developing an international consensus on DDT: a balance of environmental protection and disease control. Int. J. Hyg. Environ. Health., 206: 423-435.

WHO. (World Health Organization), 2010. Lymphatic filariasis.

Yildirim, E., Aslan, A., Emsen, B., Cakir, A., Ercisli, S., 2012. Insecticidal effect of Usnea longissima (Parmeliaceae) extracts against *Sitophilus granarius* (Coleoptera: Curculionidea). Int. J. Agric. Biol., 14: 303-306.

Zhu, J., Zeng, X., Yanma, Liu, T., Qian, K., Han, Y., Xue, S., Tucker, B., Schultz, G., Coats, J., Rowley, W., Zhang, A., 2006. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. J. Am. Mosq. Contr. Assoc., 22(3): 515-522.

Zhu, J., Zeng, X., O'neal, M., Schultz, G., Tucker, B., Coats, J., 2008. Mosquito larvicidal activity of botanical-based mosquito repellents. J. Am. Mosq. Contr. Assoc., 24: 161-168.