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Larvicidal Efficacy of *Citrus sinensis* Extracts against *Culex quinquefasciatus*

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Abstract
This study was planned to evaluate the larvicidal effect of *Citrus sinensis* on the fourth instar larvae of *Culex quinquefasciatus*. Different concentrations of aqueous extracts of peel and leaves of *Citrus sinensis* were prepared. The mortality rates of larvae were observed after each 24 h of exposure. All the graded concentrations (2%, 3%, 4%) of both peel and leaf extracts showed significant ($p<0.05$) larvicidal activity. But 4% concentration of peel extract proved to be most effective as it took least time (5 days) to give 100% mortality. No mortality was observed in control group. The results showed that *Citrus sinensis* as a potential bio control agent against *Culex quinquefasciatus* due to its larvicidal effect and could be used as an ecofriendly approach for the control of the mosquito.
Keywords: Mosquito larvae, *Citrus sinensis*, larvicidal effect, *Culex quinquefasciatus*.

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INTRODUCTION

Mosquitoes are the major cause of several diseases all over the world. Female mosquitoes nourish their eggs by biting and cause disease (Ghosh *et al.*, 2012). *Aedes* is a genus of mosquitoes naturally found in tropical and subtropical zones. The members of *Aedes* genus serve as vectors for various viral infections. The two important species that transfer viruses are *Aedes aegypti* and *Aedes albopictus* due to which virus is transmitted that result in cause of dengue fever, yellow fever along with many other, less notable diseases (Reinert *et al.* 2004) The mosquitoes that carry dengue fever virus breed in containers that hold water, and bite during the day, not mainly at dusk or evening like other types of mosquito. There is no antitoxin for the control of dengue fever, so mosquito prevention measures are essential. The World Health Organization reports (2009) that dengue disease occur in more than 100 countries.

The genus Anopheles is spreading malaria to humans. People can prevent themselves from malaria by taking antimalarial tablets, and taking measures to avoid being bitten. Gambiae is the species of Anopheles infamous for transmitting *Plasmodium falciparum* that is the most threatening form of malaria all over the world. *Culex quinquefasciatus*, generally known as the southern house mosquito that is a medium-sized brown mosquito. This night time-active, opportunistic blood feeder is a way of many of microbes, several of which affect humans. *Culex quinquefasciatus* is a major route of lymphatic filariasis in Pakistan as well as in India (Jahan and Hussain, 2011).

The sweet orange (*Citrus sinensis*) accounts for about 70% of the total citrus production in the world (Okwi and Emenike, 2006; Milind and Dev, 2012). Orange consists of Beta carotene that is a powerful antioxidant guarding the health of our cells. The saponins present in the peel possess larvicidal activity (Milind and Dev, 2012). Alkaloids, saponins, and tannins are known to have medicinal and pesticidal properties (Azmathullah *et al.*, 2011). Saponins contain a larvicidal efficacy against *Aedes aegypti* and *Culex quinquefasciatus* (Bagavan *et al.*, 2008). Many medicinal

plants are approved for their pesticide and repellent potential, as crude material, individual active ingredients (Fallatah and Khater, 2010). The importance of this is to provide alternative source of drugs for the currently used synthetic drugs (Enejoh *et al.,* 2015).

The aim of this study was to find out a cheap, easiest and non-toxic way to control mosquito population. For this larvicidal efficacy of *Citrus sinensis* was assessed against the fourth instar larvae of *Culex quinquefasciatus* by making various concentrations of aqueous extract of peel and leaf of *Citrus*.

MATERIALS AND METHODS

Selection and identification of mosquito larvae

The mosquito larvae were collected from Bagh-e-Jinnah and Humdard Park of Lahore in the 1st week of April. The samples were transferred into a beaker containing 500ml of water. The mosquito larvae were identified as *Culex quinquefasciatus* with the help of the following morphological key features. Make 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 these were confirmed as 4th instar larvae of *C. quinquefasciatus*. These 4th instar larvae were brought to the Vector biology lab for experimental use.

Collection of plant

The peel and leaves of *Citrus sinensis* (Orange) were accumulated from the local market during March 2014. They were identified and authenticated.

Preparation of leaf powder

The leaves of *C. sinensis* were separated and dehydrated under shade at room temperature (28±2°C) for 3 to 5 days. The leaves were ground to a fine powder using electric mixer or blender and stored in glass jars for experimental use.

Preparation of different concentrations of leaf extract

For 2% concentration 20 g of *Citrus sinensis* leaf powder was taken and soaked in 500ml of distilled water for 24 hours. The mixture was vigorously swirled. After 24 hours with interval stirring, the mixture was filtered with the help of muslin cloth. Distilled water was added to make final volume up to 1000ml. Similar procedure was adopted for making 3% and 4% solution by taking 30 g and 40 g of leaf powder respectively.

Preparation of peel powder

The peel was separated from the pulp and cut into small pieces and dehydrated under shade at room temperature (28±2°C) for 3 to 5 days. The dried peel was ground into a fine powder using electric mixer or blender and stored in glass jars.

Preparation of different concentrations of peel extract

For 2% concentration 20 g of *Citrus sinensis* peel powder was taken and soaked in 500ml of distilled water for 24 hours. The mixture was vigorously swirled and filtered with the help of muslin cloth. Distilled water was added to make final volume up to 1000ml. Similar procedure was adopted for making 3% and 4% solution by taking 30 g and 40 g of peel powder respectively.

Bioassay

Two sets of dissecting dishes (25cm×18cm×4.5cm) were taken. Each set consisted of four dishes. They were labeled as A, B, C and D. In dish A of first set, 2% solution of peel extract was taken. In dish B, 3% peel extract was taken and in dish C 4% extract was taken, in dish D just 1000ml of distilled water was added as it had to serve as control. In the second set of dishes similar procedure was adopted for the leaf extract. In dish A of second set, 2% solution of leaf extract was taken. In dish B, 3% leaf extract was taken and in dish C, 4% extract was taken, in dish D just 1000ml of distilled water was added as it had to serve as control. In all these eight dishes, 10 (4th instar) larvae of *Culex quinquefasciatus* were added with the dropper. A pinch of food which consisted of dog biscuit and yeast powder in a 3:1 ratio was sprinkled on each dish. All these eight dishes were tightly covered with fine net so as to avoid egg laying of any insect. The mortality or survival rate of these larvae was checked after each 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 degradation.

The mortality rate of 4th instar larvae of *Culex quinquefasciatus* was observed. The number of larvae surviving after each 24 hours was reported and the percent mortality was calculated. The percentage morality was determined by Abbott's formula:

$$\% \text{ Mortality} = \frac{\text{Number of larvae dead}}{\text{Number of larvae taken}} \times 100$$

Statistical Analysis

The analysis of mortality was done by ANOVA. 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

This study was carried out to evaluate the larvicidal activity of *Citrus sinensis*. For this different concentration of crude aqueous extracts of peel and leaf of citrus plant were prepared and their effects were tested against the fourth instar larvae of *Culex quinquefasciatus.*

Larvicidal effect of peel extracts

In the first set of experiment of peel extracts of 2%, 3% and 4% concentrations along with the control, the following results were obtained.

In 2% concentration of peel extract 50% mortality of larvae was observed after 9 days of the treatment, while 100% mortality of larvae was recorded within 11 days (Figure 1).

So the survival percentage was zero in this concentration. Aqueous peel extract of 3% concentration showed 50% mortality after 2 days and 100% mortality on 6th day (Figure 2). So, the survival was 0%. The 4% concentration of peel extract gave 50% mortality after 3 days, while 100% mortality took place within 5 days (Figure 3). None of the larvae could pupate in this concentration so survival in this concentration was zero. In the control group of peel all the larva transformed into pupa in 3 to 4 days on average and then into adult at about 7th day, so the survival was 100% (Table 1) and the mortality percentage was 0%. A comparison of all the three concentrations at 72 hours showed that 4% concentration gave 50% mortality while the 2% and 3% gave 33.3% and 20% respectively (Figure 7). Among all concentrations of peel extract 4% was found to be most effective as compared to the 2% and 3% as is clear from its P-value i.e. 0.000 (Table 3)

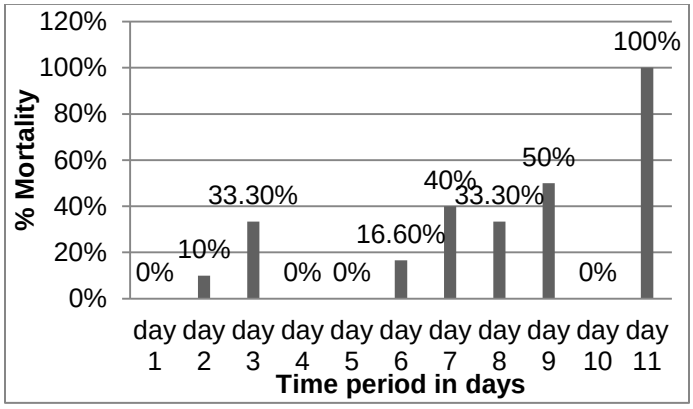

Fig. 1. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 2% peel extract of *Citrus sinensis.*

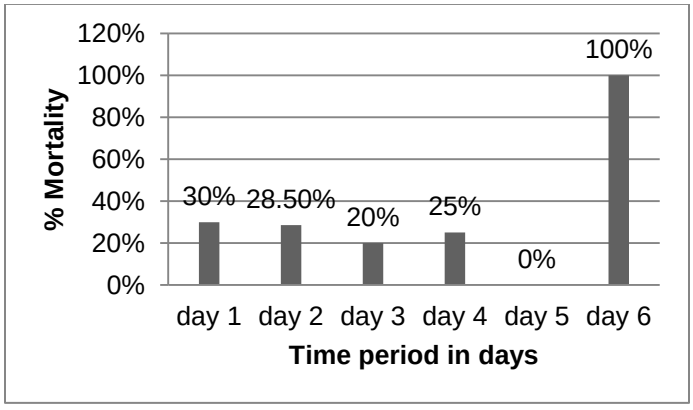

Fig. 2. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 3% peel extract of *Citrus sinensis.*

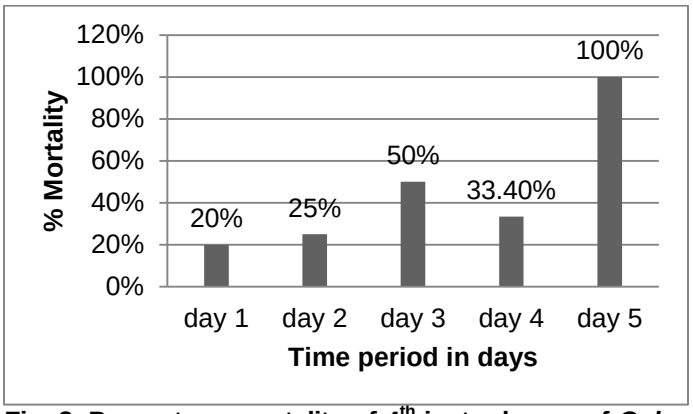

Fig. 3. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 4% peel extract of *Citrus sinensis.*

Table 1. Survival of 4th instar larvae of *Culex quinquefasciatus* in Control group of peel extract.

Time period in days(after 24 hour)	No. of larvae taken	Survival (In numbers)			% Survival		
		L	P	A	L	P	A
1st	10	10	0	-	100%	-	-
2nd	10	10	0	-	100%	-	-
3rd	10	10	0	-	100%	-	-
4th	10	5	5	-	50%	50%	-
5th	10	0	10	-	0%	100%	0%
6th	10	0	5	5	0%	50%	50%
7th	10	0	0	5	0%	0%	100%

* L= Larva, P= Pupa, A= Adult

Larvicidal effect of leaf extracts

In the second set of experiment of leaf extracts of 2%, 3% and 4% concentrations along with the control, the following results were obtained. In 2% concentration of leaf extract 50% mortality of larvae was observed after 7 days of the treatment, while 100% mortality of larvae was recorded within 12 days (Figure 4). So the survival was zero. Aqueous leaf extract of 3% concentration showed 50% mortality after 3 days and 100% mortality on 9th day (Figure 5).The survival percentage was 0%. The 4% leaf extract gave 50% mortality after 2 days. While 100% mortality took place within 5 days (Figure 6). The survival was 0%. In the control group of leaf extract all the larva transformed into pupa in 3 to 4 days on average and then into adult at about 7th day, so the survival percentage was 100% (Table 2) and the mortality percentage was 0%. A comparison of all the three concentrations at 72 hours showed that 4% concentration gave 25% mortality while the 2% and 3% gave 10% and 28.5% respectively (Figure 8). Among all concentrations of leaf extract 4% concentration was found to be most effective as compared to the 2% and 3% as its P-value is less than 0.05 i.e. .000 (Table 3).

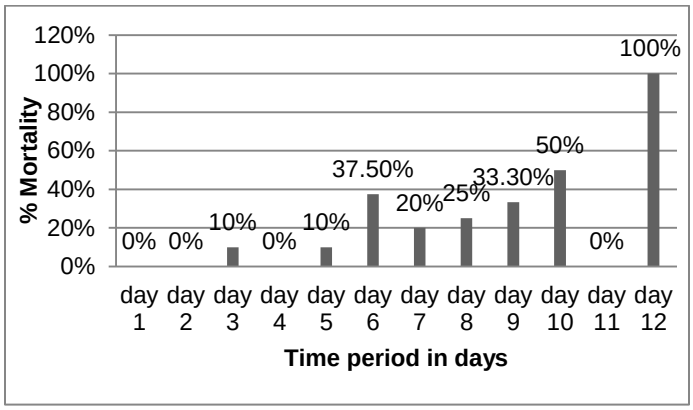

Fig. 4. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 2% leaf extract of *Citrus sinensis.*

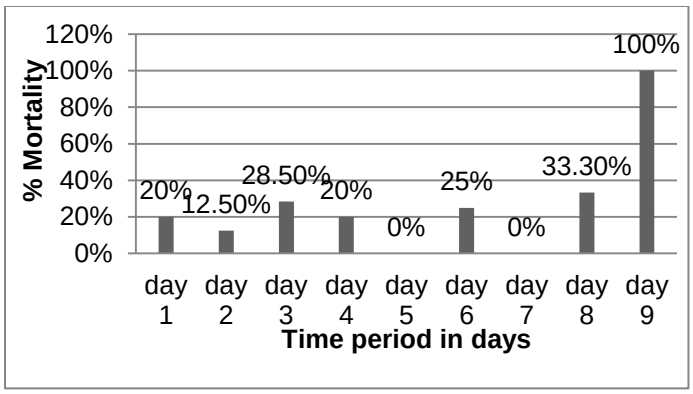

Fig. 5. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 3% leaf extract of *Citrus sinensis.*

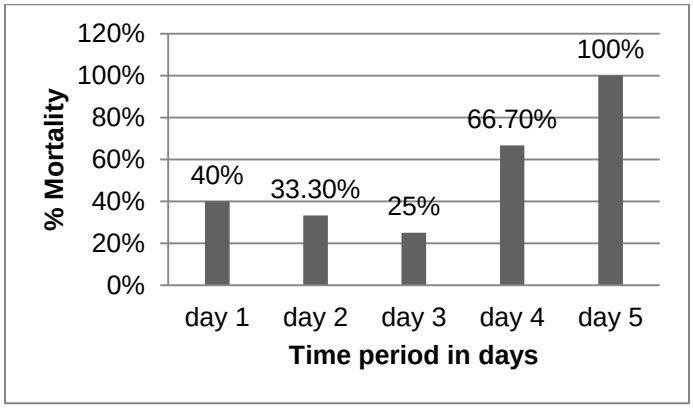

Fig. 6. Percentage mortality of 4th instar larvae of *Culex quinquefasciatus* in 4% leaf extract of *Citrus sinensis.*

Table 2. Survival of 4th instar larvae of *Culex quinquefasciatus* in Control group of leaf extract.

Time period in days(after 24 hour)	No. of larvae taken	Survival (In numbers)			% Survival		
		L	P	A	L	P	A
1st	10	10	0	-	100%	-	-
2nd	10	10	0	-	100%	-	-
3rd	10	10	0	-	100%	-	-
4th	10	6	4	-	60%	40%	-
5th	10	0	10	-	0%	100%	0%
6th	10	0	6	4	0%	60%	40%
7th	10	0	0	6	0%	0%	100%

* L= Larva, P= Pupa, A= Adult

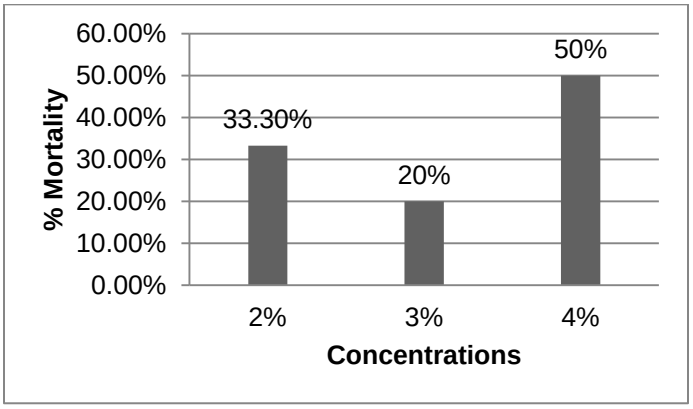

Fig. 7. Comparison between the percent Mortality of 4th instar larvae of *Culex quinquefasicatus* in different concentrations of peel at 72 hour.

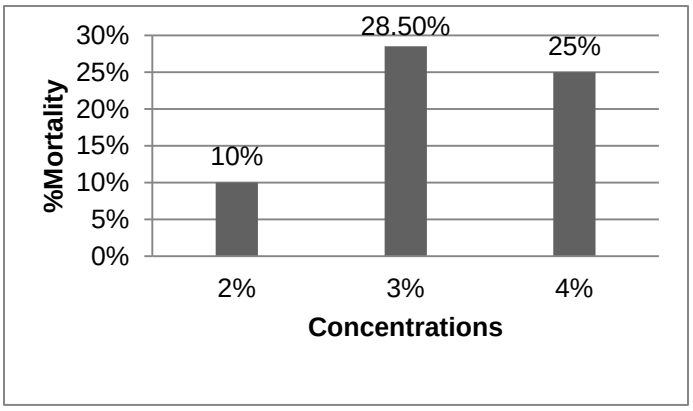

Fig. 8. Comaprison between the percent Mortality of 4th instar larvae of *Culex quinquefasciatus* in different concentrations of leaf at 72 hour.

Comparative efficacy of peel and leaf extracts

The results obtained were analyzed using One –Way ANOVA, comparing experimental and control group, with a significance level established at P<0.05. The results indicated a highly significant difference between treatments and control (Table 3). The P- values for peel extracts were lower than leaf extracts showing that peel extracts are highly toxic and more effective than the leaf extracts. From all the concentrations used for experimental work 4% concentration of peel was found to be most effective as it gave 100% mortality in 5 days. The results showed that percentage mortality was increased as the concentrations of the extract increased. All the extracts used in experiment had significant potency against the fourth instar larvae of *Culex quinquefasciatus.*

Table 3. ANOVA Results on the Mortality of Culex quinquefasciatus 4th instar larvae treated on the various concentrations of the fruits Extracts and the Control group.

Variable compared	F-value	P-value	Implication
Control vs. 2% peel	4.755	.044*	• At 0.05 level of significance.
Control vs. 3% peel	13.462	.004*	
Control vs. 4% peel	58.333	.000*	
2% peel vs. 3% peel	2.471	.137	
2% peel vs. 4% peel	2.844	.114	
3% peel vs. 4% peel	1.28	.729	
Control vs. 2% leaf	6.808	.018*	• *Implies that there is a significant difference
Control vs. 3% leaf	13.920	.002*	
Control vs. 4% leaf	23.692	.000*	
2% leaf vs. 3% leaf	.059	.811	
2% leaf vs. 4% leaf	2.140	.164	
3% leaf vs. 4% leaf	.874	.368	

DISCUSSION

Mosquitoes are responsible for public health problems as vectors of serious human diseases (El Hag *et al*., 1999). Certain species belonging to genera Aedes, *Anopheles* and *Culex* are vectors for the microbes of various diseases like Dengue fever, Dengue haemorrhagic fever, Malaria, Japanese encephalitis and Filariasis (Service, 1983). Control of these vectors is being carried out for a long time, by using synthetic chemicals but the chemicals may cause pollution problem and help to progress resistance in mosquito species (Das and Rajagopalan, 1981). 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).

The current study determined the effect of peel and leaf of *Citrus sinensis* against larvae of *Culex quinquefasciatus*. The results indicated that the different concentrations of peel and leaf extracts have significant larvicidal effect against *Culex quinquefasciatus*. The three different concentrations of peel and leaf of *Citrus sinensis* were prepared in order to check their efficacy on fourth instar larvae of *Culex quinquefasciatus*. The 2%, 3% and 4% extracts gave 100% mortality but extract with 4% concentrations gave mortality in least days. This showed that 4% concentration was more effective as compared to 2% and 3%. The increased amount of extract resulted in increased the rate of mortality. These results indicated the presence of some toxic components in the leaf and peel of *Citrus sinensis* 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 Ubulom et al. (2012) who observed increasing mortality with increase in extract concentration and increase in exposure time in all treatments. The results of our study are also in accordance with the work of Mwangi and Mukiama (1988) who observed that *Melia volkensii* extracts prolonged the lifespan of *Anopheles arabiensis* larvae but not the pupal period. The plant *Citrus sinensis* used in our study gave good results even on larval stage as it prolonged its larval duration (upto 11 days) and then caused their death.

In the current study, larval mortality in all the extracts may be attributed to the presence of flavonoids, alkaloids, tannins and saponins, present abundantly in Citrus. In our study among the various concentrations of leaf extract the 4% extract of leaf was most effective as it gave 100% mortality in just 5 days. The high mortality rate might be due to the high amount of the components in the leaf of the Citrus *sinensis*. 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). Enejoh et al. (2015) showed that various concentrations of the *Citrus sinensis* fruit juice inhibited the hatching of eggs and killed the larvae of *Heligmosomoides bakeri*. Humaid (2016) determined antimicrobial artivity of camel's urine. Kalim et al. (2016) and Hussain et al. (2016) demonstrated antifungal and antibacterial activity of methanolic extracts of *Datura inoxia*. 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.

CONCLUSION

It is concluded that leaf and peel of *Citrus sinensis* 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.

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CONFLICT OF INTEREST

There is no conflict of interest.

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