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Evaluation of the larvicidal potential of *Calotropis procera* plant extract against *Culex pipiens*

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Abstract
The aim of the present research work was to evaluate the larvicidal potential of the plant extract of *Calotropis procera* against the 4th instar larvae of the *Culex pipiens*. In the present study, it was observed that mortality of the larvae was directly proportional to the dose and time period of exposure. The larvae were treated for 24, 48 and 72 hours with concentrations of 1%, 0.5%, 0.25% and 0.125% with doses of the *C. procera* extract of 1ml, 0.5ml, 0.25ml and 0.125ml. The rates of mortalities showed that extract of *C. procera* can be effectively used for the control of mosquitoes and other insect species. No larvae survived at 1% of latex for 72 hours of treatment. The results revealed that extract of *C. procera* is a potent and efficient botanical pesticide, environment friendly and easily available so it can be effectively utilized for the control of various insect species. This will help to prevent the biodiversity from the side effects of chemical control measures. We recommend that chemical control measures for insects and other pests should be replaced with botanical and biological control measures for the protection of biodiversity.

Keywords: *Culex pipiens*, *Calotropis procera*, latex, mortality, plant extracts

Introduction
Mosquitoes are important vectors of various human and animal diseases such as malaria, encephalitis, yellow fever, dengue and filariasis [1]. A particular mosquito species acts as a vector for a specific disease like Anopheles sp. for malaria [2], Aedes sp. for yellow fever [3], Culex sp. for filariasis in humans and lumbar paralysis in cattles [4]. Prevention of mosquito population is very important because they transmit a variety of diseases by acting as primary vectors for the pathogens that cause severe infections. Chemical control measures have a number of impaired health risks by affecting the natural environment. Therefore environment friendly control measures of mosquitoes is important by means of that plant extracts having insecticidal potential. Extracts of plants, tree parts, herbs, shrubs and fruits has been used effectively against mosquito larvae [5]. Biologically active plant extracts are mostly used for the control of various species of mosquitoes [6]. Fruit extract of *Melia azadirachta* and *Azadirachta indica* has been reported to have produced a variety of disorders in insects such as reduced fecundity, moulting disorders, antifeedant, growth retardation, morphogenetic defects and changes of behaviour [7]. Seeds and leaf juices of Annonaceae family have been used to kill head and body lice while bark extracts of *Goniothalamus macrophyllus* has been used as mosquito repellents [8].
Aedes species act as vector for dengue fever and dengue hemorrhagic fever which is endemic in Southeast Asia, the Pacific Islands, Africa and America [9]. Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and *Japanese encephalitis* (JE). The global annual incidence and mortality rate for *Japanese encephalitis* has been reported to be 30,000 to 50,000 and 10,000 respectively [10]. Larvicidal measures play a vital role in controlling mosquitoes in their breeding sites but also have negative impact on beneficial and non-target organisms [11].
The present research work was aimed to ascertain the larvicidal effects of the plant extract of *Calotropis procera* against the 4th instar larvae of *Culex pipiens*. The plant extract are environment friendly and can be effectively used for mosquito control measures. The objective of the study was to replace the traditional chemical control measures with botanical control measures by using various plant extracts and to secure biodiversity form the side effects associated with chemical control measures.

Materials and Methods

A: Collection of Mosquito Larvae

The mosquito larvae were collected from Muslim Abad, a village in district Kohat of Khyber Pakhtunkhwa province of Pakistan, approximately 6km East from Kohat University of Science and Technology in September, October and November. The mosquito larvae were collected in common plastic jars and survived in normal environmental conditions.

B: Collection of Samples of the Plant Species and Preparation of Stock Solution

The leaves of the studied plant were collected locally in and around Kohat University of Science and Technology (KUST) with the help of experienced botanists and were washed with water and then dried in shadow at room temperature. The dried out leaves were crushed to powder form with Mortar and pestle. The dried powder were then dissolved in distilled water for making stock solution.

C: Bioassay Method of Treatment

Different concentrations of extracts were prepared using distilled water. The mosquito larvae were treated with various concentrations of plant extract according to the guidelines of World Health Organization. The larvae of *C. pipiens* were exposed to different test concentrations of the plant extract along with a set of control stock containing distilled water without any test solution. After adding the larvae, the petri dishes were placed in laboratory at room temperature. By counting the number of dead larvae after 24, 48 and 72 hours of exposure, the mortality rate and lethal concentrations were calculated. Five replicates of each concentration of a particular plant extract were maintained. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid mortality in remaining larvae of *C. pipiens*.

Results

The obtained results revealed that the tested botanical pesticide of the plant *C. procera* was very effective against the 4th instar larvae of *C. pipiens* as is evident from the values of mortalities (Table 1-3, Fig. 1-3). Treatment of the larvae with *C. procera* has shown progressive mortality effects in various degrees. By using the proper amount of latex at 1ml dose for 72 hours, approximately 100% mortality was achieved (Table 3, Fig. 3). A remarkable increase in mortality rate of *C. pipiens* larvae was observed with increasing concentration of *C. procera* latex. The final obtained mortality results after 24 hours of treatment of *C. pipiens* larvae with *C. procera* latex by using 1ml dose has shown a mortality rate of 96, 90, 86, 72 and 0 values (Table 1, Fig. 1). By using lower concentration of the latex, lower mortality of 64, 50, 24 and 8 was observed (Table1, Fig. 1). By extending the time duration from 24 hours to 48 hours with the same concentration of 1ml, the mortality rate was recorded as 98, 94, 88, and 82 (Table 2, Fig. 2). With the same duration but at lower concentration of the latex, different values of mortality were obtained as 88, 66, 36 and 24 (Table 2, Fig. 2). After 72 hours duration at 1ml concentration of the latex, very high mortality rate was observed as 100, 94, 84, 76 (Table 3, Fig. 3). The obtained result indicates that mortality in mosquito larvae is dependent on dose of the plant extract and duration of treatment.

By decreasing the dose from 1ml to 0.5 ml of latex for the same time duration, different values of *C. pipiens* mortality were obtained as 98, 96, 84, 72 (Table 3, Fig. 3). The treated larvae became gradually inactive due to the effects of plant extracts and started to settle down towards the bottom of the petri dishes. Microscopic study of dead larvae revealed that the extract has penetrated into larval digestive system. The treated larvae showed curling up, agitation and vigorous body movements.

Table 1: Mean percent mortalities data obtained after 24 hours of treatment with different concentrations (%) and doses (ml) of latex (*Calotropis procera*).

S. No.	Concentration (%)	Dose (ml)/ Mean (%) Mortality			
		Mean% Mortality at 1ml	Mean% Mortality at 0.5 ml	Mean% Mortality at 0.25 ml	Mean% Mortality at 0.125 ml
1	1%	96	94	78	64
2	0.5%	90	84	64	50
3	0.25%	86	62	30	24
4	0.125%	72	54	10	8
5	Control	00	00	00	00

Table 2: Mean percent mortalities data obtained after 48 hours of treatment with different concentrations (%) and doses (ml) of latex (*Calotropis procera*).

S. No.	Concentration (%)	Dose (Ml)/ Mean (%) Mortality			
		Mean% Mortality at 1ml	Mean% Mortality at 0.5 ml	Mean% Mortality at 0.25 ml	Mean% Mortality at 0.125 ml
1	1%	98	94	88	68
2	0.5%	94	90	66	56
3	0.25%	88	72	36	26
4	0.125%	82	58	24	14
5	Control	00	00	00	00

Table 3: Mean percent mortalities data obtained after 72 hours of treatment with different concentrations (%) and doses (ml) of latex (*Calotropis procera*).

S. No.	Concentration (%)	Dose (Ml)/ Mean (%) Mortality			
		Mean% Mortality at 1ml	Mean% Mortality at 0.5 ml	Mean% Mortality at 0.25 ml	Mean% Mortality at 0.125 ml
1	1%	100	98	94	92
2	0.5%	94	96	82	74
3	0.25%	84	84	64	56
4	0.125%	76	72	38	32
5	Control	00	00	00	00

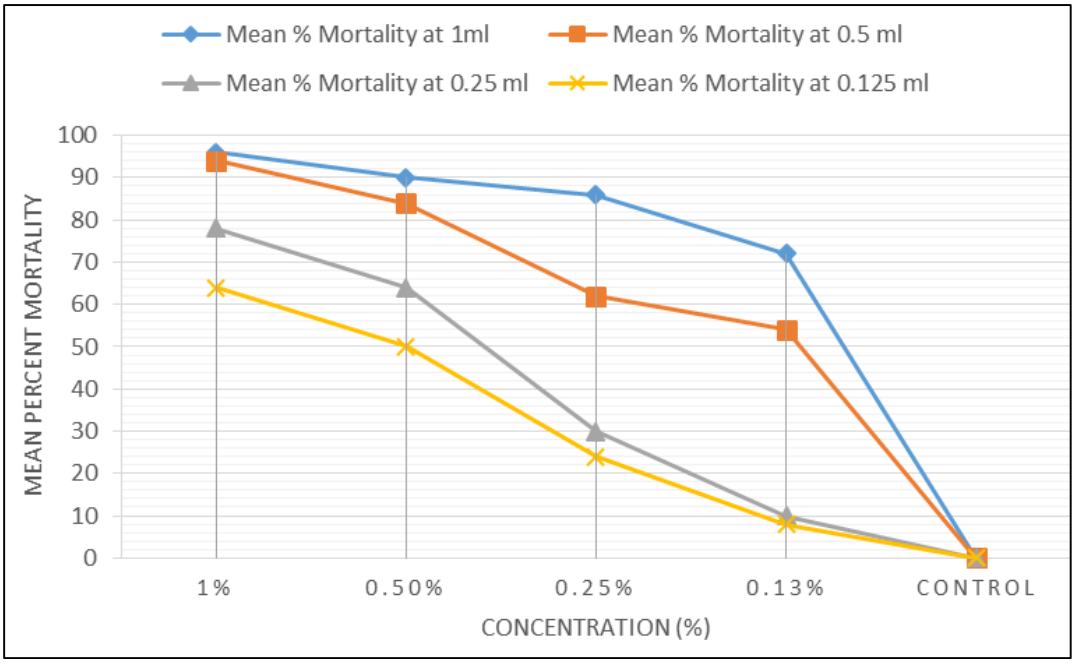

Fig 1: Mean percent mortalities after 24 hours of treatment of *C. pipiens* larvae with different concentrations (%) and doses (ml) of latex (*C. procera*)

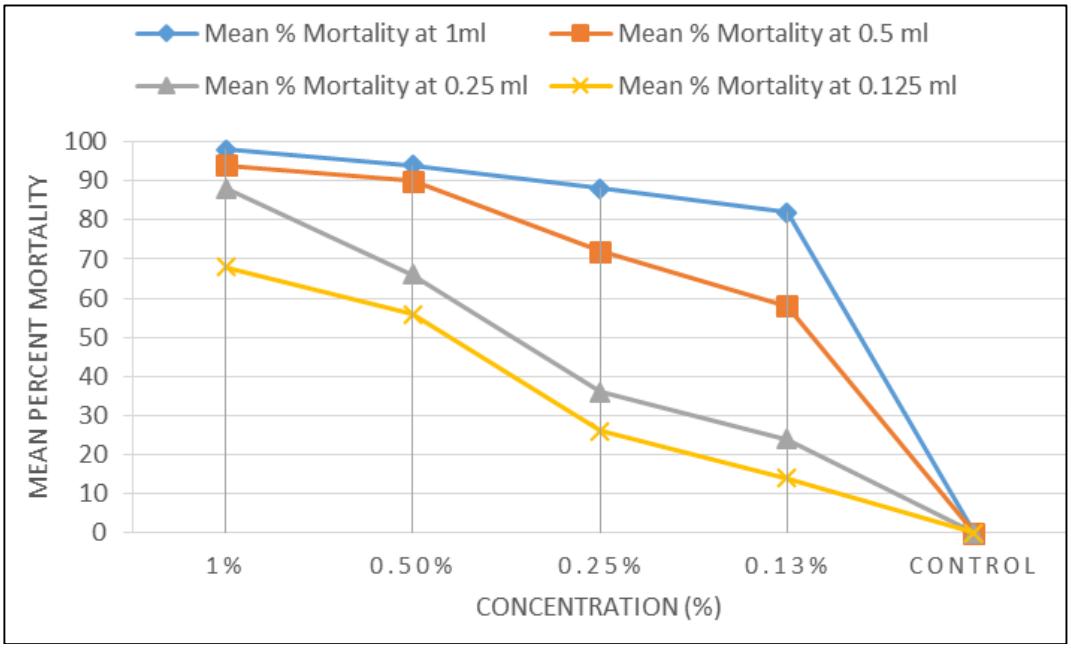

Fig 2: Mean percent mortalities after 48 hours of treatment of *C. pipiens* larvae with different concentrations (%) and doses (ml) of latex (*C. procera*)

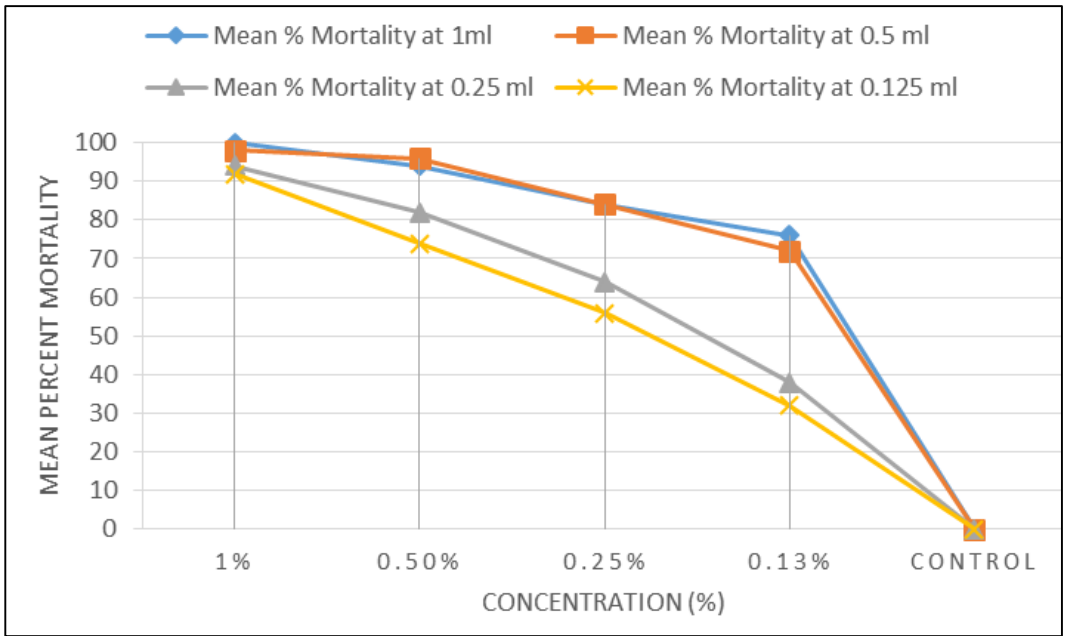

Fig 3: Mean percent mortalities after 72 hours of treatment of *C. pipiens* larvae with different concentrations (%) and doses (ml) of latex (*C. procera*)

Discussion

Botanical pesticides has shown to be effective against mosquitoes and are also environment friendly. Mosquitoes are potent vectors for the transmission of various pathogens to humans and transmit a variety of diseases in humans and other animals. The latex of *C. procera* was found effective for the control of mosquito larvae as indicated by the rate of mortalities (Table 1-3, Fig. 1-3). Extract of *Citrullus vulgaris* has been reported to have caused high mortalities in larvae of *Anopheles stephensi* [12]. The present findings are comparable with the cited study in terms of mortalities and larvicidal potential of botanical extracts.

Application of *C. procera* extract in combination with *Annona squamosa* ethanol extract against 3rd instar larvae of *Musca domestica* for 24 hours has shown lower mortality compared with 48 hours of treatment [13]. Leaf extract of *A. squamosa* at 200 ppm concentration has caused the same rate of mortality in 3rd instar larvae of *M. domestica* as has been calculated with *C. procera* extract for 48 hours of exposure in the present study. This is in agreement with the present findings that *C. procera* leaf extract has the potential to be used effectively against mosquitoes and other insect species.

The present findings can also be compared with a previous report where 5% aqueous extract of leaf, flower and root of *C. procera* resulted in 100% mortality of *Henosepilachna elaterii* larvae while 1% and 2.5% extract highly reduced fecundity and longevity [14]. This shows similarity with our present findings and proves that extract of *C. procera* can be effectively used for the control of various insect species [15-20].

In the present finding, it was observed that mortality of the larvae was directly proportional to the dose and time period of exposure. The larvae were treated for 24, 48 and 72 hours with concentrations of 1%, 0.5%, 0.25% and 0.125% with doses of the *C. procera* extract of 1ml, 0.5ml, 0.25ml and 0.125ml.The rates of mortalities authenticated the previous findings on the larvicidal potential of *C. procera* extract, and indicates that this extract can be effectively used for the control of mosquitoes and other insect species [21-26].

Conclusion

Extract of *C. procera* is a potent botanical pesticide and can be used effectively for the control of mosquito species. *C. procera* is a common weed plant of Pakistan where large quantities of different parts of the plant can easily be collected utilized for the control of different insect species. The present findings concludes that mortality of the *C. pipiens* larvae is directly proportional to the dose and duration of treatment with the studied plant extract. The most important aspect of the present finding is that the plant extracts are environment friendly and can be used effectively to replace the chemical control measures which are dangerous for the whole biodiversity. Chemical control measures has deteriorated the natural ecosystems and has made immense losses to humans, animals and plant species. In light of the obtained results, it is highly recommended that the use of botanical pesticides should be enhanced in the future and chemical control measures should be eliminated as far as possible.

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