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Potential use of *Calotropis procera* (Milk Weed) to Control *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract.- *Culex quinquefasciatus* cause severe biting nuisance and responsible for the spread of dreadful diseases among human beings. Biological researchers are trying to introduce eco-friendly products for the control of mosquitoes. The use of botanical pesticides has become very popular throughout the world as an alternative tool for the mosquito control. Present study deals with latex produced by the green parts of the *Calotropis procera* (milk weed) and aims to evaluate its toxic effects upon the mortality of *C. quinquifasciatus* larvae. Three concentrations (*i.e.*, 0.5%, 0.25% and 0.1%) were tested against the larvae. There was significantly higher mortality in the treated group compared to the control group. The whole latex showed higher larval mortality for all concentrations at 24 h in lesser time compared to the rubber free latex. The percentage mortality was increased with increasing latex concentrations, indicating a direct relationship between the dose and percent mortality. Although the effectiveness of rubber free latex was less as compared to the whole latex but both types of latex caused 100% mortality within 24 h with 0.5% and 0.25% concentrations. When both types of latex were submitted to heat-treatment, the toxic effects were diminished considerably suggesting low thermo-stability of the toxic compounds. The results suggested that latex of *C. procera* can be used in mosquito control programs as it possesses remarkable larvicidal properties. However, it is recommended that effectiveness of the latex of *C. procera* should also be evaluated against other mosquito species especially against the *Anopheles stephensi* and *Aedes agypti*,which are growing threat in the country.

Keywords: *Culex quinquefasciatus*, *Calotropis procera*, thermo-stability of latex, plants extracts.

INTRODUCTION

Culex quinquefasciatus is responsible for the transmission of encephalitis, filariasis and west nile in many parts of the world (Shahi *et al.,* 2010). In Punjab Pakistan high densities of this mosquito species especially during summer months cause severe biting nuisance (Tahir *et al.,* 2009). Insecticides are being widely used to control mosquitoes throughout the world. However, with a greater awareness of the side effects associated with the use of chemical insecticides, scientists are trying to explore alternative products for mosquito control (Begum *et al.,* 2000). Many researchers have reported the effectiveness natural plant extracts as possible alternatives to synthetic chemical insecticides (Schumutterer *et al.,* 1995; Pathak *et al.,* 2000; Rajkumar and Jebanesan, 2005, Promsiri *et al.,* 2006).

The *Calotropis procera* (locally known as Akk in Punjab, Pakistan) has attained a high repute for its various medicinal properties (Ramos *et al.,* 2006; Doshi *et al.,* 2010). *C. procera* is a shrub which belongs to the plant family, Asclepiadaceae. It is distributed in West Africa, Asia and other parts of the tropics. The plant is erect, tall, large, branched and perennial with milky latex throughout (Begum *et al.,* 2000). The latex of *C. procera* has been used as an antidysenteric, antirheumatic, a diaphoretic, an expectorant, a purgative, an anti-inflammatory, for the treatment of bronchial asthma and skin conditions and for milk coagulation in cheese making (Watt and Breyer-Brandwijik, 1962; EL`Badwi, 1998). Latex of *C. procera* has been proved to have insecticidal activity against different insects (Moursy, 1997; Morsy *et al.,* 2001). The extract of *C. procera* also has been reported to have toxic and potent growth reducing activity to mosquitoes (Singhi, 2004). The abundance of latex (containing alkaloids) in the green parts of the plant reinforces the idea that it produced and accumulated latex as a defense strategy against organisms such as virus, fungi and insects (Dubey *et al.,* 2007; Rashmi *et al.,* 2011). Phytochemical screening of the extracts of *C. procera* indicated the presence of alkaloids, carbohydrates, saponins, phenols, tannins,

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terpenoids and flavanoids which are known to possess medicinal and pesticidal properties (Doshi *et al.,* 2010). The leaves extracts of the milkweed *Calotropis procera* show oviposition deterrant, larvicidal and ovicidal activities against mosquito (Singh *et al.,* 2005; Kabir *et al.,* 2010; Sripongpun 2008; Doshi *et al.,* 2010; Giridhar *et al.,* 1987) found compounds with larvicidal activity in the latex of *C. procera* for the first time.

Keeping in view of wide range of biological activity of *C. procera* present study was undertaken. Objective of the study were: (i) to evaluate the insecticidal activity of whole latex of *C. procera,* against *C. quinquefasciatus;* (ii) to test the larvicidal activity of latex without rubbery material against *C. quinquefasciatus*; (iii) to check the thermo-stability of latex without rubbery material; and (iv) to compare the effectiveness of fresh latex and 20 days old latex (without rubbery material).

MATERIALS AND METHODS

Study was conducted during February through May, 2012. Healthy and non-cultivated plants of *C. procera* (Family Ascelpiadaceace), growing in the vicinity of University of Sargodha were selected. The taxonomic identification was confirmed by Dr. Abdul Ghani, Assistant Professor of Botany, Department of Biological Sciences, University of Sargodha. To collect the latex of *C. procera* petioles of the young leaves were cut and left to flow off in the glass vials (100ml). The latex was gently agitated during collection to overcome the tendency of the coagulation-like effect (Ramos *et al.,* 2011). Larvae of *C. quinquefasciatus* were collected from the stagnant water area of Sargodha 46 SB (32º 04' 46.06" N, 72º 40' 19, 34"E elevation 627 ft). The larvae were identified by one of us (SYK). Collected larvae were transferred into a vessel containing 400 ml water and provided with biscuit and yeast powder in the ratio of 3:2 as a nutrient (Arunpandiyan, 2011).

Larvicidal activity of whole extract of C. procera

Bioassays for the larvicidal activity were carried out following the method described by WHO (1981) with slight modifications. From the extract of *C. procera* different concentrations (i.e., 0.5%, 0.25% and 0.1%) were made. To test the larvicidal activity against each concentration eight plastic cups were used (six for treated group and two for the control group). Each cup of treated group contained 100 ml of 0.1%, 0.25% or 0.5% of latex material and 25 larvae (3^{rd} to 4^{th} instars). However, only the simple water (100 ml) and 25 larvae were present in each cup of the control group. Each batch of treated group was exposed to one of the three concentrations *i.e.,* 0.5%, 0.25% and 0.1%. No food was offered to the larvae in the treated or control group throughout the experiment. Larval mortality was recorded at different time intervals till 24 h after exposure. Dead larvae were identified when they failed to move after probing with the needle in a siphon or cervical region. Bioassay experiment for each concentration was repeated thrice.

Larvicidal activity of extract of C. procera *without rubbery material*

Fresh latex was collected from healthy plants by small incisions near the youngest leaves and left to flow off into a bottle (100 ml). This latex was transported to the laboratory. The latex was centrifuged at 2000 rpm at room temperature (25°C) in a non-refrigerated bench top centrifuge for 5 minutes. The precipitated material, showing rubber aspect, was pooled apart while the supernatant was used for bioassay tests against the larvae. Different concentrations (*i.e.*, 0.5%, 0.25% and 0.1%) were prepared from the supernatant following the method described in the first experiment. Similarly rest of the procedure was also similar to the previous experiment.

Thermo-stability of latex without rubbery material

To evaluate the effect of temperature on the stability of latex, the latex was divided into two groups. One group was heated at 50°C for 5 minutes while the second group was heated at 100°C for the same duration. The lavicidal activity of both groups was assessed by the same methods as described above.

Effectiveness 20 days old latex (without rubbery material)

To test the insecticidal activity of 20 days old

latex with and without rubbery material fresh whole latex and latex devoid of rubbery material was taken and kept at room temperature for 20 days. After 20 days different concentrations of latex were prepared and the insecticidal activity of each concentration was determined.

Statistical analyses

The normality of the data was checked using Kolmogorov-Smirnov test. Analysis of variance was used to compare the mortality of *C. quinquefasciatus* larvae treated with different concentrations (*i.e.*, water, 0.5%, 0.25% and 0.1%) of latex of *C. procera*. The mortality of *C. quinquefasciatus* treated with the latex heated at 50ºC or 100ºC was compared using Fisher Exact Test. Similarly Fisher Exact Test was also used to compare mortality of *C. quinquefasciatus* larvae treated with 20 days old whole latex and latex without rubbery material. All statistical analyses were performed consulting SPSS (Version 16).

RESULTS

Larvicidal activity of whole extract of C. procera

When the larvae were exposed to the 0.5% leaf extract of *C. procera* 60% mortality was observed just after two hours (Fig. 1A). After eight hours the mortality rate reached the 100%. When larvae were exposed to the 0.25% extract the mortality rate observed after two hours was 16%. After four hours the mortality was 47% and reached to the 92% after eight hours. The 100% mortality was observed at 12 hours after the exposure (Fig. 1B). When the larvae were exposed to 0.1% extract initially the mortality was low. The mortality recorded at 24 h was 90% (Fig. 1C). No mortality was recorded in the control group. Significant difference was observed in mortality at different concentrations. Results of ANOVA and Tukey's test are given in Table I.

Larvicidal activity of extract of C. procera without rubbery material:

When the larvae were exposed to 0.5% the extract without rubbery material there was no mortality after two hours of exposure. However, the mortality rate gradually increased with time. The

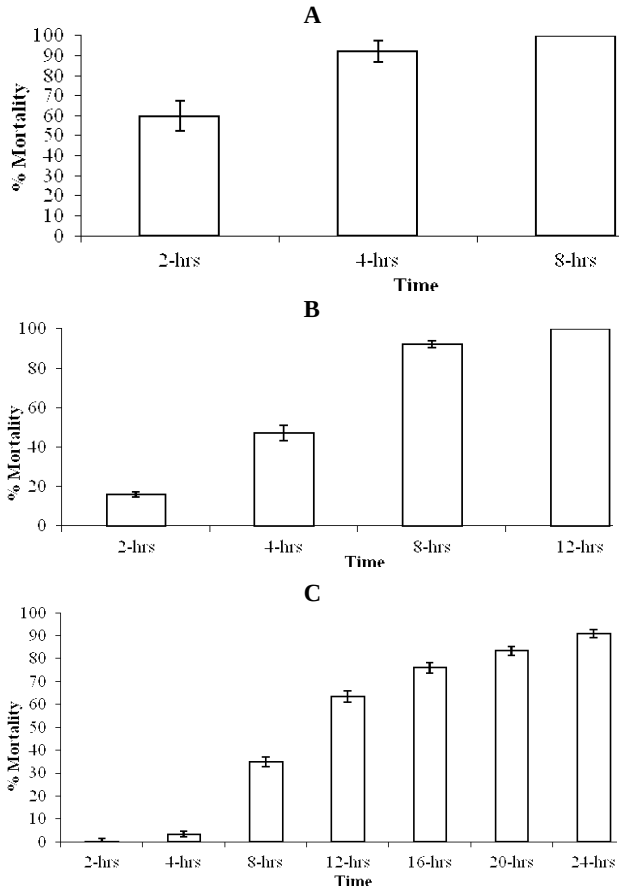

Fig. 1. Mortality (%) of *C. quinquefasciatus* larvae caused by different concentrations of crude extract of latex (whole) of *C. procera*; A, 0.5%; B, 0.25%; C, 0.1%. Error bars in the figure indicate standard error.

mortality was 100% after 24 hours (Fig. 2A). However in the group of larvae exposed to the 0.25% extract, there was no mortality after eight hours after exposure. Only 23% mortality was observed after 24 h (Fig. 2B). With 0.1% extract there was only 9% mortality after 24 h (Fig. 2C). No mortality was recorded in the control group. Results of ANOVA and Tukey's test are given in Table II.

Thermo-stability of latex without rubbery material

Results of thermo-stability experiment are given in the Figure 3. When 0.5% extract was heated at 50ºC the mortality was only 48%, however by heating the extract at 100ºC, the % mortality was declined to 25% only. The mortality rate of the larvae with 0.25% extract heated at 50°C and 100°C

Table I.- Comparison of mortality of *C. quinquefasciatus* larvae treated with different concentrations (*i.e.*, water, 0.5%, 0.25% and 0.1%) of whole latex.

a) ANOVA

	Sum of squares	df	Mean square	F	P-value
Between groups	17408.917	3	5802.972	904.359	< 0.001
Within groups	51.333	8	6.417		
Total	17460.250	11			

b) Tukey's HSD

Concentration (%)	N	Subset for alpha = .05			
		1	2	3	4
Control	3	.0000			
0.1	3		32.0000		
0.25	3			84.3333	
0.5	3				92.6667

Table II.- Comparison of mortality of *C. quinquefasciatus* larvae treated with different concentrations (*i.e.*, water, 0.5%, 0.25% and 0.1%) of latex devoid of rubbery material.

a) ANOVA

	Sum of squares	df	Mean square	F	P-value
Between groups	15830.917	3	5276.972	917.734	< 0.001
Within groups	46.000	8	5.750		
Total	15876.917	11			

b) Tukey's HSD

Concentration (%)	N	Subset for alpha = .05			
		1	2	3	4
Control	3	.0000			
0.1	3		9.6667		
0.25	3			23.3333	
0.5	3				92.6667

were 35% and 17% respectively. Similarly with 0.1% extract the mortality rates were 23% (at 50ºC) and 9 (at 100ºC) respectively. Significant difference in mortality was observed at two different temperature treatments (Fig. 3, $P < 0.01$).

Effectiveness of 20 days old latex (with and without rubbery material)

The effectiveness of the latex is diminished with time as indicated in the Figure 4. The mortality

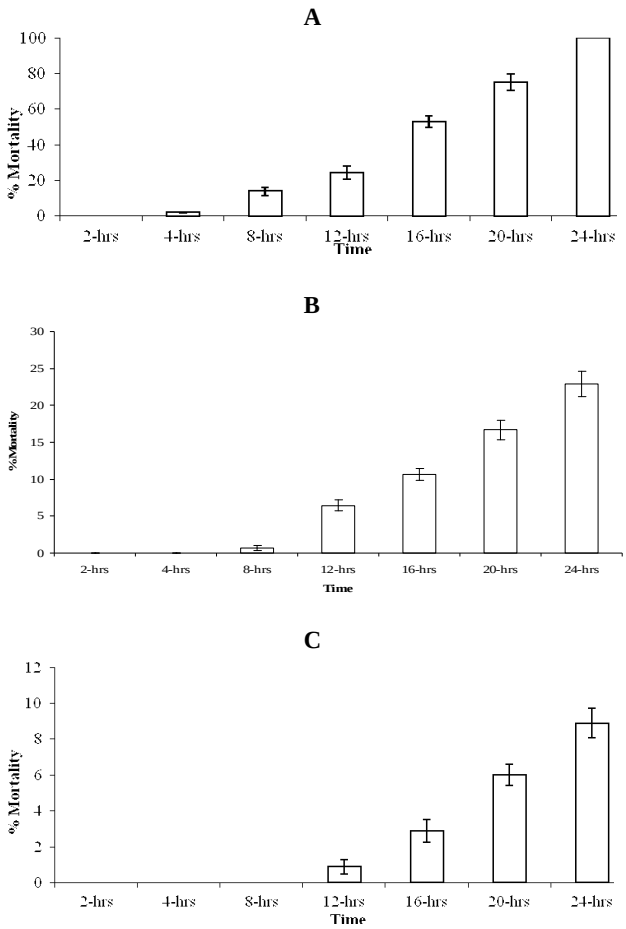

Fig. 2. Mortality (%) of *C. quinquefasciatus* larvae caused by different concentrations of crude extract (without rubbery material) of latex of *C. procera*; A, 0.5%; B, 0.25%; C, 0.1%.
Error bars in the figure indicate standard error.

was 100% with 20 days old 0.5% whole latex after 24 h of exposure. However, only 38% mortality was caused by latex without rubbery material. The mortality rates with 0.25% and 0.1% of 20 days old whole latex were 100 % and 67 % respectively after 24 h. Similarly with 0.1% 20 days old without rubbery material latex the mortality rates were 15% and 8%, respectively. Mortality was significantly higher in the mosquitoes treated with latex with rubbery material (Fig. 4, $P < 0.01$).

DISCUSSION

Mosquitoes are the vectors of many diseases

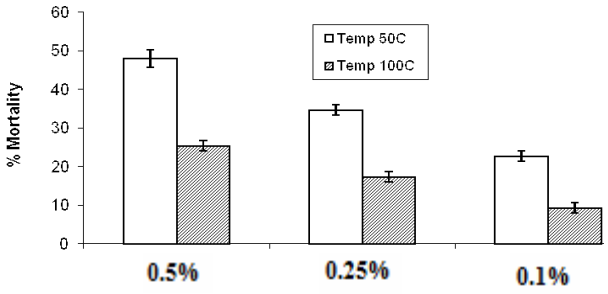

Fig. 3. Mortality (%) of *C. quinquefasciatus* larvae at 24 h caused by different concentrations of extract of *C. procera* heated at 50°C and 100°C.

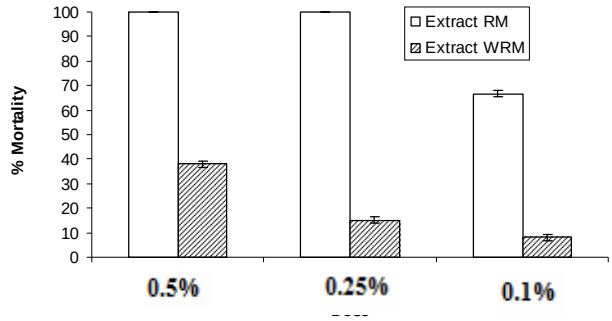

Fig. 4. Comparison of mortality (%) of *C. quinquefasciatus* caused by 20 days old latex with and without rubbery material.

throughout the world and botanical derivatives may be the future of mosquito control programs (Shahi *et al.*, 2010). Many researchers have tested the phytochemicals against various life stages of mosquitoes and reported that these plant products have potential to inhibit the growth and reproduction of mosquitoes (Alencar *et al.*, 2004). They also act as repellents and oviposition deterrents (Doshi *et al.*, 2010; Kabir *et al.*, 2010; Singh *et al.*, 2005). The plant extracts should be preferred as they are cheaper, degrade after sometime, have a different mode of action, have least effects of the non target organisms and chance of resistance among the insects is limited (Kabir *et al.*, 2010). Phytochemicals are considered the good alternative of pesticides. Some botanical compounds such as alkaloids, nicotine, anabasin and lupitin produced high mortality against mosquito larvae (Jain *et al.*,1996; Sharma and Sharma, 2000; Dewan *et al.*, 2000). Organic solvent extracts of some plant species and their oil cakes have also been reported to be quite effective against larvae of *An. stephensi*, *Ae. aegypti* and *C. quinquefasciatus* (Srivastava *et al.*, 2008; Maurya *et al.*, 2008; Shanmugasundaram *et al.*, 2008).

Present study showed that the complete latex of *C. procera* was highly effective against larvae of *C. quinquefasciatus* as it caused more than 90% mortality after 24 h at all the tested concentration (*i.e.*, 0.1%, 0.25% and 0.5%). The latex of *C. procera* latex contains the larvicidal compounds, which caused high mortality in the larvae of mosquitoes within a short period of time (Giridhar *et al.*, 1987; Markouk *et al.*, 2000; Ramos *et al.*, 2006; Shahi *et al.*, 2010). Shahi *et al.* (2010) showed in his study that larvae of *C. quinquefasciatus* is more susceptible against the latex of *C. procera* than *An. stephensi* at the same concentration. However, efficacy of a phytochemical depends on mosquito species, life stage, the plant parts and solvent used for extraction, phototoxic activity and the geographical origin of a plant compound. Latex of *C. procera* has antibacterial, analgesic or schizontocidal activities. Giridhar *et al.* (1987) found compounds with larvicidal activity in the latex of *C. procera* for the first time.

In the current study it has been observed that although latex without rubbery material was effective in killing the mosquito larvae but its effectiveness was less compared to the complete latex. So it can be predicted that the rubbery material has harmful effects upon larvae however, its low water solubility diminishes its effectiveness in mosquito control programs (Ramos *et al.*, 2006).

After heating the latex toxic effects were diminished, indicating low thermo-stability of the toxic compounds. This finding corresponds to the work of Ramos *et al.* (2006) who also observed low thermo-stability of latex of *C. procera*. However, it was observed that even heating the latex at 100 ºC, the effectiveness of the latex was not completely lost. About 25% mortality was recorded after 24 h with 0.5% of latex even if it was heated at 100 ºC (Figure 3). From the results it can be predicted that the components of latex which are the cause of larval mortality even heating at high temperature may involve non protein molecules, as protein

components must have been denatured at this high temperature.

The effectiveness of the latex also diminished with time (Figure 4). However, in this study we did not investigate the cause and recommend further studies. Studies should also be focused to introduce chemicals which would be helpful to enhance the stability of latex so that it can be effective for a longer period of time. Further more studies should also be conducted to characterize latex of *C. procera* to isolate different fractions and to evaluate the susceptibility of *C. quinquefasciatus* against each fraction of latex so that we would be able to pinpoint those fractions which are the actual cause of mortality.

From the results of study it can be concluded that *C. procera* extracts possess good larvicidal activity against *C. quinquefasciatus* and could be a good alternative to the insecticide for the control of this mosquito species in the study area. As *C. procera* is abundant in Sargodha, it is suggested to perform more studies to produce natural insecticide/larvicide from this native plant.

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