Larvicidal, pupicidal and adulticidal activities of non-polar solvent extract of *Cymbopogon nardus* (Linn.) whole plant against a mosquito, *Culex quinquefasciatus* (Say.)

Ikram Ilahi[1,2]* and Ali Muhammad Yousafzai[1]
[1]Department of Zoology, Islamia College University, Peshawar, Khyber Pakhtunkhwa, Pakistan
[2]Department of Zoology, University of Malakand, Chakdara, Dir Lower, Khyber Pakhtunkhwa, Pakistan

Abstract: The present research is aimed to investigate the susceptibility of *Cx. quinquefasciatus* larvae (2nd and 4th instars), pupae and adults during exposure to various concentrations of *C. nardus* n-hexane extract. Increase in mortality of larvae and pupae was observed with increase in concentration of the extract solution (R^2>0.90). LC_{50} values of extract for 2nd and 4th instar larvae were 451.8 and 599.6 ppm, respectively. The LC_{90} values of extract for 2nd and 4th instar larvae were 2519.6 and 3017.7 ppm, respectively. The LC_{50} and LC_{90} values of extract during pupicidal activity were 1307.7 and 2257.9 ppm, respectively. During CDC bottle bioassay, mosquito adult knockdown was observed after exposure to various concentrations (0.15% to 1.25%) of *C. nardus* n-hexane extract. The KDT_{50} values at highest (1.25 %) and lowest (0.15%) concentrations were 100.9 and 184.2 minutes, respectively. After 24 hours exposure period, the highest extract concentration (1.25%) caused 62.5±6.5% mortality. During filter paper impregnation bioassay, the KDT_{50} value at highest concentration (0.13 mg/cm^2) was 94.4 minutes and at lowest concentration (0.017 mg/cm^2) was 178.9 minutes. KDT_{90} values at these concentrations were 176.7 and 290.1 minutes, respectively. After 24 hours of recovery period, the filter papers impregnated with highest extract concentration (0.13mg/cm2) caused 60±8.2% mortality.

Keywords: 2nd and 4th instars, pupae, adults, knock-down, mortality.

INTRODUCTION

Culex quinquefasciatus (Say.) is a culicine mosquito species, breed in a wide range of larval habitats (Muturi *et al*. 2006) and is the vector of *Wuchereria bancrofti*. *W. bancrofti* is the lymphatic filarial parasite of humans that causes lymphatic filariasis commonly known as elephantiasis, affecting more than 100 million people in Asia, Africa, Central- and South America and the Pacific (Simonsen, 2009). Beg *et al*. (2001) reported confirmed cases of tropical pulmonary eosinophilia in indigenous patients however this disease is very rare in Pakistan. In Pakistan, *Cx. quinquefasciatus* is the most abundant and widely distributed mosquito species (Ilahi and Suleman, 2013). Being abundant, *Cx. quinquefasciatus* causes serious nuisance through its irritating biting. Control of mosquitoes is essential and several synthetic insecticides have been used for decades. Synthetic insecticides have created serious problems including the development of insecticide resistance and undesirable effects on humans, mammals, and other non-target organisms (Lee *et al*., 2001). Being effective, easily degradable and eco-friendly, plant-based insecticides have got attention (Jacobson, 1941). During the present research, the insecticidal efficacy of *Cymbopogon nardus* (Linn.) was studied against *Cx. quinquefasciatus*. *C. nardus* belongs to the genus *Cymbopogon*, locally known as Sargarai and its essential oil is used in the production of citronella, food, drink, perfumery, soap, body care products and pharmaceutical products (Wei and Wee, 2013). Many studies have reported the antifungal and antimicrobial property of *C. nardus* essential oil (Oussalah *et al*., 2006). The essential oil of *C. nardus* possesses efficient insecticidal activity against stored food products beetles and its analysis has shown the presence of citronellal, citronellol, geraniol, elemol and limonene (Doumbia *et al*., 2014).

MATERIALS AND METHODS

Preparation of non-polar solvent extract and Susceptibility bioassays

Whole plant of *Cymbopogon nardus* (Linn.) was collected from hill terrains in surrounding of Chakdara, Dir Lower and was authenticated by an expert in plant taxonomy and converted into powder form by electric grinder. Non-polar compounds are more penetrating to the cuticle of insects than polar compounds, therefore n-hexane; a non-polar solvent was used for the extraction as it would preferably dissolve non-polar constituents (Khader, 1999). Extract in paste form was obtained and stored at 4ºC for use in various mosquitocidal bioassays. Larvae of *Cx. quinquefasciatus* were collected from breeding sites and reared till adulthood in 500ml plastic jars in the Entomology laboratory at the University of Malakand. Yeast and dog biscuit in 2:1 ratio were provided to the larvae as food. The adults obtained were initially provided with 10% sucrose and later blood fed periodically by allowing mice for eggs development. Larvae, pupae and

*Corresponding author: e-mail: ikramilahi@yahoo.com

adults were obtained for experiments. The bioassays for susceptibility of larvae and pupae of *Cx. quinquefasciatus* were performed by taking guidance from WHO standard procedures (WHO, 1981a). 4000 mg of n-hexane extract was dissolved in 32ml acetone and emulsified through addition of 2ml Tween 80 and then tap water was added to obtain 2000 ml stock solution of 2000 ppm. 100ml solution each of 1000, 500, 250, 125, 62 and 31.5 ppm was prepared in disposable plastic boxes of 200 ml volume. Control solutions for each treatment were also prepared. Twenty 2nd and 4th instar larvae and pupae of *Cx. quinquefasciatus* were separately put into each plastic box. The experiment was conducted in four replicates. The percent mortalities of larvae and pupae were calculated after 24 hours of exposure period.

Susceptibility of Cx. quinquefasciatus adults exposed to n-hexane extract of C. nardus

The susceptibility of *Cx. quinquefasciatus* to n-hexane extract of *C. nardus* was studied by two procedures; (1) Centres for Disease Control and Prevention (CDC) bottle bioassay and (2) filter paper impregnation bioassay. During CDC bottle bioassay, stock solutions of 1.25, 0.625, 0.31 and 0.15% concentrations were prepared. 1 ml of stock solution of each concentration was poured into CDC bottles and immediately sealed with caps. Only 1 ml of acetone was added to the control bottle. Each bottle was gently rotated to swirl the solution and thus all the insides of the bottle become coated with the solution. The caps were removed and the bottles were rolled continuously to make the insides of bottles dry. Bottles were covered with aluminum foil for protection from light and left for 24 hours in horizontal position. After the bottles became dry completely, 20 adult blood starved and glucose fed female mosquitoes (2-5 days old) were aspired out through mouth aspirator and introduced into each test bottle including the control bottle. Mosquitoes knock-down was recorded after every 15 minutes for 90 minutes. Percent mortality was noted after 24 hours. During filter paper impregnation bioassay, the susceptibility of *Cx. quinquefasciatus* adults exposed to filter papers impregnated with various concentration (0.15 % to 1.25%) of n-hexane extract of *C. nardus* was studied by following WHO protocol (WHO, 1981). 2ml of each solution was applied on Whatman no.1 filter papers of 12 x15 cm size and impregnated filter papers with 0.13, 0.069, 0.034 and 0.017mg/cm^2 concentration of extract were prepared. Control filter papers were also arranged by applying 2 ml of acetone only. 20 female blood starved glucose fed and 2 to 5 days old mosquitoes were introduced into the holding tube. After 1 hour of acclimation, the mosquitoes were then exposed to test paper in exposure tubes for 90 minutes. Mosquitoes knock-down was recorded at 15 minutes intervals. Mosquitoes were then transferred back to the holding tube at the end of exposure period and kept for a recovery period of 24 hours. A cotton pad soaked in 10 % glucose solution was placed on the mesh screen. After 24 hours recovery period, mortality of mosquito was recorded.

Analysis of data

The percent mortality was presented as mean and standard deviation of mean of four replicates. Percent mortality in treatments was corrected by applying Abbott's formula (Abbott, 1925) if 5% or more than 5% mortality was observed in control. R square values were calculated by applying linear regression test in Microsoft Excel 2010. LC_{50} and $LC_{90,}$ KDT_{50} and KDT_{90} values were calculated by applying log probit analysis test (Finny, 1971).

RESULTS

Larval and pupal susceptibility

Table 1 shows the susceptibility of *Cx. quinquefasciatus* larvae (2nd and 4th instars) and pupae after 24 hours exposure to various concentrations (31.2 to 1000 ppm) of *C. nardus* n-hexane extract. The highest concentration of extract caused 77.5±11.9% mortality of 2nd instar larvae whereas the lowest concentration caused 3.8±4.8% mortality of 2nd instar larvae. Similar trend was observed for 4th instar larvae. LC_{50} values of extract for 2nd and 4th instar larvae were 451.8 and 599.6 ppm respectively. The LC_{90} values of extract for 2nd and 4th instar larvae were 2519.6 and 3017.7 ppm respectively. During the susceptibility study of pupae, the highest concentration caused 30±8.2% mortality whereas the lowest concentration of extract caused no pupal mortality with. The R^2 value was 0.96. The LC_{50} and LC_{90} values of extract during pupicidal activity were 1307.7 and 2257.9 ppm respectively.

Adult susceptibility

The susceptibility of adult *Cx. quinquefasciatus* during exposure to various concentrations (0.15 % to 1.25 %) of *C. nardus* n-hexane extract was studied by CDC bottle bioassay and impregnated filter paper bioassay (table 2). During CDC bottle bioassay, the KDT_{50} values of the extract solution at 1.25%, 0.625%, 0.31% and 0.15% concentrations were 100.9, 121.9, 140.4 and 184.2 minutes respectively. The KDT_{90} values at 1.25%, 0.625 %, 0.31% and 0.15 % concentrations were 194.9, 223.3, 233.5 and 312.9 minutes, respectively. The mean percent mortality after 24 hours exposure period at 1.25%, 0.625 %, 0.31% and 0.15% concentrations were 62.5±6.5, 47.5 ±11.9, 32.5±2.9 and 22.5±2.9, respectively. During filter paper impregnation bioassay, the KDT_{50} values of 0.13 mg/cm^2, 0.069 mg/cm^2, 0.034 mg/cm^2 and 0.017mg/cm^2 impregnated filter papers were 94.4, 124.6, 147.2 and 178.9 minutes, respectively whereas the KDT_{90} values were 176.7, 200.9, 249.4 and 290.1 minutes respectively. The mean percent mortality after 24 hours of recovery period were 60±8.2, 47.5±14.4, 30±9.1 and 20±4.1 for 0.13, 0.069, 0.034 and 0.017 mg/cm^2 impregnated filter papers, respectively.

Table 1: Susceptibility of *Cx. quinquefasciatus* larvae after exposure to various concentration of n-hexane extract of *C. nardus* for 24 hours

Larval Stage	%Mortality						R^2	LC_{50} (L-U)	LC_{90} (L-U)
	Concentration (ppm)								
	1000	500	250	125	62.5	31.2			
2st	77.5± 11.9	46.3 ± .3	33.8 ±6.3	16.3 ±2.5	6.3 ±2.5	3.8 ± 4.8	0.96	451.8 (380.3-549.6)	2519.6 (1788.7-3990.3)
4^{th}	71.3± 6.3	41.3 ±4.9	26.3 ±6.4	12.5 ± 5	6.3 ±6.2	1.3 ±2.5	0.98	599.6 (467.8-693.7)	3017.7 (2097.5-4960.2)
Pupae	30.0± 8.2	20.0 ±8.1	8.7 ±2.5	7.5 ±5.0	3.8 ±4.7	0.0 ±0.0	0.96	1307.7 (946.6-2600.7)	2257.9 (1566.6-4947.3)

L-U stands for lower and upper bound of 95% Confidence Limits for extract concentration

Table 2: Susceptibility of *Cx. quinquefasciatus* adults exposed to various concentration of n-hexane extract of *C. nardus* during CDC bottle and filter paper impregnation bioassays

Bioassay	Conc	KDT_{50} (L-U)	KDT_{90} (L-U)	24 hour % Mortality
CDC bottle	1.25%	100.9 (93.7110.6)	194.9 (175.2-221.9)	62.5±6.5
	0.625%	121.9 (111.0- 137.4)	223.3 (197.1-261.4)	47.5±11.9
	0.31%	140.4 (123.2-168.8)	233.5 (197.8-293.4)	32.5±2.9
	0.15%	184.2 (156.0-233.8)	312.9 (256.9-412.6)	22.5±2.9
Filter paper	1.25 mg/cm^2	94.4 (88.6 – 101.7)	176.7 (161.3-197.1)	60±8.2
	0.625 g/cm^2	124.6 (115.1-137.7)	200.9 (180.9-228.5)	47.5±14.4
	0.31 mg/cm^2	147.2 (131.1-171.4)	249.4 (215.9-300.5)	30±9.1
	0.15 mg/cm^2	178.9 (147.7-242.1)	290.1 (230.8-411.6)	20±4.1

L-U stands for lower and upper bound of 95% Confidence Limits for time

DISCUSSION

C. nardus belong to the family Poacea and its essential oil is known for its mosquito repellent property (Fradin, 1998) and is toxic against the cowpea weevil (Ketoh, 2000). Doumbia *et al*. (2014) reported that essential oil of *C. nardus* caused higher mortality of stored food products insect pests. Other plants of the genus *Cymbopogon* that possess insecticidal activity include *C. martini*, *C. citratus* (Hernandez-Lambraño *et al*., 2015). During the present research, the n-hexane extract of *C. nardus* showed efficient larvicidal and pupicidal activity against *Cx. quinquefasciatus*. LC_{50} values of extract for 2^{nd} and 4^{th} instar larvae were 451.8 and 599.6 ppm, respectively. The LC_{90} values of extract for 2nd and 4th instar larvae were 2519.6 and 3017.7 ppm, respectively. From this data it is evident that 2^{nd} instar larvae were more susceptible than 4^{th} instar larvae. Similar trend has been reported (Abdalla *et al*., 2009). The LC_{50} and LC_{90} values of extract for pupicidal activity were 1307.7 and 2257.9 ppm, respectively. These data indicate that pupae were not as susceptible to extract solution as the larvae. The possible reason is the presence of much heavier cuticle in pupae than larvae. Such trend can be seen in the results of research work of other researchers (Jayanthi *et al*., 2012).

During the present research, the knockdown and mortality of *Cx. quinquefasciatus* adults exposed to various concentration of *C. nardus* n-hexane extract were studied by applying filter paper impregnation and CDC bottle bioassays. During both bioassays, the highest concentration caused maximum knockdown of mosquitoes. During CDC bottle bioassay, the KDT_{50} and KDT_{90} values for highest concentration (1.25%) of *C. nardus* n-hexane extract were 100.9 and 194.9 minutes, respectively. After 24 hours exposure period, 62.5 ± 6.5 % adults showed mortality. During filter paper impregnation bioassay, the KDT_{50} and KDT_{90} values for filter papers impregnated with highest extract concentration (0.13 mg/cm^2) were 94.4 and 176.7 minutes respectively. After 24 hours recovery period, 60±8.2% adult mosquitoes showed mortality. Ajaegbu *et al*. (2016) reported the efficient adulticidal activity of the leaf extracts of *Spondias mombin* (Linn.) against *Aedes aegypti* (Linn.) by conducting CDC bottle bioassay. Dua *et al*. (2010) reported the adulticidal potential of essential oil of *Lantana camara* leaves against mosquitoes by applying filter paper impregnation bioassay.

The larvicidal/pupicidal and adulticidal potential of plant extracts is due to the presence of secondary metabolites. The essential oil of *C. nardus* is mainly composed of monoterpenes, sesquiterpenes and alcohols which are responsible for insecticidal property. The presence of citronellal, citronellol, geraniol, elemol and limonene in essential oil of *C. nardus* has been reported (Doumbia *et*

al., 2014). The potent mosquitocidal activity of *C. nardus* n-hexane extract may be attributed to the presence of such phytochemicals.

CONCLUSION

From the finding of the present research it is concluded that the n-hexane extract of *C. nardus* has good larvicidal, pupicidal and adulticidal activity against *Cx. quinquefasciatus* mosquito.

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