Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in

September 2009, 30(5) 883-888 (2009)
For personal use only
Commercial distribution of this copy is illegal

Evaluation of larvicidal efficacy of *Solanum xanthocarpum* storage against vector mosquitoes in north - western Rajasthan

S.K. Bansal*[1], Karam V. Singh[1] and M.R.K. Sherwani[2]

[1]Desert Medicine Research Centre (ICMR), New Pali Road, Jodhpur - 342 005, India
[2]Department of Chemistry, JNV University, Jodhpur - 342 005, India

(Received: June 11, 2008; Revised received: October 08, 2008; Accepted: October 11, 2008)

Abstract: *Efficacy of the methanol extracts from fruits without seeds, whole yellow ripe fruits and seeds of the plant Solanum xanthocarpum was evaluated against larvae of Anopheles culicifacies, Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus, the important vector mosquitoes prevalent in the arid region. Experiments were carried out on late 3rd or early 4th instar larvae of these mosquitoes using standard WHO technique. 24 and 48 hr LC_{50} and LC_{90} values along with their fiducial limits, regression equation, chi-square (x^2)/ heterogeneity of the response have been determined by log probit regression analysis. The 24 hr LC_{50} values as observed for fruit without seeds, whole fruits and seeds after six months were 79.6, 91.7 and 131.7; 131.4, 186.9 and 195.6; 273.4, 290.9 and 377.6 and 384.9, 450.6 and 520.0 mgl^{-1} for An. culicifacies, An. stephensi, Ae. aegypti and Cx. quinquefasciatus respectively. However, the 24 hr LC_{50} values for fresh yellow ripe fruits and seeds determined previously were 51.6 and 66.9; 52.2 and 73.7; 118.3 and 123.8 and 157.1 and 154.9 mgl^{-1} for the above four vector species respectively showing that the efficacy was decreased two to three times more after six months of storage. The 24 hr LC_{50} values were also determined after one year of storage for fruit without seeds, whole fruits and seeds. The values were 103.3, 120.1 and 195.7; 146.3, 224.3 and 251.2; 316.9, 336.5 and 426.2 and 393.0, 500.3 and 656.7 mgl^{-1} as compared to the 24 hr LC_{50} values of 47.4,51.6 and 66.9; 52.3,52.2 and 73.7; 108.0,118.3 and 123.8 and 141.1, 157.1 and 154.9 mgl^{-1} of all the three fresh preparations for the above four vector species respectively showing that the efficacy was further decreased after one year of storage. Further the extracts should be used when they have been prepared from the fresh plant parts instead of the old stored parts. Larvae of anophelines were found more susceptible as compared to culicine. HPLC and IR studies showed that active component might be present in the fruits and seeds of this plant species. The study would be of great importance while planning vector control strategy based on alternative plant derived insecticides.*

Key words: *Larvicidal, Solanum xanthocarpum, Vector mosquitoes, Arid region*
PDF of full length paper is available online

Introduction

Among the arthropods, mosquitoes are responsible for many dreaded diseases than any other group of insects. Mosquito-borne diseases, such as malaria, filariasis, dengue / dengue haemorrnagic fever (DHF), yellow fever and Japanese encephalitis contribute significantly to morbidity and mortality in many tropical and sub-tropical regions of the world. However, control of malaria and other mosquito-borne diseases is becoming increasingly difficult because the effectiveness of vector control has declined due to the development of resistance in mosquitoes against currently used insecticides in vector control operations (Chandre *et al*., 1998). Secondly, the synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Therefore, there is a need to find out alternatives to these synthetic insecticides. Plants may be an alternative source of mosquito-control agents because they constitute a rich source of bioactive chemicals, which inhibit growth (Sharma *et al*., 2006), development and metamorphosis of insects (Mwangi and Rembold, 1986; Sukumar *et al*., 1991). Much effort has been focused on phytochemicals and their essential oils (Dharmagadda *et al*., 2005) as potential sources of mosquito-control agents as they are environment friendly and easily biodegradable.

Anopheles culicifacies and *An. stephensi* are the important vectors of malaria while *Aedes aegypti* and *Culex quinquefasciatus* are the vectors of dengue and filariasis respectively. All these mosquito species have been identified as primary vectors of the above diseases in this arid region (Bansal and Singh, 1993; Bansal *et al*., 1994). Hence, their control either by biological or chemical means is the basic requirement for planning an effective vector control strategy. Although several studies have been done on the insecticide susceptibility status of adult (Bansal and Singh, 1995, 1996) and larval (Bansal and Singh, 2004, 2006, 2007) mosquitoes in this region, the level of susceptibility of their larvae to different phyto-derivatives after different period of storage is not yet assessed.

Materials and Methods

Solanum xanthocarpum/ S. surrattense/ S. virginianum (family: solanaceae), the 'Indian nightshade' is widely distributed throughout the country but more abundantly in the arid region. It was collected from different habitats in and around district Jodhpur and help from Botanical Survey of India (BSI), Jodhpur was taken

* **Corresponding author:** bansalshivk@hotmail.com

Online Copy

for identification after depositing the sample specimen in the herbarium. The samples of fruits were washed with distilled water and shade dried separately at a temperature between 30-40°C for 10-15 days. Dried raw fruits were stored for 6 and 12 months for doing the experiments after different period of storage. Seeds were taken out from the dried fruits, powdered separately and stored at a temperature range of 15-20°C. 100 g of the powdered material each from whole fruits, seeds and fruit without seeds was extracted separately using 400 ml of methanol at room temperature for two days with constant stirring and finally filtered, air dried and weighed. The percent extraction yield for whole fruits, fruit without seeds and seeds was 10.5, 9.0 and 7.5% respectively. Stock (5 g /50 ml) from the residual extracts was prepared in ethanol and test concentrations from 25-400 mg l^{-1} were used during the experiments.

Bioassays on different mosquito larvae were done as per the method described by WHO (1981). Serial dilutions of the stock solution were prepared and added to 249 ml of tap water in a 500 ml beaker to obtain the test concentrations. Different larval stages from 1st to 4th instar were reared in the laboratory and used for the efficacy tests. 20-25 healthy late 3rd or early 4th instar larvae were kept in different test concentrations and mortality noted after 24 and 48 hr. Controls with the same amount of solvent were kept side by side. All treatments were replicated 3-4 times and carried out at a room temperature of 28±2°C and Relative Humidity of 75±5%. The percent corrected mortality was calculated using Abbott's formula (Abbott, 1925), if mortality was between 5-20% in control experiments. The LC_{50} and LC_{90} values were computed using probit regression analysis (Finney, 1971). For HPLC and Infra Red (IR) spectroscopic studies, cleaned and dried seeds were powdered and a known amount was extracted with petroleum ether (40-60°C) as a solvent. The solvent was evaporated and the oil so obtained was subjected to saponification using KOH and ethanol as solvent for the preparation of mixtures of fatty acids. The fatty acids were subjected to esterification using diethyl ether. The upper organic layer was removed and methyl ester was obtained after drying on Na_2SO_4 that was finally subjected to HPLC and IR analysis in sophisticated analytical instrument facility at CDRI, Lucknow.

Results and Discussion

The result of the relative efficacy of different extracts from fruits and seeds of *S. xanthocarpum* after six and twelve months of storage with all the four vector mosquitoes are given in Tables 1-4 along with the results of HPLC and IR studies (Fig. 1, 2). Efficacy tests carried out with methanol extracts of fruits without seeds, whole fruits and seeds after six months of storage showed that these were quite effective to all the four mosquito species *viz. An. culicifacies, An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus,* the 24 hr LC_{50} being 79.6, 91.7 and 131.7; 131.4, 186.9 and 195.6; 273.4, 290.9 and 377.6 and 384.9, 450.6 and 520.0 mgl^{-1} respectively (Tables 1- 3). These values are 2-3 times higher when compared with the LC_{50} values obtained by us with fresh fruits of this plant species (Bansal *et al*., 2009) showing that efficacy goes down with the period of storage. 48 hr LC_{50} value was much less as compared to the 24 hr LC_{50} value indicating that efficacy was much pronounced after 48 hr showing its delayed activity. *An. culicifacies* was found to

Table - 1: Efficacy of methanol extracts of S. *xanthocarpum* (fruit without seeds) on different mosquito larvae after 6 and 12 months of storage

Mosquito species	Regression coefficient (Slope)	Regression equation	Intercept	Chi-square (D.F)	LC_{50} values 24 hr (Fiducial limits)	48 hr	LC_{90} (Fiducial limits)
Larvicidal efficacy after 6 months of storage							
An. culicifacies	2.83	Y=-0.52+2.83x	-0.52	0.72(2)	79.6 (70.3-114.1)	60.0	253.8 (157.4-409.2)
An. stephensi	2.63	Y=-0.57+2.63x	-0.57	1.92(2)	131.4 (100.7-171.2)	67.3	402.9 (217.3-746.9)
Ae. aegypti	2.17	Y=-0.29+2.17x	-0.29	3.32(2)	273.4 (196.0-381.4)	108.7	1063.0 (490.5-2305.0)
Cx. quinque-fasciatus	1.82	Y=-0.28+1.82x	-0.28	3.13(2)	384.9 (256.8-576.9)	150.0	1936.0 (730.0-3140.0)
Larvicidal efficacy after 12 months of storage							
An. culicifacies	2.31	Y=-0.56+2.31x	-0.56	0.81(2)	103.3 (62.2-111.5)	62.3	297.6 (167.2-563.3)
An. stephensi	2.28	Y=-0.06+2.28x	-0.06	1.28(2)	146.3 (115.2-185.8)	80.4	533.1 (303.0-938.0)
Ae. aegypti	1.70	Y=-0.74+1.70x	-0.74	5.71(2)	316.9 (216.2-466.5)	126.0	1091.0 (600.0-2358.0)
Cx. quinque-fasciatus	3.01	Y=-2.81+3.01x	-2.81	0.99(2)	393.0 (308.4-500.7)	222.2	1046.0 (593.1-1844.0)

All values of LC_{50} and LC_{90} along with their fiducial limits are in mg l^{-1}, D.F. = Degree of freedom

Online Copy

Table - 2: Efficacy of methanol extracts of *S. xanthocarpum* (whole fruits) on different mosquito larvae after 6 and 12 months of storage

Mosquito species	Regression coefficient (Slope)	Regression equation	Intercept	Chi-square (D.F)	LC_{50} values 24 hr (Fiducial limits)	48 hr	LC_{90} (Fiducial limits)
Larvicidal efficacy after 6 months of storage							
An. culicifacies	2.08	Y=0.92+2.08x	0.92	1.17(2)	91.7 (59.4-141.9)	66.5	379.0 (152.3-942.4)
An. stephensi	3.06	Y=-1.94+3.06x	-1.94	4.50(2)	186.9 (151.1-231.2)	87.9	490.3 (296.1-811.7)
Ae. aegypti	2.70	Y=-1.66+2.70x	-1.66	0.44(2)	290.9 (161.3-263.7)	144.1	865.8 (486.8-1539.0)
Cx. quinque-fasciatus	2.06	Y =-0.47+2.06x	-0.47	0.25(2)	450.6 (302.6-671.0)	220.4	1881.0 (676.9-3228.0)
Larvicidal efficacy after 12 months of storage							
An. culicifacies	1.93	Y=0.98+1.93x	0.98	0.61(2)	120.1 (89.7-161.4)	83.3	552.5 (230.5-1324.0)
An. stephensi	2.13	Y=-0.01+2.13x	-0.01	0.40(2)	224.3 (170.1-295.5)	152.5	893.5 (444.5-1796)
Ae. aegypti	2.18	Y=-0.51+2.18x	-0.51	2.00(2)	336.5 (260.4-434.8)	213.4	1301.0 (547.5-2090)
Cx. quinque-fasciatus	1.95	Y=-0.25+1.95x	-0.25	2.17(2)	500.3 (356.5-702.5)	331.8	2277.0 (835.4-3205)

All values of LC_{50} and LC_{90} along with their fiducial limits are in mg l^{-1}, D.F> = Degree of freedom

Table - 3: Efficacy of methanol extracts of *S. xanthocarpum* (seeds) on different mosquito larvae after 6 and 12 months of storage

Mosquito species	Regression coefficient (Slope)	Regression equation	Intercept	Chi-square (D.F)	LC_{50} values 24 hr (Fiducial limits)	48 hr	LC_{90} (Fiducial limits)
Larvicidal efficacy after 6 months of storage							
An. culicifacies	2.54	Y=-0.39+2.54x	-0.39	2.07(2)	131.7 (102.2-169.7)	79.3	419.9 (214.6-821.3)
An. stephensi	1.89	Y=0.80+1.89x	0.80	1.89(2)	195.6 (124.3-220.8)	95.6	787.4 (356.1-1741.0)
Ae. aegypti	2.01	Y=-0.19+2.01x	-0.19	0.94(2)	377.6 (252.2-565.4)	131.1	1633.0 (601.7-2434.0)
Cx. quinque-fasciatus	1.73	Y=-0.30+1.73x	-0.30	0.28(2)	520.0 (304.7-887.8)	222.7	2860.0 (782.7-3450.0)
Larvicidal efficacy after 12 months of storage							
An. culicifacies	2.13	Y=0.12+2.13x	0.12	1.02(2)	195.7 (145.8-262.7)	112.4	781.1 (352.2-1732)
An. stephensi	1.86	Y=0.53+1.86x	0.53	1.70(2)	251.2 (177.4-357.3)	172.4	1225.0 (442.6-2389)
Ae. aegypti	2.25	Y=-0.92+2.25x	-0.92	2.03(2)	426.2 (326.0-557.2)	252.0	1548.0 (760.7-3280)
Cx. quinque-fasciatus	1.78	Y=-0.02+1.78x	-0.02	1.69(2)	656.7 (432.8-996.8)	431.5	2440.0 (1134-3430)

All values of LC_{50} and LC_{90} along with their fiducial limits are in mg l^{-1}, D.F. = Degree of freedom

Online Copy

Table - 4: Comparative 24 and 48 hr LC_{50} values (mg l^{-1}) of extracts from fruits and seeds of *S. xanthocarpum* to larvae of mosquito vectors after different period of storage

Mosquito species	Fruit without seeds 24 (48 hr) LC_{50}			Whole fruit 24 (48 hr) LC_{50}			Seeds 24 (48 hr) LC_{50}		
	FP	6 m	12 m	FP*	6 m	12 m	FP*	6 m	12 m
Anopheles culicifacies	47.4	79.6 (60.0)	103.3 (62.3)	51.6	91.7 (66.5)	120.1 (83.3)	66.9	131.7 (79.3)	195.7 (112.4)
Anopheles stephensi	52.3	131.4 (67.3)	146.3 (80.4)	52.2	186.9 (87.9)	224.3 (152.5)	73.7	195.6 (95.6)	251.2 (172.4)
Aedes aegypti	108.0	273.4 (108.7)	316.9 (126.0)	118.3	290.9 (144.1)	336.5 (213.4)	123.8	377.6 (131.1)	426.2 (252.0)
Culex quinquefasciatus	141.1	384.9 (150.0)	393.0 (222.2)	157.1	450.6 (220.4)	500.3 (331.8)	154.9	520.0 (222.7)	656.7 (431.5)

FP = Fresh preparation, 6 m = Six months storage, 12 m = Twelve months storage, * = Data from Bansal *et al*. (2009)

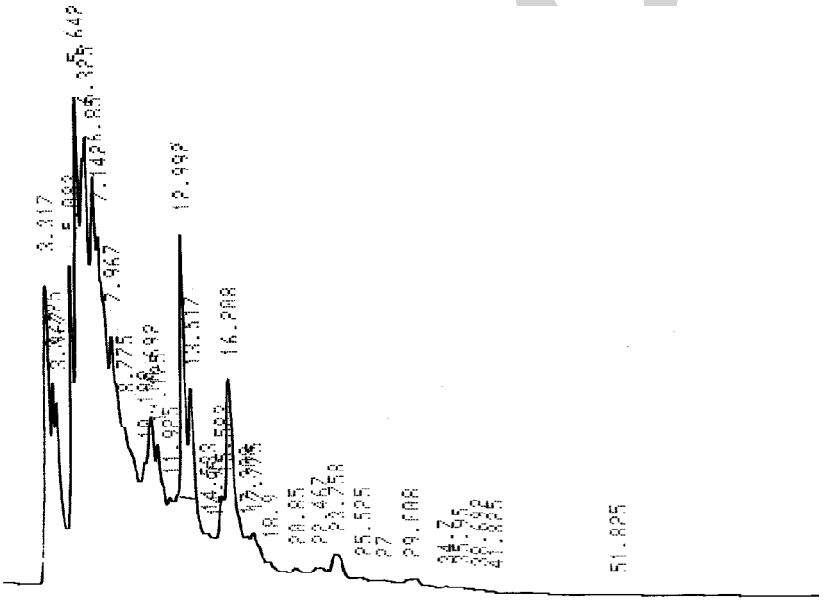

Fig. 1: HPLC analysis of extracts of seeds of *S. xanthocarpum*

be the most susceptible followed by *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus.* Experiments were also carried out with methanol extracts of these samples after twelve months of storage (Tables 1- 3), the 24 hr LC_{50} being 103.3, 120.1 and 195.7; 146.3, 224.3 and 251.2; 316.9, 336.5 and 426.2 and 393.0, 500.3 and 656.7 mgl^{-1} for the above four mosquito species respectively, showing that the efficacy has gone down 2-3 times as compared to the fresh extracts (Table 4). Anophelines were found much more susceptible as compared to the culicines, the efficacy being almost double the culicines. Efficacy was found almost comparable in the three different samples, although a little bit less in seeds, suggests that active larvicidal component may be present only in the fruits and seeds of this plant species. Singh and Bansal (2003) and Bansal *et al.* (2009) also observed that extracts from fresh green and yellow fruits of this plant were very much effective to the vectors of malaria and dengue. Mohan *et al.* (2005) also observed that the fruits of this plant were very effective against the larvae of *An. stephensi* (24 hr LC_{50} of CCl_4 extract being 5.1ppm) and *Cx. quinquefasciatus* (24 hr LC_{50} of petroleum ether extract being 62.2 ppm). They also observed that the petroleum ether root extracts of this plant showed synergistic effects with cypermethrin when evaluated against larvae of *Cx. quinquefasciatus* and *An. stephensi* (Mohan *et al*., 2007). Sukumar *et al*. (1991) have also stated the existence of variations in the toxicities of phytochemicals on target species *vis-à-vis* plant parts from which they are extracted, species response to the specified extract, solvent of extraction, geographical origin of the plant,

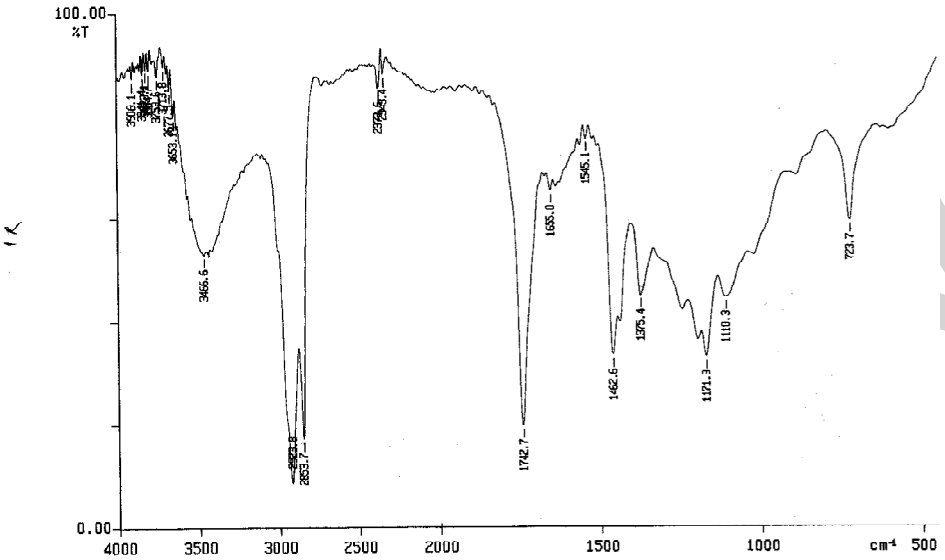

Fig. 2: Infra red spectroscopic analysis of extracts from seeds of *S. xanthocarpum*

photosensitivity of some of the compounds in the extract and effect on growth (Sharma *et al*., 2006) and reproduction.

Results of this study with extracts from different parts of this plant have exhibited variable toxicity to all the four vector species and warrants further investigations on its active components. HPLC and Infra Red spectroscopic analysis carried out on fresh seeds suggest that it contains total saturated acids (30.16%) (palmitic acid, 8.58; stearic acid, 18.44 and aracidic acid, 3.14%), total unsaturated fatty acids (66.02%) (oleic acid, 28.74, linoleic acid, 32.96 and linolenic acid, 4.32%), polyunsaturated fatty acids (PUFA) (37.28%) (linoleic acid, 32.96 and linolenic acid, 4.32%), and hydroxy acid (03.72%). All the above components are present in edible oils (Deora *et al*., 2003) except the hydroxy group that may contain the larvicidal components which requires further elution by chromatography and NMR studies. Keeping in view the above variations, it will be of importance to study the variations in toxic effects of extracts and also to characterize the active ingredients which either alone or in combination is responsible for the toxicity in different plants (Jacobson, 1989; Sivagnaname and Kalyanasundaram, 2004). Various compounds including phenolics, terpenoids and alkaloids exit in plants (Wink, 1993) which may jointly or independently contribute to the generation of larvicidal activities in mosquitoes (Hostettmann and Potterat, 1997).

The findings of the present study suggest that chemical composition of extracts from different parts of the same or different plants may differ and require thorough understanding of the active ingredients present in these plants. Further work on these plant-derived derivatives is needed for developing them into effective formulations to be utilized in integrated vector control measures through community participation. This plant is easily available to the local people and being an ayurvedic herb with multiple medicinal properties is easily acceptable to them, since during application it would neither cause any toxic effects nor any additional economic burden. Further research is in progress to identify the key components present in this plant species.

Acknowledgments

The authors are grateful to Officer-In-Charge, DMRC, Jodhpur for providing necessary research facilities for the present investigations. They are also thankful to Dr. R. S. Sindal, former Head, Department of Chemistry, JNV University, Jodhpur for providing facilities for chemical analysis of the samples. Sophisticated Analytical Instrument Facility at CDRI, Lucknow is thankfully acknowledged for HPLC and IR spectroscopic analysis of the samples.

References

Abbott, W.S.: A method of computing the effectiveness of an insecticide. *J. Economic Entomol.,* **18**, 265-267 (1925).

Bansal, S.K. and Karam V. Singh: Prevalence and seasonal distribution of anopheline fauna in district Bikaner (Rajasthan). *Ind. J. Malariol*., **30**, 119-125 (1993).

Bansal, S.K., V. Joshi and Karam V. Singh: A survey of the mosquito fauna with special reference to vectors of Japanese Encephalitis (JE) in district Bikaner. Part I - The culicine fauna. *Proc. Acad. Environ. Biol*., **3**, 9-15 (1994).

Bansal, S.K. and Karam V. Singh: Susceptibility status of two species of Japanese encephalitis vectors to insecticides in the Thar Desert, district Bikaner (Rajasthan). *Ind. J. Med. Res.,* **101**, 190-192 (1995).

Bansal, S.K. and Karam V. Singh: Insecticide susceptibility status of anophelines in district Bikaner, Rajasthan. *Ind. J. Malariol*., **33**, 1-6 (1996).

Bansal, S.K. and Karam V. Singh: Efficacy of different organophosphate and synthetic pyrethroid insecticides to the larvae of malaria vector *Anopheles stephensi*, Liston. *J. Environ. Biol*., **25**, 485-488 (2004).

Bansal, S.K. and Karam V. Singh: Laboratory evaluation for comparative insecticidal activity of some synthetic pyrethroids against vector mosquitoes in arid region. *J. Environ. Biol*., **27**, 251-255 (2006).

Bansal, S.K. and Karam V. Singh: Relative susceptibility of some common mosquito vector larvae to synthetic insecticidal compounds in north-western Rajasthan. *J. Environ. Biol*., **28**, 829-832 (2007).

Bansal, S.K., Karam V. Singh and Suresh Kumar. Larvicidal activity of the extracts from different parts of the plant *Solanum xanthocarpum* against important mosquito vectors in the arid region. *J. Environ. Biol.*, **30**, 221-226 (2009).

Chandre, F., F. Darriet, M. Darder, A. Cuany, J. M.C. Doannio, N. Pasteur and P. Guillet: Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. *Med. Vet. Entomol.*, **12**, 359-366 (1998).

Deora, M.A., P. Jaiswal, A. Mathur and M.R.K. Sherwani: Fatty acid composition of some minor seed oils from arid zone of Rajasthan. *J. Ind. Chem. Soc*., **80**,141-142 (2003).

Dharmagadda, V.S.S., S.N. Naik, P.K. Mittal and P. Vasudevan: Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. *Bioresource Technol.*, **96**, 1235-1240 (2005).

Finney, D.J.: Probit Analysis, 3rd Edn., Cambridge Univ. Press,Cambridge, London (1971).

Hostettmann, K. and O. Potterat: Strategy for the isolation and analysis of antifungal, molluscicidal, and larvicidal agents from tropical plants. *In*: Phytochemicals for pest control (*Eds*.: P.A. Hedin, R.M. Hollingworth, E.P. Masler, J. Miyamoto and D.G. Thompson). ACS Symp. Ser. No. 658, Am. Chem. Soc., Washington D.C., USA. pp. 14-26 (1997).

Jacobson, M.: Botanical pesticides: Past, present, and future. *In*: Insecticides of plant origin (*Eds*.: J.T. Arnason, B.J.R. Philogene and P. Morand). ACS Symp. Ser. No. 387, Am. Chem. Soc., Washington D.C., USA. pp. 1-10 (1989).

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

Mohan, L., P. Sharma and C.N. Srivastava: Comparative efficacy of *Solanum xanthocarpum* extracts alone and in combination with a synthetic pyrethroid, cypermethrin, against malaria vector, *Anopheles stephensi*. *Southeast Asian J. Trop. Med. Public Hlth*., **38**, 256-260 (2007).

Mwangi, R.W. and H. Rembold: Growth regulating activity of *Melia volkensii* extracts against the larvae of *Aedes aegypti*. Proc. 3rd Int. Neem Conf., Nairobi, Kenya. pp. 669-681 (1986).

Sharma, P., L. Mohan and C.N. Srivastava: Phytoextract-induced developmental deformities in malaria vector. *Bioresource Technol.*, **97**, 1599-1604 (2006).

Singh, Karam V. and S.K. Bansal: Larvicidal potential of a Perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/DHF. *Curr. Sci.,* **84**, 749-751 (2003).

Sivagnaname, N. and M. Kalyanasundaram: Laboratory evaluation of methanolic extract of *Atlantia monophylla* (Family: Rutaceae) against immature stages of mosquitoes and non-target organisms. *Mem. Inst. Oswaldo Cruz. Rio de Janerio*, **99**, 115-118 (2004).

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

WHO: Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides. WHO/VBC 81.807 (1981).

Wink, M.: Production and application of phytochemicals from an agricultural perspective. *In:* Phytochemistry and agriculture (*Eds*.: T.A. Van Beek and H. Breteler). Clarendon Press, Oxford, UK. pp. 171-213 (1993).

Online Copy