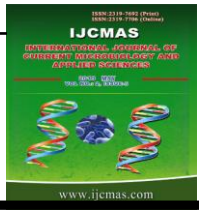
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 5 (2013) pp. 423-432**
http://www.ijcmas.com



Original Research Article

A Study on the Larvicidal Activity of Some Medicinal Plant Extracts From Western Uttar Pradesh India, Against the Filarial Vector, *Culex quinquefasciatus* Say

Amitabh Shad* and J. Andrew

Department of Zoology and School of Entomology, St. John's College, Agra, 282002, India

**Corresponding author*

A B S T R A C T

Mosquitoes are vector for various disease including malaria, yellow fever, filariasis Japanese encephalitis and chikungunya. *Culex quinquefasciatus* Say is known to transmit filariasis, Japanese encephalitis and other mosquito borne diseases. Harmful effects of using synthetic chemical insecticides in mosquito control including vector resistance, environmental pollution and health hazards have necessitated the current significance in the search for plant-based insecticide products that are environmentally safe and effective to control mosquito. As a part of a programme on possible utilization of indigenous plant extracts in vector management practices, ethanolic extracts of three plant species collected in the state of Uttar Pradesh, India, were tested for their larvicidal activity against the filarial vector, *Culex quinquefasciatus* Say. Leaves of *Tagetus minuta*, *Ageratum conyzoides and Jatropha curcas* were collected locally, shade dried and extracted in the soxhlet apparatus. The result revealed that the plant extracts of *Tagetus minuta, Ageratum conyzoides and Jatropha curcas* were promising larvicide to control mosquito larvae. *Tagetus minuta* ethanolic extracts showed highest larvicidal activity within the range of 0.05-0.25 mg/ml concentration. The results reported in the present study open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extract as larvicides to control mosquitoes.

Keywords

C.quinquefasciatus, Tagetusminuta, Ageratum conyzoides, Jatrophacurcas

Introduction

In many tropical and subtropical countries diseases transmitted by arthropods are among the major causes of illness and deaths (WHO, 1992). Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human health (WHO, 1998). The most common disease associated with mosquitoes are Japanese encephalitis, chikungunya, filariasis, malaria, dengue and yellow fever (Hag *et al.*, 1999) World health organization has declared the

mosquito as "public enemy number one" (WHO, 1996). Among these communicable diseases, Japanese encephalitis is endemic in about 21 countries (Service, 1983).It is the leading cause of viral encephalitis in Asia with 30000-50000 cases annually. In India, an estimated 378 million people are living at the risk of Japanese encephalitis in 12 states including Uttar Pradesh which is highly endemic contributing 52.40% of the total deaths due to Japanese encephalitis in the country (Gubler, 1998). Lymphatic filariasis commonly known as elephantiasis is considered as another major public health problem due to its morbidity and social stigma caused by the symptoms of this disease. It is the second most common vector borne parasitic disease after malaria and is found in 81 tropical and subtropical countries. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia and pacific islands. India alone contributes around 40% of global filariasis burden and the estimated annual loss is about 720 crore INR. The transmission of *Wuchereria bancrofti* by the mosquito *Culex quinquefasciatus* Say, accounts for 95% of the total lymphatic filariasis cases in India (WHO, 1996; Service, 1983; Gubler, 1998).*Culex quinquefasciatus* Say, a principle vector for filariasis and transmission of Japanese encephalitis is one among the cosmopolitan mosquitoes which occurs in human habitations in the tropics and subtropics of the world. Medically the most important species, *C. quinquefasciatus* breeds in dirty waters polluted with organic debris, soak pits, drains, ditches, septic tanks and other such places. *C. quinquefasiciatus* commonly rest indoors both before and after feeding and may also shelter in outdoor resting places (Service, 2000).

In the past several decades, a number of synthetic chemical insecticides have been developed and effectively used to control mosquitoes. Unfortunately the application of such chemical insecticides has resulted in long term harmful effects on the environment and non-target organism including human beings. In addition the management of these disease vectors using synthetic insecticides has failed in part due to their efficiency in attaining physiological resistance (ICMR, 2002).Majority of the mosquito control programs target on the larval stage in their breeding sites. (5,6) Medicinal plants are rich source of alternative agents for control of mosquitoes because they contain bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly (ICMR, 2003). Several plant extracts and isolated botanicals from some plant families have been evaluated for their promising larvicidal activity. Plant based products does not have any hazardous effects on ecosystem. Recent researches have proved the effectiveness of plant derived compounds, such as saponins, steroids, isoflavonoids, alkaloids and tannins as potential larvicides (ICMR, 2003; Perich *et al.,* 1994).

The present investigation was carried out to establish the larvicidal efficiency of a few medicinal plants belonging to western Uttar Pradesh namely, *Tagetus minuta, Ageratum conyzoides* and *Jatropha curcas*. *Tagetus minuta* also known as southern cone Marigold is an erect annual herb having slightly glossy green leaves. The under surface of their leaves bear a number of small punctate, multicellular glands which exudes a liquorice-like aroma when ruptured.

Tagetus minuta is rich in many secondary compounds including terpenes, flavonoids, thiophenes and aromatics (Perich *et al.,* 1994; Sharma and Saxena, 1994). *Ageratum conyzoides*, commonly called as 'Neelaphulna', is an annual herb having a wide range of chemical compounds including alkanoids, flavonoids, chromenes and benzofurans. Extracts and metabolites from this plant have been found to possess pharmacological, bactericidal and insecticidal properties (Ming, 1999; Vyas and Mulchandani, 1986; Weidenfield and Roeder, 1991). *Jatropha curcas* L. known as 'Physic nut' is currently used primarily for the production of biodiesel (Thomas *et al.,* 2008). Besides, it is also used as a medicinal plant. Most parts of the plant are used for the treatment of various human and veterinary ailments (Goel *et al.,* 2007).

The white latex serves as a disinfectant in mouth infections in children. Its latex contains alkaloids including jatrophine, jatropham and curcain with anti-cancerous properties (Kumar and Sharma, 2008). It is also used externally against skin diseases, piles and sores among the domestic livestock (Adebowale and Adedire, 2006). The leaves of *J. Curcas*also contain apigenin, vitexin and isovitexin which along with other factors enable them to be used against malaria, rheumatic and muscular pains (Thomas *et al.,* 2008). Antibiotic activity of *Jatropha*has been observed against organisms including *Staphylococcus aureus* and *Escherichia coli* (Matsuse *et al.,* 1998).

Materials and Methods

Screening and collection of plant materials

The medicinal plants namely *Tagetus minuta, Ageratum conyzoides and Jatropha curcas* were selected on the basis of ethanopharmacological information, aromatic smell and ethanobotanical literature surveyed. The leaves of selected plants were collected locally in and around Agra city (26° 44' N to 27° 25' N latitude; 77° 26' E to 78° 32' E longitude). The leaves were plucked early in the morning and brought to the laboratory in plastic sampling bags. The taxonomic identification was done with the help of experienced botanists at the Department of Botany, St. Johns College, Agra, India. The voucher specimens were numbered and kept in our research laboratory for further reference.

Preparation of plant extracts

The leaves of different plants were washed using tap water; shade dried for 7 – 10 days at room temperature (27-37 °C) during day time and powdered individually using electrical blender (Bajaj, India). Each powdered plant material was sieved using strainer (40 mesh). The obtained dried powder (200 grams) of each plant were extracted with 600 ml of Ethanol (Qualigens, Fine chemicals Mumbai, India) with a minimum of 8 hours up to 48 hours in Soxhlet apparatus at 55°C to 60 °C (Vogel, 1978). The extracts thus obtained, were filtered using Whatmann Filter Paper (12.5 cm) and concentrated using rotatory evaporator to remove ethanol. The solidified plant extracts thus obtained were stored in sterilized amber coloured bottles and maintained at 4°C in refrigerator.

Mosquito culture

The larvae and adults of the mosquito, *Culex quinquefasciatus* were collected from various mosquito genic regions of Agra. Sampling of larvae and pupae were done using aquatic dipnet. Egg rafts, if

any, were collected in the filter paper and kept in the specimen box. Collected larvae were numbered, recorded and identified using standard keys (Barraud, 1934).

The colony was maintained from the egg rafts kept in white enamelled breeding bowls 10 inches in diameter, 5 inches deep and half filled with dechlorinated tap water. The water in the breeding bowls, on the onset of the first instars larvae was supplemented with yeast at an interval of every twenty four hours, till the larvae transformed into pupae. Each bowl contained about 150- 200 larvae. The scum, if formed, was removed using a glass rod. Pupae were collected daily and transferred into empty 18″×18″×18″ mosquito breeding cages (Fig:1).

The metamorphosed adults were fed with 10 % sugar solution or honey soaked in a piece of cotton for three to four days. Each cage contained about 200♀ and 100♂ adults which were allowed to mate. The females were then given blood meals from a healthy but immobilized pigeon during nights on alternate days. For egg laying suitable containers half filled with water were introduced inside the breeding cages which contained fertilized females. Each container was lined with filter paper to prevent the egg rafts from adhering to the containers walls. The egg rafts were processed again to yield adults as described above. Deposited egg rafts were collected daily, numbered, marked and incubated for experimental usage.

Larvicidal bioassay

In the larvicidal bioassay early fourth instar larvae of C. *quinquefasciatus* were exposed to different concentration of extracts. Standard WHO (2005) protocol with slight modification was adapted for the study. 100 ml of tap water was taken in a series of 250 ml glass beakers. The measured amounts of extracts were dissolved in 1 ml of solvent (ethanol).

The dissolved plant extracts were added to the water in the beakers. A control was also maintained by adding 1 ml of solvent to 100 ml of water in beaker. Five replicates along with a control were run simultaneously with each trial. 10 fourth instar larvae of the mosquito *C. quinquefasciatus,* obtained from laboratory colonized mosquitoes of F_1 generation, were introduced in different test concentrations of plant extracts along with the control at room temperature.

The larvae were fed dried yeast powder (50mg/litre) on the water surface. The number of dead larvae at 24 hours, 48 hours and 72 hours were recorded. The dead larvae were removed soon after the mortality in order to prevent decomposition, which may cause rapid death of the remaining larvae. A total of three such trials were carried out. The corrected mortality was analysed using Abbot Formulae, if required (Abbott, 1925).The behavioural changes, if any, were also observed and recorded.

Statistical analysis

The collected data pertaining to the toxicity assay were analysed statistically using SPSS, MS Excel 07 and Sigmaplot 12.5 software.

Results and Discussion

The ethanolic extract of the medicinal plant *Tagetus minuta* was taken in five different concentration of 0.05, 0.10, 0.15, 0.20 and 0.25 mg/ml (Figure: 1,4). The result showed the larval mortality of 1.6,

3, 5.6 , 8.6 and 10 in 24 hours 5.6, 5.6, 7.3, 9.6 and 10 in 48 hours and 7, 6.6, 8.3, 9.6 and 10 in 72 hours (Figure:1,4). In control the larval mortality was record as 0, 0 and 1 for 24, 48 and 72 hours respectively. *Ageratum conyzoides* ethanolic extract was taken in five different concentrations of 2.50, 5.00, 7.50, 10.0 and 12.5 mg/ml. Control was also maintained. The result recorded the larval mortality of 2.6, 3, 4.9, 7 and 10 in 24 hours ,4, 5 , 7, 9 and 10 in 48 hours and 6, 8, 10, 10 and 10 in 72 hours of exposure to the respective concentration (Figure.2,5). The control did not record any larval mortality. The ethanolic extract of *Jatropha curcas* was taken in five different concentration of 0.20, 0.40, 0.80, 1.00 and 1.20 along with the control (Figure:3,6). The larval mortality was 1.6, 3.6, 5, 8.3 and 9.3 in 24 hours; 2, 5.6, 5.6, 10 and 10 in 48 hours and 2.3, 6.3, 9.6, 10 in 72 hours respectively for each concentration (Figure:3,6). In control the larval mortality was 0, 1 and 1 in 24, 48 and 76 hours of exposure respectively (Figure:3,6). In the present study, the ethanolic extract of *Tagetus minuta* plant recorded the highest larvcidal activity against the *C. quinquefasciatus* larvae while the plant extract of *Ageratum conyzoides* was least effective among all three plant extracts tested for the larvicidal efficacy against the fourth instar larvae of mosquito, *C. quinquefasciatus* (Figure:4,5&6). The 24 hours LC50 values of *Tagetus minuta* (0.14), *Ageratum conyzoides* (7.52) and *Jatropha curcas* (0.80), reveals their order of larvicidal activity against the fourth instar larvae of the mosquito, *C. quinquefasciatus* (Figure:4,5&6).

The result revealed that the plant extracts of *Tagetus minuta, Ageratum conyzoides and Jatropha curcas* were promising larvicide to control mosquito larvae. *Tagetus minuta* ethanolic extracts showed highest larvicidal effects within the range of 0.05-0.25 mg/ml concentration. The treated larvae could not pupate. The larvae showed abnormal movement and become immovable. *Tagetus minuta* essential oil terpenes were found to be responsible for the toxic effects reported in *Dipterans* and possibly that is revealed by the present study also (Marcombe *et al.*,2012). It should be noted that *T. minuta* aqueous leaf extracts had less fatal effects against *C. quiquefasciatus* larvae and pupa. Similar trends were observed in previous larvicidal activity studies, where ethanol and hexane leaf extracts of *Cassia occidentalis* and *Lantana camara*dis played better larvicidal activities toward *Anopheles stephensi*and *Aedesa egypti*larvae, respectively than aqueous extracts (Dhandapani and Kadarkarai, 2011; Marcombe *et al.,* 2012; Murugan *et al.,* 2012). The aquatic immature life stages (larvae and pupa) prefer to inhabit water pools that are polluted and such behaviour may act to assist larvae and pupa to develop metabolic defence strategies against plant aqueous extracts that they might have been exposed to previously (Dhandapani and Kadarkarai, 2011). This observation may be attributed to the differences in polarity of the extractants used in this study. The essential oil of *Tagetus minuta*have also been reported to possess larvicidal efficacy over *Anopheles stephensi* and *Aedesa egypti* (Perich *et al.,* 1994). The petroleum ether extracts of *Tagetes erectus* is reported to have significant effect on larval mortality. The hydrogenated fraction of *Tagetus minuta* isalso reported to have mosquitocidal effects(Sharma and Saxena, 1994).

Jatropha curcas was the second most effective plant extract against *C.*

quinquefasciatus larvae with the range of 0.20-1.20 mg/ml of concentration and LC50 value of 0.80 for 24 hours mortality. Most parts of *J. Curcas* plant is reported to be toxic, probably explaining the larvicidal activity shown in the present study. Leaves of *Jatropha* contains insecticidal compound such as apigenin, histamine, isovitexin and vitexin etc. *Jatropha* species were reported to have larvicidal and antifeedent activity against *Spodoptrea litura* (Bhagat and Kulkarni, 2012). Toxic activity of plant *Jatropha curcas* against larvae of Schistosomonas was also reported (Rug and Ruppel, 2000). No publicized reports were encountered on the toxicity of *Jatropha* phorbol esters on non-target aquatic organisms, suggesting their potential role in the control of disease vector mosquitoes. Furthermore, some aquatic organisms are known to nullify the toxic effects of *Jatropha* phorbol esters (Goel *et al.*, 2007).

FIGURE :1 Larvicidal efficacy of *Tagetus minuta* extract over fourth instar larvae of *C.quinquefasciatus* Say.

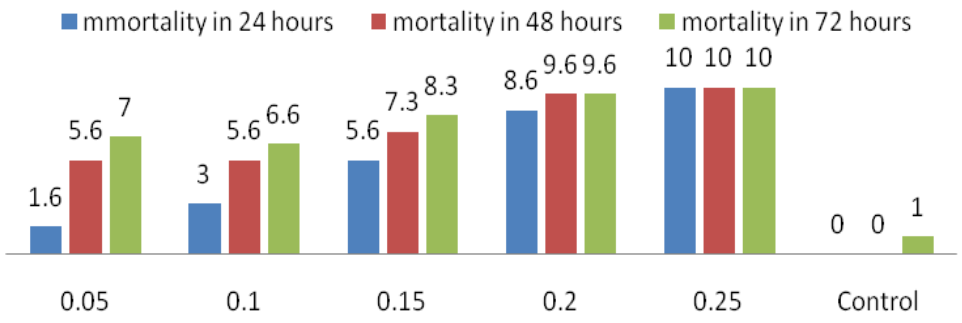


FIGURE :2 Larvicidal efficacy of *Ageratum conyzoides* extract over fourth instar larvae of *C.quinquefasciatus* Say.

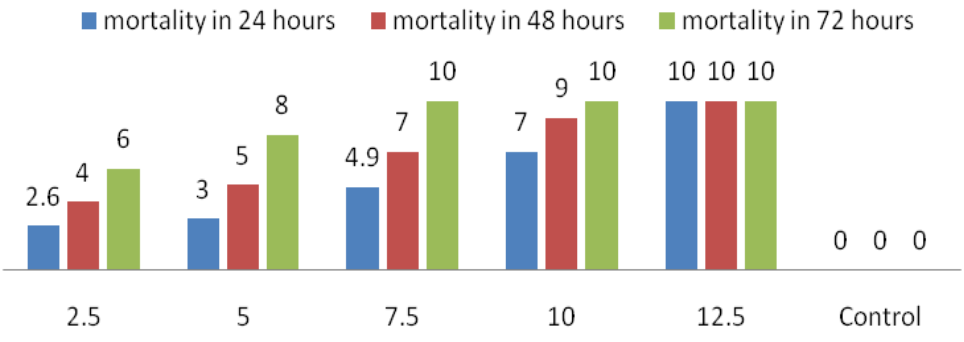


FIGURE :3 Larvicidal efficacy of *Jatropha curcas* extract over fourth instar larvae of *C.quinquefasciatus* Say.

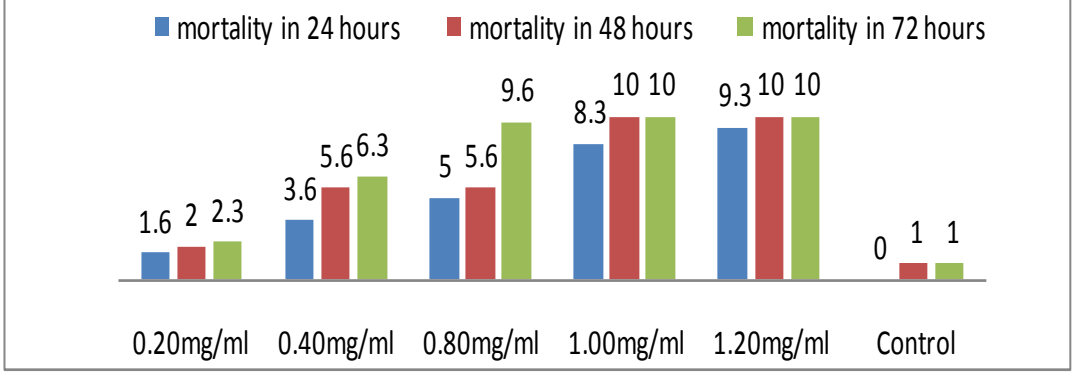


FIGURE :4 *Culex quiquefasciatus* larval mortality by *Tagetus minuta* extracts in 24 hours

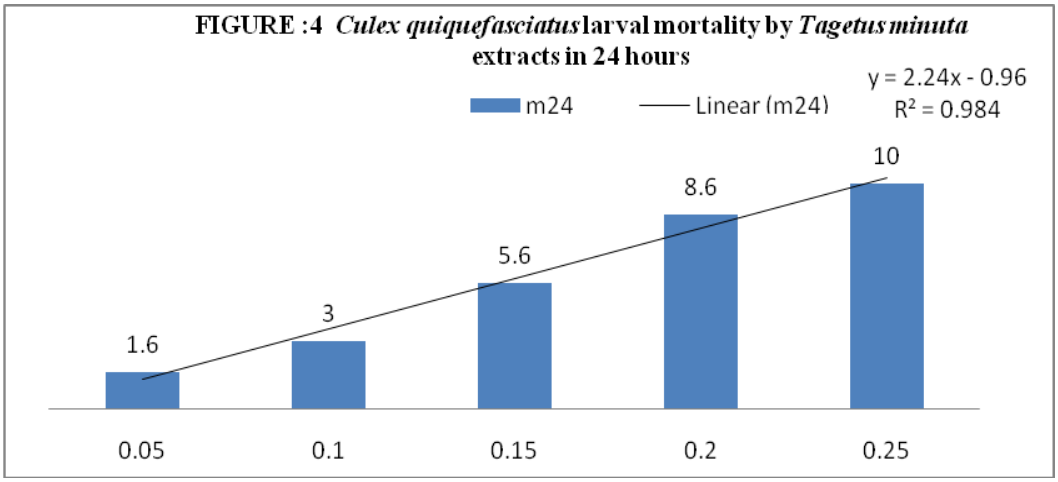
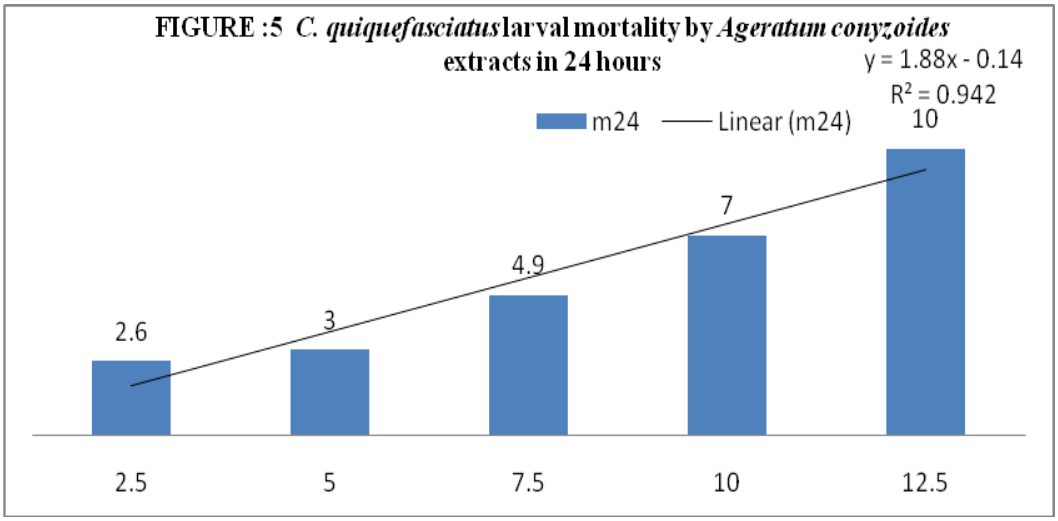


FIGURE :5 *C. quiquefasciatus* larval mortality by *Ageratum conyzoides* extracts in 24 hours



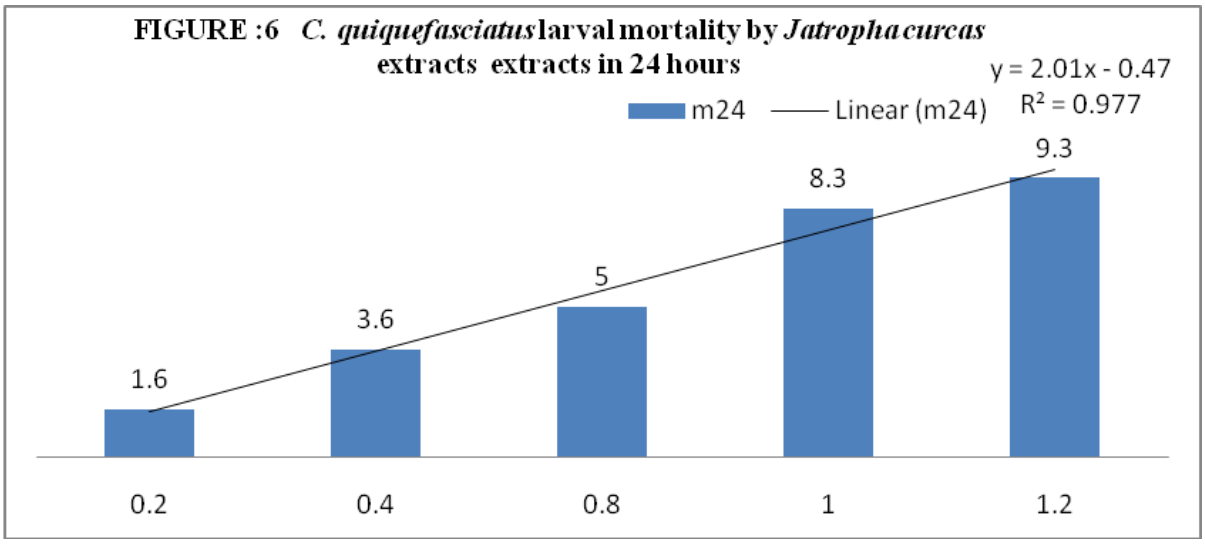


FIGURE :6 ***C. quiquefasciatus*** **larval mortality by** ***Jatropha curcas*** **extracts extracts in 24 hours**

Ageratum conyzoides extracts were also taken in five different concentrations ranging from 2.50 – 125 mg/ml against the *C. quinquefasciatus* larvae. In this study, *Ageratum conyzoides* extracts recorded the least larvicidal efficacy among all the locally available plants used in larvicidal experiments. The larvae treated with *Ageratum conyzoides* extracts showed abnormal random movement biting their siphon. The extract affected the air tube of *C. quinquefasciatus* larvae. Although the larvicidal activity of *Ageratum conyzoides* was least among the three plants still its combination with other botanicals may be tried for the synergistic effect on mosquito larvae.

In conclusion, Plant could be an excellent alternative source for mosquito larvicides because they constitute a potential sourceof bioactive chemicals and generally free from harmful effects to the environment and non-target organisms. Use of these plant extracts as larvicides in mosquito control instead of synthetic insecticides could reduce the cost, adverse environmental effects and pollution. This study reveals the excellent mosquitocidal potentiality of the locally available plants namely *Tagetus minuta, Jatropha curcas* and *Ageratum conyzoides*. Further studies on identification of active compounds and field trials are needed to recommend theactive fraction of these plant extracts for development of eco-friendly larvicides for control of insect vectors.

Acknowledgements

The authors are grateful to the University Grant Commission, New Delhi for providing the financial assistance through the Project F. No. 34-481/2008 (SR).

References

Abbott, W.S., 1925.A method of computing the effectiveness of insecticides. J. Econ. Entomol. 18:267–269.

Adebowale, K.O., and Adedire, C.O. 2006. Chemical composition and insecticidal properties of the underutilized *Jatropha curcas* seed oil. African Jour. of Biotech.5: 901-906.

Bhagat, R.B. and Kulkarni, D.K. 2012. Evaluation of larvicidal and antifeedant potential of three *Jatropha species* against *Spodopteralitura* (*Lepidoptera:*

Noctudae) and two predators (*Coleoptera:Coccinellidae*). Ann. of Biol. Res. 3 (6):2911-2916

Dhandapani, A. and Kadarkarai, M. 2011. HPTLC quantification of flavonoids, larvicidal and smoke repellent activities of *Cassia occidentalis* L. (*Caesalpiniaceae*) against malarial vector *Anopheles stephensi* Liston (*Diptera: Culicidae*). Jour.Phyto.3: 60-72.

Goel, G., Makkar, H.P.S., Francis, G. and Becker, K. 2007. Phorbol esters: structure, biological activity and toxicity in animals. Int. Jour. of Toxicol.26: 279-288.

Gubler, D.J. 1998. Resurgent vector-borne diseases as a global health problem. Em. Inf. Dis. 4(3): 442-450.

Hag, E., Nadi, E.A.A.H. and Zaitoon, A.A. 1999. Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (*Diptera: Culicidae*). Phyto. Res.13: 388-392.

I.C.M.R.,2002.Chemical insecticides in malaria vector control in India. ICMR Bull. 32:0377- 4910.

Indian Council of Medical Research., 2003.Prospects of using herbal products in the control of mosquito vectors.ICMRBull.33: 1-12.

Kumar, A. and Sharma, S. 2008. An evaluation of multipurpose oil seed crop for industrial uses *Jatropha curcas* L.: a review. *Indian Crop. Prod.*66-71.

Marcombe, S., Mathieu, R.B., Pocquet, N., Riaz, M.A., Poupardin, R.,Sélior, S., Darriet, F., Reynaud, S., Yébakima, A., Corbel, V., Jean-Philippe, D. and Chandre F.2012. Insecticide resistance in the dengue vector *Aedesa egypti* from Martinique: distribution, mechanisms and relations with environmental factors. 7(2): 309-89.

Matsuse, I.T., Lim, Y.A., Hattori, M., Correa, M. and Gupta, M.P. 1998.A search for anti-viral properties in Panamanian medicinal plants: the effects on HIV and its essential enzymes. Jour. of Ethnopharma.64: 15-22.

Ming, L.C. 1999. *Ageratum conyzoides*: A tropical source of medicinal and agricultural roducts. In Janick J.*Perspectives on new crops and new uses*. American Society for Horticultural Science (ASHS) Press, Alexandria VA, USA. 469-473.

Murugan, K., Kumar, P.M., Kovendan, K., Amerasan, D., Subrmaniam, J. and Hwang J.S.2012. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi*, *Aedesa egypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol.Res.111: 1757–1769.

Perich, M.J., Wells, C., Bertsch, W. and Tredway, K.E.1994. Toxicity of extracts from three *Tagetes* against adults and larvae of yellow fever mosquito and *Anopheles stephensi* (Diptera: Culicidae). J. Med. Entomol. 31: 833-7.

Rug, M. and Ruppel, A. 2000.Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of schistosomes. Trop. Med. and Inter. Health.5: 423-430.

Service, M.W. 1983. Management of vectors. In: Youdeowei, A. and M.W. Service (Eds.), Pest and Vect. Manag.in Trop. 265-280.

Service, M.W. 2000.Medical entomology for students, 2nd edn. Cambridge University Press, Cambridge. 52–55.

Sharma, M. and Saxena, R.C. 1994.Phytotoxicological evaluation

of *Tagete serectes* on aquatic stages of *Anopheles stephensi*. Indian. J Malariol.31:21–26.

Thomas, R., Sah, N.K. and Sharma, P.B. 2008. Therapeutic biology of *Jatropha curcas*: a mini review. Curr. Pharma. Biotech. 9: 315-324.

Vogel. 1978. Textbook of practical organic chemistry. The English Language Book Society and Longman, London.

Vyas, A.V. and Mulchandani, N.B. 1986. Polyoxygenated flavones from *Ageratum conyzoides*. Phytochem. 25: 2625-2627.

World Health Organization.1992. Vector resistance to pesticides. Fifteenth report of the WHO expert committee on Vector biology and control. Tech. Rep. Ser. 818: 1-62.

World Health Organization.1996. The World Health Report, Geneva.

World Health Organization.1998. Malaria prevention and control. WHO Report. Geneva.

W.H.O., 2005. Guidelines for laboratory and field testing of mosquito larvicides. WHO/CDC/WHOPES/ GCDPP.13.

Wiedenfeld, H. and Roeder, E. 1991. Pyrrolizidine alkaloids from *Ageratum conyzoides*. Planta. Med. 57:578-579.