International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2018; 5(2): 47-51

Received: 09-01-2018
Accepted: 10-02-2018

Zia Ullah
(1) Department of Entomology, University of Agriculture, Faisalabad, Pakistan
(2) Public Health Pest Laboratory, Jeddah Municipality, Kingdom of Saudi Arabia

Atif Ijaz
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Talha Karamat Mughal
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Khurram Zia
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Correspondence
Zia Ullah
(1) Department of Entomology, University of Agriculture, Faisalabad, Pakistan
(2) Public Health Pest Laboratory, Jeddah Municipality, Kingdom of Saudi Arabia

Larvicidal activity of medicinal plant extracts against *Culex quinquefasciatus* Say. (Culicidae, Diptera)

Zia Ullah, Atif Ijaz, Talha Karamat Mughal and Khurram Zia

Abstract
Mosquito is a vector of different diseases like Malaria, Filariasis and Dengue fever. It has been increasing development of resistance to synthetic chemicals, so plant extracts may be the alternate source of mosquito control agent. This study was conducted on five different plant extracts as *Nicotiana tabacum*, *Ocimum basilicum*, *Datura stramonium*, *Chenopodium album* and *Cassia fistula* against *Culex quinquefasciatus* Say. to evaluate them as an efficient larvicide potential. Plant extracts offered a virtually untapped chemical compounds with different potential uses. Study was focused on laboratory determination of LC_{50} and LC_{90} values by a bioassay test. These plant extracts were tested on 3rd instar of mosquito larvae. The plant extracts were applied with different concentrations as 400, 200, 100, 50, 25 ppm with acetone as control and mortality was counted after 24, 48 and 72 hours. The efficiency of plant extracts was recorded in term of % age mortality. From these extracts *Cassia fistula* gave 100% mortality and *Nicotiana tabacum* gave 98% mortality after 72 hours of application. In sense of LC_{50} and LC_{90} values *Cassia fistula* showed 50.272ppm and 203.994ppm respectively. LC_{50} and LC_{90} values of *Nicotiana tabacum* were 17.772ppm and 206.485ppm respectively.

Keywords: Mosquito, vector, diseases, chemicals, resistance**,** plants extract

1. Introduction
Mosquitoes are creating many serious issues from years in several countries, e.g. in Africa, Canada, Thailand, America, Srilanka and Pakistan. They are known to transmit many diseases to human beings, e.g. dengue, encephalitis, chikungunya, filariasis, yellow fever, malaria and cause deaths. There are several genera of mosquitoes, In Pakistan six genera, forty five species exist [1]**.** Mosquitoes lay eggs in standing water or showing a little movement. Mosquito's larvae and pupae nourish in water. Larvae feed on the either dead or living fauna present in water. There are three blood feeding genera *Aedes*, *Anopheles*, *Culex*, make their breeding sites in stagnant water in our surroundings like tires and tree holes [2]. To regulate the mosquito's population is very difficult because resistance has developed against chemical insecticides which result in the rebounding vectorial capability [3]. The most economical method to control mosquitoes is to eradicate their breeding sites by application of environment friendly larvicides [4]. Larval treatment is more effective to control this notorious insect due to its localization and low mobility [5].
To control the mosquito there are traditional chemical insecticides, larvicides, pupaecides and adulticides. The use of these traditional insecticides have harmful effects as; resurgence and resistance in insect populations, mammalian toxicity, residual effect, non-specificity in action which kill all insects, beneficial and harmful collectively. So keeping in view these side effects some alternate option should be chosen [6]. The severe effect of using synthetic chemical insecticides in environment has received wide public awareness [7]. Many problems have resulted due to overuse of synthetic insecticides in Agriculture and Public health programs, environmental pollution toxic hazards to human and non-target organisms [8, 9]. As a result, W.H.O. has facilitated replacement of the traditional insecticides with bacterial and phytochemicals through the development of standards by their use and registration. Plants contain different biochemicals in them which act as insects feeding deterrent, disturb adult emergence capacities and can be repelled by plant extracts such as *Nicotiana tabacum* and pesticidal effect of *Datura stramonium* also kill the insect pests by feeding on limonoids.

These chemicals possibly extract from plants by various ways. These chemicals show effective results in controlling of mosquitoes and are safe for environment, no health hazardous to mammals. Pyrethrin was the first pyhytochemical which was extracted from chrysanthemum. Plant extractions of *Chenopodium album, Cassia fistula, Nicotiana tabacum, Datura stramonium* and *Ocimum basilicum* have been used against populations of mosquitoes. In mosquito control programmes these all extracted botanicals play an important role. These are oviposition deterrent and female irritant [10]. The acetone tobacco extract has effective larvicidal potential in controlling the immature instars of mosquito. Tobacco gives best results against larvae and act as repellent against adults. *Datura stramonium* has good larvicidal potential against mosquitoes [11]. It is assumed that the tropical areas are more susceptible to parasitic diseases and so the risk has been increased due to change in climate and increasing globalization [12].Allergic responses are also caused by mosquitoes in humans which may be local skin or systematic reactions like angioedema [13]. The purpose of present study was to observe the larvicidal effect of plant extracts which may be helpful in future to manage the mosquito population.

2. Materials and Methods

The study was conducted in Department of Agri. Entomology, University of Agriculture Faisalabad. For our experiment, in the first part of research the ovicidal traps were installed at probable breeding sites. Traps were filled with water manually. This all was checked daily and observe the mosquito population if present there. Number of days for how long these traps hold and store the water was recorded. In other condition at the same sites when rainfall occur these traps were self-filled with water and these were kept in appropriate level with subsequent filling of water after few days [14].

2.1 Procedure

The larvae were collected from the mosquito breeding sites in University of Agriculture Faisalabad. The larvae after identification were shift into separate trays in Entomological laboratory of department of University of Agriculture Faisalabad for rearing. When pupae were developed then these were transferred to beaker in rearing chamber. Females were fed on blood of albino rats that were trapped in cage. Females were laid eggs on surface of water; eggs were frozen for off season experiments.

Male mosquitoes were fed on solution prepared from sugar (sucrose 10%). Albino rats were shifted in cages so that mosquitoes can feed easily. At last after full meal after emergence they were transferred to trays where they were provided with measured amount of fish diet. Laboratory culture of mosquito larvae was maintained at 27 ± 2 C, 60 – 70% relative humidity [15].

Leaves of plants like Niazbo (*Ocimum basilicum*), Amaltas (*Cassia fistula),* Datura *(Datura stramonium),* Tobacco (*Nicotiana tabacum*), Bathu (*Chenopodium album*) were taken. Fruit of Amaltas was collected and dried in an oven. These were dried at appropriate temperature in an oven for recommended time till no moisture is left in the collected leaves.

After dried process, leaves were grinded to convert into powder form. Powder was used for extraction of oil in the soxhlet apparatus [16]. First of all powder was loaded in soxhlet apparatus then its funnel was filled with petroleum ether and the oven turn on. By this method oil was extracted from selected plants. Different concentrations were sorted out from these oils. Preparation of stock solution with 10% concentration (crude oil) was prepared by dissolving 10ml of extract into 90ml of petroleum ether. Concentrations were prepared from this solution as 400, 200, 100, 50, 25 ppm respectively. The experiment was carried under CRD design in laboratory conditions. In beaker of 250ml the 100ml volume of particular concentration was used, 15 larvae of 3rd instar were placed in the beakers. Three replicates of each treatment were used and petroleum ether as control. Three concentrations of five treatments were applied on 3rd instars of mosquito larvae and data was taken by counting dead larvae or rest of living larvae after 24, 48 and 72 hours. Mortality percentage was the efficacy of a plant extract against mosquito larvae Mortality percentage was calculated by using Abbot's formula [17] and data was analyzed by Probit analysis [18].

3. Results

The present research was carried out for the management of *Culex quinquefasciatus* mosquitoes. Our results were recorded in term of % age mortality against the 3rd larval instars of mosquito species at different concentrations for 24, 48, 72 hours. Results for LC_{50}, LC_{90}, P- values and egression equations are given in (Table 1). The graphically representation of % mortality, LC_{50} and LC_{90} values are shown in the (fig. 1, fig. 2 and fig. 3)

The LC_{50} and LC_{90} values of Bathu *(Chenopodium album)* extract against 3rd instar larvae of *Culex spp.* after 24 hours interval were 277.796 and 739.425ppm respectively. The regression equation was Y= -.771 +.003x with 15.449 chi-square value and P value was 0.452. After 48 hours interval the LC_{50} and LC_{90} values recorded was 258.027 and 717.820 ppm respectively. The regression equation was Y= -.791 +.003x with 11.289 chi-square value, the P value was 0.752. After 72 hours the LC_{50} and LC_{90} values were 136.232 and 488.558 ppm respectively.

Table 1: LC_{50} and LC_{90} values of plant extracts with P-values and Regression Equations at different time intervals against the 3rd instar larvae of *Culex spp.*

Plant Extracts	Time (hours)	LC_{50} Values (ppm)	LC_{90} Values (ppm)	P-value	Chi-Square	Regression equations Y= a + bx
Amaltas (*Cassia fistula*)	24	203.492	542.804	0.653	12.695	-.769 +.004x
	48	124.154	443.420	0.225	19.891	-.498 +.004x
	72	50.272	203.994	0.305	18.337	-.419 +.008x
Datura (*Datura stramonium*)	24	409.870	919.868	0.96	5.043	-1.030+.003x
	48	272.865	670.489	0.95	7.730	-.879 +.003x

	72	177.180	515.552	0.82	10.750	-.671 +.004x
Tobacco(*Nicotiana tabacum*)	24	223.868	645.459	0.438	16.027	-.681 +.003x
	48	148.505	554.868	0.266	19.050	-.468 +.003x
	72	17.772	206.485	0.000	60.299	-.121 +.007x
Niazbo (*Ocimum basilicum*)	24	299.176	645.919	0.901	9.289	-1.106+.004x
	48	244.273	592.041	0.966	7.336	-.900 +.004x
	72	162.884	475.524	0.841	10.463	-.668 +.004x
Bathu (*Chenopodium album*)	24	277.796	739.425	0.452	15.449	-.771 +.003x
	48	258.027	717.820	0.752	11.289	-.791 +.003x
	72	136.232	488.558	0.542	14.082	-.496 +.004x

The regression equation was Y= -.496 +.004x with chi-square value 14.082 and P value 0.542.

The LC_{50} and LC_{90} values for Amaltas *(Cassia fistula)* leaves extract against 3rd instar larvae of *Culex spp.* after 24 hours interval were 203.492 and 542.804 ppm respectively. The regression equation was Y= -.769 +.004x with 12.695 chi-square value and P value was 0.653. After 48 hours interval the LC_{50} and LC_{90} values were recorded as 124.154 and 443.420 ppm respectively. The regression equation was Y= -.498 +.004x with 19.891chi-square value, the P value was 0.225. After 72 hours interval the LC_{50} and LC_{90} values were 50.272 and 203.994 ppm respectively. The regression equation was Y= -.419 +.008x with 18.337 chi-square value, the P value was.305.

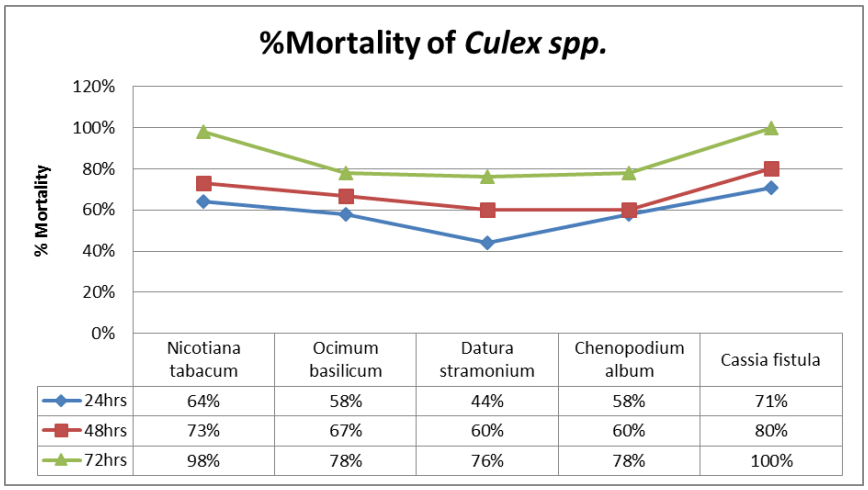

Fig 1: % Mlortality of 3rd instar larvae of *Culex spp* at different time intervals

The LC_{50} and LC_{90} values for Datura *(Datura stramonium)* leaves extract against 3rd instar larvae of *Culex spp.* after 24 hours were 409.870 and 919.868 ppm respectively. The regression equation was Y= -1.030 +.003x with chi-square value 5.043 and P value 0.096. After the interval of 48 hours LC_{50} and LC_{90} values were 272.865 and 670.489 ppm respectively. The regression equation was Y=-.879 +.003x with 7.730 and 0.095 chi-square value and P value respectively. After the interval of 72 hours LC_{50} and LC_{90} values were 177.180 and 515.552 ppm respectively. The regression equation was Y= -.671 +.004x with 10.750 chi-square value and the P value was 0.082.

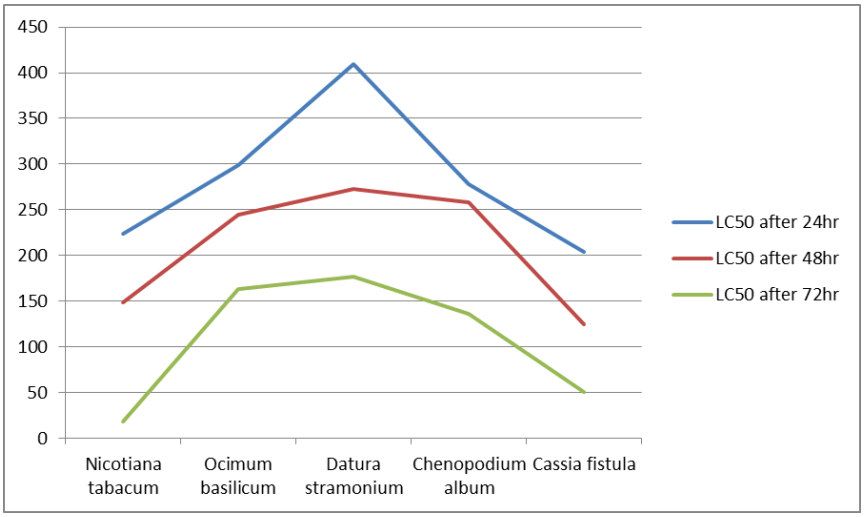

Fig 2: % Mortality of *Cules spp.* for different Plant Extracts after 24, 48 and 72 hours

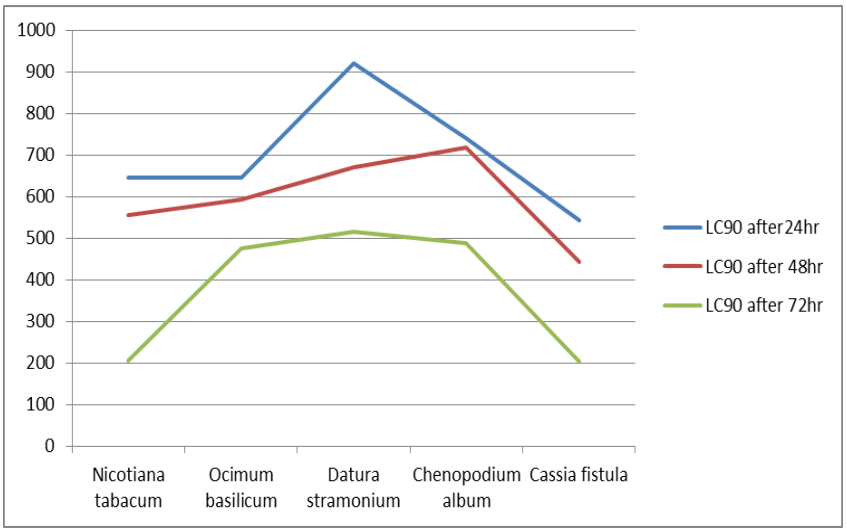

Fig 3: LC_{90} values for different Plant Extracts against *Cules spp* after 24, 48 and 72 hours

The LC_{50} and LC_{90} values for Tobacco *(Nicotiana tabacum)* leaves extract against 3rd instar larvae of *Culex spp.* after the interval of 24 hours were 223.868 and 645.459 ppm respectively. The regression equation was Y= -.681 +.003x with 16.027 chi-square value, the P value was 0.438. After the interval of 48 hours LC_{50} and LC_{90} values were 148.505 and 554.868 ppm respectively. The regression equation was Y= -.468 +.003x with 19.050 chi-square value, the P value was 0.266. After the interval of 72 hours LC_{50} and LC_{90} values were 17.772 and 206.485 ppm respectively. The regression equation was Y= -.121 +.007x with 60.299 chi-square value, the P value was 0.000.

The LC_{50} and LC_{90} values for Niazbo *(Ocimum basilicum)* leaves extract against 3rd larvae of *Culex spp.* after the interval of 24 hours were 299.176 and 645.919 ppm respectively. The regression equation was Y= -1.106 +.004x with 9.289 chi-square value, the P value was 0.901. After the interval of 48 hours LC_{50} and LC_{90} values were 244.273 and 592.041 ppm respectively. The regression equation was Y= -.900 +.004x with 7.336 chi-square value, the P value was 0.966. After the interval of 72 hours LC_{50} and LC_{90} values were 162.884 and 475.524 ppm respectively. The regression equation was Y= -.668 +.004x with 10.463 chi-square value, the P value was 0.841.

5. Discussion

Our results were almost similar to the scientists who worked with different plant extracts against mosquitoes. Bigoga *et al.*, studied the Larvicidal and repellent effect of the essential oil from the seeds and leaves of *Chenopodium ambrosioides* Linn against the larvae and adults of *Anopheles gambiae* at concentrations of 0, 50, 75, 100, 200, 300 and 400 ppm. They concluded that 100% larval mortality was observed at 200ppm and 300ppm for the essential oils from seeds and leaves respectively [19]. Our results differ because of the spp. difference as we use *Culex spp.* but they use *Anopheles gambiae*.

Govindarajan *et al*., conducted an experiment to check the larvicidal and ovicidal activity of *Cassia fistula* against mosquito larvae of *Anopheles spp*. They applied different concentrations on Anopheles larvae and their mortality percentage was satisfactory, so they concluded that *Cassia fistula* can be used as larvicide against mosquito larvae [20]. Govindarajan *et al.*, conducted another experiment on the larvicidal property of *C. fistula* and concluded that it had good larvicidal potential for controlling of *C. tritaeniorhynchus* and *A. subpictus* mosquitoes larvae. They used methanol, acetone and petroleum ether extractions of *C. fistula* against 4th instar larvae of C. *tritaeniorhynchus* and *A. subpictus* were 36.43 ppm, 33.76 ppm, 48.55 ppm and 52.17 ppm, 45.57 ppm, 39.01 ppm, respectively [21]. The difference of the LC_{50} values with our may be due the exposure time, difference in concentrations mosquitoes spp. and may be the larval instar.

Olofintoye *et al.*, worked over the toxicity of extracts of the two members of the *Solanacae* family; *Nicotiana tabacum* and *Datura stamonium* on the larvae of *culicinae* and *anophilinae*. They found that both extracts were efficient in controlling the mosquito larvae, but tobacco extract proved the best in controlling anopheles larvae. They found 50% mortality of culicine larvae in first 24 hours, at 100% concentration mortality was also 100%. On another spp. anophilinae the mortality was 70%, at 100% concentration, mortality was 90% in anophilinae. They found high correlation of 0.93 and 0.68 with time interval 24 hour and 48 hours [22]. The findings of Olofintoye *et al.*, were also similar to our results and some differences may be due to the difference in concentrations.

Our results were close to Tennyson *et al*., they investigated on petroleum ether leaf extracts of *Nicotiana tabacum* to check their larvicidal property. They evaluated the *Nicotiana tabacum* as larvicidal potential against the *A. aegypti* at different concentrations of 62.5, 125, 250, 600 and 1000 ppm. Mortality percentage was observed for 24-48 hours. *Nicotiana tabacum* with LC_{50} values 95.75, 124.28 and 236.73ppm after 24, 48 and 72 hours respectively [23]. The difference in results may be due to the difference in the spp.

Murugan and Jeyabalan studied the *Ocimum basilicum* for their larvicidal property against *Culex quinquefasciatus.* They applied different concentrations of *Ocimum basilicum* on 4th instar larvae of *Culex quinquefasciatus* and counted mortality percentage was satisfactory. Their results showed that *Ocimum basilicum* can be used as insecticide [24]. The observations of Murugan and Jeyabalan were also same to our study.

6. Conclusion

Plant extracts can play an important role in the management of the mosquitoes as they have less hazardous effects on human health. In our study it was proved that *Cassia fistula* and *Nicotiana tabacum* extracts were considered the best in terms of LC_{50} values, LC_{90} values as well as in terms of percent mortalities as compared to other extracts. So according to our findings it is encouraged to add these extracts in mosquitoes control program for the effective and safe results.

7. Reference

1. Suleman J, Amer A, Mehlhorn H. Larvicidal effects of various essential oils against *Aedes*, *Anopheles* and *Culex* larvae (Diptera: Culicidae). Parasitol. Res. 1993; 99:466-472.
2. Yenoviak SP. The macrofauna of water-filled tree holes on Barro Colorado island, Panama. Biotropica. 2001; 33(1):110-120.
3. Liu M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd MY. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). J Vec. Born. Dis., 2006; 47:185-189.
4. Certin H, Erler F, Vanikoglu A. Larvicidal activity of botanical natural products, Akse Bio, against *Culex pipens*. Fitoterapia. 2004; 75:724-728.
5. Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. B.M.C Pub. Hlth. 2007; 7:199-204.
6. Singhi M, Joshi V, Sharma RC, Sharma K. Ovipositioning behaviour of *Aedes aegypti* on different concentrations of latex of *Calotropis procera*: studies on refractory behaviour and its sustenance across gonotrophic cycles. Deng. Bull. 2004; 28:185-188.
7. St. legar RJ, Joshi L, Bidochka MJ, Roberts DW. Constriction of and improved mycoinsecticides over expressing a toxic protease. Proceed. National Acad. Sci., USA. 1996; 93:6349-6354.
8. Severini C, Rom R, marinucci M, Rajmond M. Mechanism of insecticide resistance in field population of *Culex pipiens* from Italy. J Amer. Mosq. Contr. Assoc. 1993; 9:164-168.
9. Forget O. Pesticides, necessary but dangerous poison. The IDRC reports. 1989; 18:7-13.
10. Koul O, Walia S, Dhaliwal GS. Essential oils as green pesticides: potential and constraints. Biopestic. Int. 2008; 4(1):63-84.
11. Thavara U, Twatsin A, Chompoosri J. Phytochemicals as repellents against mosquitoes in Thailand. Ntl. Inst. Health, Depart. Medical Science Ministry of Public Health. Proceed. Intern. Conf. Biopest. April 21-26, Kuala Lumpur, Malaysia, 2002, 244-250.
12. Karunamoorthy K, Ilango K, Murugan K. Laboratory evaluation of traditionally used plant-based insect repellents against the malaria vector *Anopheles arabiensis* Patton. Parasitol Res., 2010; 106:1217-1223.
13. Peng Z, Yang J, Wang H. Simons FER Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. Insect Biochem. Mol. Biol. 1999; 29:909-914.
14. Chen CD, Lee HL, Steela-Wong SP, Lau KW, Sofian-Arizon M. Container survey of mosquito breeding sites in a university campus in Kuala Lumpur, Malaysia. Center Tropical Biodiversity Res., Inst. Biol. Sci. 2009; 2(3):83-87.
15. Adebayo TA, Gbolade AA, Olaifa J. Comparative study of toxicity of some essential oils to larvae of three mosquito species. J Am. Mos. Cont. Assoc. 1999; 12:406-408.
16. Handa C, Kolaczinski JH, Conway DJ, Carnevale P, Curtis CF. The pyrethroid resistance gene in *Anopheles gambiae*: tests of non pyrethroid insecticides and a new detection method for the gene. Parasitol. 2008; 41:323-326.
17. Abbott WS. A method of computing the effectiveness of an insecticide. J Econ. Entomol. 1925; 18:265-267.
18. Finney DJ. Probit analysis, 3rd ed. Cambridge Great Britain University press, UK, 1971.
19. Bigoga JD, Saakem PA, Ndindeng SA, Ngondi JL, Nyegue M, Oben JE *et al*. Larvicidal and Repellent Potential of *Chenopodium ambrosioides* Linn Essential Oil against *Anopheles gambiae* Giles *(*Diptera: Culicidae)., The Open Entomol. J. 2013; 7:16-22.
20. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol. Res. 2008; 102:289-292.
21. Govindarajan M, Sivakumar R, Rajeswari D. Larvicidal efficacy of *Cassia fistula* Linn. leaf extract against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). Department of Zoology Asian Pacific J Trop. Dis. 2011; 34:295-298.
22. Olofintoye LK, Simon-Oke IA, Omoregie OB. Larvicidal Properties of *Datura stramonium* (Jimson Weed) extracts against the larvae of (*Anopheles* and *Culex*) mosquitoes. African Research Review AJOL. 2011; 5(1):24-29.
23. Tennyson S, Arivoli S, Raveen R, Bobby M, Dhinamala K. Larvicidal Activity of *Areca catechu*, *Nicotiana tabacum* and *Piper betle* leaf extracts against the dengue vector *Aedes aegypti* (L.) (DIPTERA: CULICIDAE). Int. J Res. Bio. Sci. 2012; 2(4):157-160.
24. Murugan K, Jeyabalan D. Mosquitocidal effect of certain plants extracts on *Anophels stephensi.* Curr. Sci. 1999; 76:631-633.