ADULTICIDAL AND LARVICIDAL ACTIVITY OF *CASSIA FISTULA* AND *PIPER NIGRUM*AGAINST MALARIA VECTOR

Sara Mehmood[1], Muhammad Lateef[1], Muhammad Ovais Omer[2], Aftab Ahmed Anjum[3], Muhammad Imran Rashid[1*], WasimShehzad[4],

[1]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Punjab.
[2]Department of Pharmacology, University of Veterinary and Animal Sciences, Lahore, Punjab.
[3]Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Punjab.
[4]Institute of Biochemistry & Biotechnology (IBBT), University of Veterinary & Animal Sciences, Lahore, Punjab.
***Corresponding author: Imran.rashid@uvas.edu.pk**

ABSTRACT*: The current study was carried out to evaluate the activity of methanol extract of leaves of C. fistula and ripened fruits of P. nigrum against Anopheles mosquito. Among both of these plants, the methanolic extracts of Piper nigrum (black pepper) exhibited remarkable Adulticidal and Larvicidal potentials. The percentage mortalities were increased by gradual increase in extracts concentrations. Larvae were more susceptible to the methanolic pepper extracts than adults. Cassia fistula (golden shower) leaf extracts were also showed promising mosquitocidal efficacy against Anopheles stephensi. It is concluded that methanolic extracts of these plants Cassia fistula and Piper nigrum have high potential of Adulticidal and Larvicidal activities. So the extracts of these plants can be used as an alternative to the conventional insecticides for long lasting mosquito problems.*

Key words: *Adulticidal, larvicidal, Cassia Fistula, Piper Nigrum, Anopheles, Malaria*

INTRODUCTION

Vector-borne diseases (VBD's)are among the major causes of illness. death and economic losses in the world [1]. These vector transmitting diseases show a significant public health problem today[2]. VBD's are generally responsible for a significant fraction of the global disease burden and have very severe effects not only on human lives but also on the socioeconomic development of affected countries. Mosquitoes are most important single group of insects, transmitting wide range of human diseases like Malaria, Japanese encephalitis, Dengue fever, Yellow fever, Filariasis and several other infectious diseases[3]. Asia comprises of both temperate and tropical regions, so many diseases like malaria, dengue fever, dengue haemorrhagic fever and schistomiasis are endemic in many developing countries of Asia[4]. In a study conducted in Khyber-Pakhtunkhwa of Pakistan, it is reported that there is high incidence of *P. falciparum* malaria due to increased temperature, rainfall and humidity etc. [4].

MATERIALS AND METHODS

The adult mosquitoes were collected with hand catch method from different areas of Lahore as per described by Manual on Practical Entomology in Malaria[5]. Mosquitoes were identified and reared as per described in Michigan Mosquito Manual, (2002). Collected *C. fistula* powdered leaves and dried fruits of *P. nigrum* were extracted with Methanol by the method described by Kumar et al.,[6]. The Larvicidal activity of methanolic extracts of both plants on *An. Stephensi* larvae was evaluated as per method described by WHO 2005. Adulticidal bioassays were performed with 4-6 days old male and female mosquitoes to check the efficacy of both plant extracts as per standard procedure of WHO [7, 8]with slight modification. The LC50 and LC90 were calculated according to probit analysis[9]. Statistical Package of Social Sciences (SPSS) was used for data analysis. Mortalities were corrected using Abbott's formula, if the mortality %age in control test were ranged between 5-20%.

$$\text{Corrected Mortality} = \frac{\%\text{ Test Mortality} - \%\text{ Control Mortality} \times 100}{100 - \%\text{ Control Mortality}}$$

RESULTS AND DISCUSSION

Treatments of different doses of both plant extracts have shown good results. The larvae and adult of *A. stephensi* showed gradual increase in mortality by increasing the concentrations of extracts. In Larvicidal bioassays, some concentrations were resulted to show complete mortality and there were no pupal or adult emergence. The untreated or control groups did not show any mortality after 24 hr and 48 hr. Within 48-72 hr the larvae were completed their metamorphosis and developed into pupae and then adults. Behavioral changes of Anopheles larvae were also observed during larvicidal assays. Larvae showed normal movements, but when they were exposed to different concentrations of extracts, some abnormality in their behavior was observed. After 5 min of exposure, larvae showed some listlessness, abnormal evidence of excitation and restiveness which persisted for 10-30 min. and was followed by other anomalous motions such as a coiling movement. After 1-2 hours, some larvae showed more toxic symptoms, they were paralyzed and had sunk to the bottom of the jar. Moribund or dead larvae were increasingly found from 2 to 7 hours. At the end of 48 hours, all larvae were subsequently died in highest concentrations of extracts. Like larvae, adult mosquitoes also showed some signs of abnormality in behavior like unable to fly, stay at the bottom of test kits. Mosquitoes were knocked down at the end of one hour exposure and when they were shifted to holding tubes, they were almost recovered. Mosquitoes were observed for 24 to 48 hours and mortalities were recorded. Table No. 1 and 2 showed adulticidal efficacy of *Piper nigrum* against malaria vector *Anopheles stephensi* after 24 and 48 hours respectively.

Table No. 3 and 4 showed Adulticidal efficacy of *Cassia fistula* against malaria vector *Anopheles stephensi* after 24

and 48 hours respectively.Table No. 5 and 6 showed larvicidal efficacy of *Piper nigrum* against malaria vector *Anopheles stephensi* after 24 and 48 hours respectively.Table No. 7 and 8 showed larvicidal efficacy of Cassia fistula against malaria vector Anopheles stephensi after 24 and 48 hrs.respectively.

Govindarajan *et al.*, (2008) reported the ovicidal and Larvicidal efficacy of methanolic leaf extracts of *Cassia fistula* against*Anopheles stephensi* and *Culexquinquefasciatus* [10]. Similarly efficacy of crude extract of *Cassia fistula* was evaluated against Culextritaeniorhynchusand Anopheles subpictus. Results shown excellentlarvicidalpotential against both mosquitoes [11]. This study was in accordance with our study. In our study methanolic extracts of *Cassia fistula* showed 89% mortality against adult mosquitoes at 70 ppm dose rate after 48 hrs. In another study, Govindarajan et al. (2009) reported the bioefficacy of Cassia fistula leaf extracts with different solvents like benzene, acetone and methanol against dengue vactor*Aedesaegypti*[12]. The larvicidal activity of methanolic leaf extracts of *Cassia fistula* showed highest efficacy in dengue vector. Kumar et al. (2010) reported larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different instars of dengue fever mosquito, *Aedesaegypti*[6]. The ethanolic extracts of three species of peppercorns were long pepper, black pepper and white pepper. Ethanolic extracts of all the three pepper species were 11-25 times more toxic against 3rd instar larvae as compared to the early 4th instar larvae. It showed the extracts of *piper nigrum* have compounds which are potentially active against insects. Nath et al. (2006) reported the Larvicidal activities of methanolic extracts of 19 different indigenous plants against 3rd instar larvae of Aedesalbopictus and Culexquinquefasciatus. Among these tested plants, piper nigrum showed 2nd highest Larvicidal mortality against 3rd instar larvae of Aedesalbopictus and Culexquinquefasciatus[13]. Several other studies had also reported that plant extracts are good alternatives to insecticides [14, 15].

Table No. 1. Adulticidal efficacy of *Piper nigrum* against Malaria vector *Anopheles stephensi* after24hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	9.25 ± 0.25	37%				
30 ppm	13.50 ± 0.29	54%	25.05	78.63	Y= 0.598 + 0.024x	0.070
50 ppm	17.75 ± 0.25	71%	(5.34 - 36.04)	(61.76-125.60)		(2)
70 ppm	21.75 ± 0.25	87%				
Control	0.0	0%				
Permethrin (0.75%)	24 ± 0.25	96%				

Table No. 2. Adulticidal efficacy of Piper nigrum against Malaria vector *Anopheles stephensi* after 48 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	13.00 ± 0.41	52%				
30 ppm	16.50 ± 0.29	66%	12.04	53.06	Y= 0.376 + 0.031x	1.128
50 ppm	21.50 ± 0.29	86%	(-9.93 -- 22.34)	(42.11-77.90)		(2)
70 ppm	24.75 ± 0.25	99%				

Table No. 3. Adulticidal efficacy of *Cassia fistula* against Malaria vector *Anopheles stephensi* after 24 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	7.25 ± 0.25	52%				
30 ppm	11.50 ± 0.29	66%	35.13	94.57	Y= 0.757 + 0.0216x	0.008
50 ppm	15.75 ± 0.48	86%	(18.80 – 47.95)	(72.71-163.76)		(2)
70 ppm	19.25 ± 0.25	99%				
Control	0.00	0%				
Permethrin (0.75%)	23 ± 0.40	92%				

Table No. 4. Adulticidal efficacy of *Cassia fistula* against Malaria vector *Anopheles stephensi* after 48 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	11.50 ± 0.29	46%				
30 ppm	15.25 ± 0.25	61%	16.18	76.43	Y= 0.344 + 0.0212x	0.158
50 ppm	18.50 ± 0.29	74%	(-17.59 - 29.32)	(58.38-136.20)		(2)
70 ppm	22.25 ± 0.25	89%				

Table No. 5. Larvicidal efficacy of *Piper nigrum* against Malaria vector *Anopheles stephensi* after 24 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
3 ppm	10.75 ± 0.25	43%				
5 ppm	14.50 ± 0.29	58%	3.87	10.63	Y= 0.735 + 0.189x	0.052
7 ppm	18.50 ± 0.29	74%	(0.54 -5.29)	(8.54-17.46)		(2)
10 ppm	21.75 ± 0.25	87%				
Control	0.00	0%				

Table No. 6. Larvicidal efficacy of *Piper nigrum* against Malaria vector *Anopheles stephensi* after 48 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
3 ppm	16.00 ± 0.41	64%				
5 ppm	19.00 ± 0.41	76%	2.07	6.56	Y= 0.591 + 0.285x	0.696
7 ppm	22.75 ± 0.25	91%	(-2.24 – 3.54)	(5.39 - 9.58)		(2)
10 ppm	25.00 ± 0.0	100%				

Table No. 7. Larvicidal efficacy of *Cassia fistula* against Malaria vector *Anopheles stephensi* after 24 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	5.75 ± 0.25	23%				
30 ppm	9.50 ± 0.29	38%	44.99	106.12	Y= 0.943 + 0.0209x	0.002
50 ppm	13.50 ± 0.29	54%	(31.79 - 62.07)	(80.64-191.60)		(2)
70 ppm	17.50 ± 0.25	70%				
Control	0.00	0%				

Table No. 8. Larvicidal efficacy of *Cassia fistula* against Malaria vector *Anopheles stephensi* after 48 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
10 ppm	9.50 ± 0.29	38%				
30 ppm	13.50 ± 0.29	54%	24.40	76.30	Y= 0.602 + 0.0247x	0.240
50 ppm	17.75 ± 0.25	71%	(5.34 - 35.16)	(60.24-119.46)		(2)
70 ppm	22.25 ± 0.48	89%				

Table No. 9. Larvicidal efficacy of Permethrin against Malaria vector Anopheles stephensi after 24 hours

Concentrations (ppm)	Adult Mortality (Mean ± S.E)	% Mortality	LC_{50}	LC_{90}	Regression Equation	Chi square X^2 (df)
			95% Confidence limits			
0.10 ppm	12.00 ± 0.41	48%				
0.20 ppm	15.75 ± 0.41	63%	0.13	0.38	Y= 0.671 + 5.186x	1.356
0.30 ppm	19.00 ± 0.28	76%	(0.03 - 0.19)	(0.31 - 0.49)		(3)
0.40 ppm	22.75 ± 0.25	91%				
0.50	25.00 ± 0.00	100%				

REFERENCES

1. Rivero A, Vezilier J, Weill M, Read AF, Gandon S. Insecticide control of vector-borne diseases: when is insecticide resistance a problem? *PLoS pathogens*; **6**(8):e1001000.(2010)
2. Ellis BR, Wilcox BA. The ecological dimensions of vector-borne disease research and control. *Cadernos de saude publica*; **25 Suppl 1**:S155-67.(2009)
3. Akram W, Khan H, Hafeez F, Bilal H, Kim YK, Lee JJ. Potential of citrus seeds against dengue fever mosquito, Aedes albopictus. *Pak J Bot*; **42**(4):3343-8.(2010)
4. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. Climate change and vector-borne diseases: a regional analysis. *Bulletin of the World Health Organization*; **78**(9):1136-47.(2000)
5. World Health Organization. Manual on Practical Entomology in Malaria.Test procedures for insecticide

resistance monitoring in malaria vectors; bio-efficacy and persistence of insectidides on treated surfaces.(1975)
6. Kumar S, Warikoo R, Wahab N. Larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different instars of an indian strain of dengue fever mosquito, Aedes aegypti L. (Diptera: Culicidae). *Parasitology research*. Sep; **107**(4):901-7.(2010)
7. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vectors; bio-efficacy and persistence of insectidides on treated surfaces.(1998)
8. WHO. Guidelines for laboratory and field testing of mosquito larvicides. Geneva, World Health Organization.(2005)
9. Finney D. Probit analysis. London: Cambridge University Press. .68-72.(1979)
10. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of Cassia fistula Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitology research*. Jan; **102**(2):289-92.(2008)
11. Govindarajan M, Sivakumar R, M. R. Larvicidal efficacy of Cassia fistula Linn. leaf extract against Culex tritaeniorhynchus Giles and Anopheles subpictus Grassi (Diptera: Culicidae). *Asian Pac J Trop Dis*:295-8.(2011)
12. Govindarajan M. Bioefficacy of Cassia fistula Linn. (Leguminosae) leaf extract against chikungunya vector, Aedes aegypti (Diptera: Culicidae). *European review for medical and pharmacological sciences*. Mar-Apr; **13**(2):99-103.(2009)
13. Nath, D.R., Bhuyan M, Goswami S. Botanicals as Mosquito Larvicides. (2006).
14. Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, A. A. Ovicidal and repellent activities of botanical extracts against Culex quinquefasciatus, Aedes aegypti and Anopheles stephensi (Diptera: Culicidae) *Asian Pac J Trop Biomed*:43–8.(2011)
15. Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of Cassia obtusifolia Linn (Family: Leguminosae) leaf extract against malarial vector, Anopheles stephensi Liston (Diptera: Culicidae). *Parasitol Res*. Jan; **104**(2):337-40.(2009)