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Comparative larvicidal efficacy of six different agricultural insecticides against a *Culicine mosquito*

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This study aimed to investigate the mosquito larvicidal efficacy of pyrethroids (deltamethrin, cypermethrin and lambda cyhalothrin), organophosphates (chloropyrifos and dichlorvos) and a neonicotinoid (acetamiprid) against *Culex quinquefasciatus* of Chakdara, Dir Lower, Khyber Pakhtunkhwa Pakistan. Fourth instar larvae of *Culex quinquefasciatus* were initially exposed to various concentrations (0.001 to 1.0 ppm) of these insecticides to determine the activity concentration ranges. The highest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chloropyrifos, dichlorvos and acetamiprid that showed no insecticidal activity against *Culex quinquefasciatus* 4th instar larvae were 0.001, 0.0005, 0.000015, 0.001, 0.0005 and 0.001 ppm, respectively. The lowest concentration of these insecticides that were capable of insecticidal activity against mosquito larvae were 0.002, 0.001, 0.000031, 0.002, 0.001 and 0.002 ppm, respectively. The lowest concentrations of these insecticides that showed 100 % insecticidal activity against mosquito larvae were 0.5, 0.015, 0.008, 0.0625, 0.031 and 0.25 ppm, respectively. The significantly lowest ($P<0.05$) LC_{50} and LC_{90} values were observed for lambdacyhalothrin (LC_{50}=0.001 ppm, LC_{90}= 0.006 ppm). After lambda cyhalothrin, cypermethrin (LC_{50}=0.005 ppm, LC_{90}=0.017 ppm) and dichlorvos (LC_{50}=0.006 ppm, LC_{90}=0.027 ppm) were found more effective larvicidals against *Cx. quinquefasciatus* 4th instar larvae. From the findings of the present study, it was concluded that among deltamethrin, cypermethrin, lambda cyhalothrin, chloropyrifos, dichlorvos and acetamiprid, the most effective larvicides against the 4th instar larvae of *Cx. quinquefasciatus* mosquito of Chakdara, Dir Lower, are lambda cyhalothrin, cypermethrin and dichlorvos. Such information could be helpful in effective control of mosquito borne diseases in the area through integrated pest management.

Keywords: Deltamethrin, Cypermethrin, Lambda cyhalothrin, Chloropyrifos, Dichlorvos, Acetamiprid

INTRODUCTION

Culex quinquefasciatus (Say.) is the most widespread mosquito species in tropical and subtropical countries (Kramer et al. 2008; Andreadis, 2012) and can breed in a wide range of larval habitats such as ditches, cemented drains, rice fields, agricultural and construction pools, river margins, ponds, well, rice fields, stagnant water channels and marshes (Muturi et al. 2006; Ilahi and Suleman, 2013). *Cx. quinquefasciatus* causes serious nuisance through its irritating biting and acts as the vector of *Wuchereria bancrofti* that causes filariasis (also called elephantiasis) in different parts of the world (Rajasekariah et al. 1991; Ramaiah et al. 2003). Filariasis is affecting more than 100 million people in Asia, Africa, Central- and South America and the Pacific (Simonsen, 2009). In Pakistan, cases of human filariasis were reported from Sindh province during 1969 (Wolfe and AslamKhan, 1969). Beg et al. (2001) reported confirmed cases of tropical pulmonary eosinophilia in indigenous patients however this disease is very rare in Pakistan. *Cx. quinquefasciatus* is also the vector of avian malaria (Glad and Crampton, 2015), vector of West Nile virus and Saint Louis encephalitis virus in the southern United States of America (Rutledge et al. 2003; Hribar et al. 2003) and the vector of rift valley fever virus in Africa (Sang et al. 2010; Khan et al. 2015).

Control of mosquitoes is very important for the prevention of transmission of mosquito-borne diseases and protection of people and livestock from their nuisance biting. There is always a need for the control of disease transmitting mosquitoes. Generally, the application of chemical insecticides is practiced for the control of mosquitoes. The chemical agents which are used to kill the insect at its larval stage are known as larvicides. Similarly, the chemical agent toxic to eggs of the insect are called ovicides, those lethal to pupae are called pupicides and those lethal to adults or imagos are called adulticides or imagicide. Various chemical larvicides, pupicides and ovicides are applied to the breeding sites parallel with habitat reduction (Amer and Mehlhorn, 2006a; Amer and Mehlhorn, 2006b; Semmler et al. 2009; Benelli, 2015).

The synthetic chemical insecticides used against insect pests include carbamates e.g. carbofuran, methomyl etc., organophosphates e.g. dichlorvos, malathion, chlorpyrifos, parathion etc. and organochlorines e.g. DDT, endosulfan, dieldrin etc. (Gullan and Cranston, 2005). Pyrethroids are the organic compounds related to the natural pyrethrin, while the new generation nicotinoids or neonicotinoids are modeled on natural nicotine (Gullan and Cranston, 2005). Pyrethroids include deltamethrin, cypermethrin, lambda cyhalothrin etc. Neonicotinoids include imidacloprid and acetamiprid etc.

Dichloro Diphenyl Trichloroethane (DDT), an organochlorine, played major role in killing mosquito but its continuous and frequent use has damaged wild life and has negative impact on human health as it accumulates in human, therefore the application of such insecticides is no more recommended (Valdimir et al. 2002). Constant use of organochlorines has also resulted in the development of insecticide resistance in insect pests (Zacharia, 2011). Development of resistance to organophosphates in insect pests has also been reported (Siegfried and Scharf, 2001), however due to low mammalian toxicity, organophosphate such as temephos has been used widely as larvicides against mosquito (WHO, 2002). Development of resistance to different insecticides in different mosquito species has been reported. For example, Khan et al. (2011) reported moderate to high level of resistance in *Ae. albopictus* to agricultural insecticides in Pakistani for the first time. Thanispong et al. (2008) reported insecticide resistance to different insecticides in *Aedes aegypti* and *Cx. quinquefasciatus* from different localities in Thailand. Yadouléton et al. (2015) investigated the resistance status of *Cx. quinquefasciatus* to carbamate, pyrethroids and organochlorine in four different localities of the Republic of Benin.

Contamination of larval habitats by agricultural insecticides results in development of insecticide resistance in mosquitoes (Yadouleton et al. 2009; Chouaïbou et al. 2016). There is increasing trend of agricultural pesticides use in Swat valley (Nafees, 2008). The watershed of the Swat River is called Swat valley which comprises three main districts of Khyber Pakhtunkhwa (KPK): Swat, Malakand (Swat Ranizai Tehsil), and Lower Dir (Adenzai Tehsil). The main fruit plants in the study area are apple and peaches, both require heavy input of pesticides. In Swat Valley, till 1990, apple was grown on 44.4% area especially in the middle and upper parts (Inam, 2000), but now peaches grown on 60 % area of the Swat Valley (Nafees, 2008). The cereal crops of the area are wheat, maize and rice. A number of vegetables are also grown in the study area. To increase crop production, farmers use pesticides. Pesticides are applied in all crops but peaches need maximum use of pesticides, ranging from one spray in a month to two sprays (during flowering and fruit) (Nafees, 2008). Insecticides from agricultural sites reach to mosquito breeding sites through runoff during rainy season, and thus may affect larval population. Frequent application of agricultural pesticides for many years can exerts a huge selection pressure on larval stages of mosquitoes that can result in development of resistance to insecticides. *Cx. quinquefasciatus* is the most common and economically important mosquito species in Swat valley where it breeds in a wide range of larval habitats (Ilahi and Suleman, 2013). The present study aimed to investigate the activity concentration ranges and comparative efficacy of different agricultural insecticides against *Cx. quinquefasciatus* mosquito of Chakdara (part of

Swat valley), Dir Lower, Khyber Pakhtunkhwa Pakistan.

MATERIALS AND METHODS

Laboratory rearing of *Cx. quinquefasciatus*

Laboratory colonies of *Cx. quinquefasciatus* were maintained during May-October, 2016. For establishing the colonies, larvae of *Cx. quinquefasciatus* were collected by using a rectangular plastic dipper (38 cm length, 28 cm width and 6.5 cm height) from a stagnant water ditch near the campus of University of Malakand, Chakdara, Dir Lower, Khyber Pakhtunkhwa, Pakistan. The larvae were brought in 500 ml plastic containers with water from the collection site to the laboratory at the University of Malakand and reared for establishing a colony. Maximum temperature inside the laboratory was 29^0C to 35^0C. The larvae were provided with larval food comprising of dog biscuit and dry yeast powder in the ratio of 3:2. The pupae emerged were transferred to a 500 ml plastic jars containing 400 ml non-chlorinated tap water and placed in mosquito cage (45 cm × 45 cm × 45 cm). The adults emerged were initially fed with carbohydrate food by providing cotton pad soaked in 10% sucrose solution and later blood fed periodically by allowing mice for eggs development. The female adult mosquitos laid eggs in the water containing jars inside the cage which then hatched into larvae (1^{st} instar). Such colony of *Cx. quinquefasciatus* was existing during experiments. Thus, for experimentation, larvae of each instar, pupae and adults were available. For confirmation of species proper literature was used for identification of both larvae and adults (Harbach, 1988).

Selection of insecticides

During the present study, the insecticide dealers and the farmers who apply the insecticides on fruit trees, vegetables and cereal crops, were interviewed. It was found that the farmers mostly apply three pyrethroids i.e., deltamethrin, cypermethrin and lambda cyhalothrin, two organophosphates i.e., chlorpyrifos, dichlorvos and one neonicotinoid i.e., acetamiprid. During interview, the details of manufacturer of these insecticides which the farmers mostly apply were also inquired, both from the insecticide dealers and farmers. Farmers mostly apply deltamethrin (25 % w/w) of HERANBA Industries Limited, India, cypermethrin (10 % w/v) of M/S Halex (M) SDN (BDH), Malaysia, lambda cyhalothrin (2.5 % w/v) of Jiangsu Fengshan Group Co. Ltd, China, chloropyrifos (40 % w/v) of M/S Halex (M) SDN (BDH), Malaysia, dichlorvos (100 % w/v) of Insecticides India Limited and acetamiprid (20 % w/w) of Jiangsu Fengshan Group Co. Ltd, China. The reason of choice of insecticides of the above manufacturers by the farmers is the lower price. Therefore, the susceptibilty of *Culex quinquefasciatus* larvae to these agricultural insecticides were studie in the laboratory. Following are the details of the insecticides used and stock solution preparation:

Deltamethrin

Deltamethrin, a pyrethroid, was procured in packet as 25 % w/w powder (25 g active ingredient/100 g powder), manufactured by HERANBA Industries Limited, India. As the formulation shows, four grams (4000 mg) of the powder was containing one gram (1000 mg) active ingredient (ai). A 500 ml stock solution of 100 ppm concentration was prepared by dissolving 200 mg of the formulation in 500 ml volumetric flask in which some tape water was already added. Then further water was added to make a volume of 500 ml.

Cypermethrin

Cypermethrin, a pyrethroid, was procured as 10 % w/v (10 g active ingredient/100 ml solution) EC (emulsified concentration), manufactured by M/S Halex (M) SDN (BDH), Malaysia. As the formulation shows, the solution concentration is 100000 ppm (100000 mg/1000 ml). A 500 ml stock solution of 100 ppm was prepared by taking 0.5 ml (500 µL) of chloropyrifos 10 EC and putting it in 500 ml volumetric flask in which some water was already added. Then further water was added to make a volume of 500 ml.

Lambdacyhalothrin

Lambdacyhalothrin, a pyrethroid, was procured as 2.5 % w/v (2.5 g active ingredient/100 ml solution) EC (emulsified concentration) solution, Jiangsu Fengshan Group Co. Ltd, China. As the formulation shows, the solution concentration is 25000 ppm (25000 mg/1000 ml). A 500 ml stock solution of 100 ppm was prepared by taking 2 ml (2000 µL) of Lambdacyhalothrin 2.5 EC and putting it in a 500 ml volumetric flask which some non-chlorinated tap water was already added. Then further water was added to make a volume of 500 ml.

Chlorpyrifos

Chloropyrifos, an organophosphate, was procured as 40 % w/v (40 g/100 ml solution) EC (emulsified concentration) solution, manufactured by M/S Halex (M) SDN (BDH), Malaysia. As the formulation shows, the solution concentration is 400000 ppm (400000 mg/1000 ml). A 500 ml stock solution of 100 ppm was prepared by taking 125 µL (0.125 ml) of chloropyrifos 40 EC and putting it in 500 ml volumetric flask in which some tap water was already added. Then further water was added to make a total volume of 500 ml.

Dichlorovos

Dichlorvos (2.2-dichlorovinyl dimethyl phosphate), an organophosphate, was procured as 100 % w/v (100 g/100 ml solution) EC (emulsified concentration) solution, trade name Naogas Super, manufactured by Insecticides India Limited. As the formulation shows, the solution concentration is 10, 00000 ppm. I had to prepare 1000 ml stock solution of 2 ppm. A 500 ml stock solution of 100 ppm was prepared by taking 50 µL (0.02 ml) of dichlorovos 100 EC and putting it in 500 ml volumetric flask in which some tap water was already added. Then further water was added to make a total volume of 500 ml.

Acetamiprid

Acetamiprid, a neonicotinoid, was procured in packet as 20 % w/w powder (20 active ingredient/100 g), manufactured by Jiangsu Fengshan Group Co. Ltd, China. As the formulation shows, five grams (5000 mg) of the powder was containing 1gram (1000 mg) acetamiprid. A 500 ml stock solution of 100 ppm concentration was prepared by dissolving 250 mg of the formulation in 500 ml volumetric flask in which some tap water was already added. Then further water was added to make a total volume of 500 ml.

Activity concentration range bioassay

During this experiment a series of dilutions were prepared from stock solution of each insecticide and then tested against 4th instar larvae in the laboratory for determining the concentration ranges to be used in definitive bioassay for the determination and comparison of LC50 and LC90 values of different agricultural insecticides. During the experiment, WHO guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005) were followed with some modifications. The following are the details:

Preparation of solutions

According to Pakistan EPA (1997), the maximum permissible concentration of insecticides in municipal and liquid industrial effluents is 0.15 ppm. During this experiment, testing solutions of each insecticide were prepared. Their concentrations were above and below the permissible limit of Pakistan EPA (1997) for insecticides in municipal and liquid industrial effluents. For preparation of testing solutions (of different concentrations) of each insecticide, 200 ml solution of 2 ppm of each insecticide was separately prepared from the respective stock solution. This solution was then serially diluted several times by factor of 2 into 100 ml solutions of 1, 0.5, 0.25, 0.125, 0.0625, 0.03, 0.015, 0.008, 0.004, 0.002 and 0.001 ppm in 250 ml polyethylene cups. Control cups containing 100 ml non-chlorinated tap water were also arranged.

Exposure of mosquito larvae

Sufficient number of mosquito larvae were initially transferred frpm the mosquito breeding containers to a 400 ml polyethylene container containing 200 ml non-chlorinated tap water with the help of wide-mouthed plastic dropper. From this container, 10 4th instar larvae of *Culex quinquefasciatus* were transferred into each cup through small and porous plastic spoon. The purpose of use of porous spoon was to prevent the unecessary addition of water into the testing cup during introduction of mosquito larvae. The mortality of larvae was checked after 24 hours of exposure period. The criterion for death was lack of response to prodding. The aim of this experiment was to determince the concentartion range to be used in definitive test for dtermining the LC_{50} and LC_{90} values of each insceticide against the 4th instar larvae of *Cx quinquefasciatus*. The highest concentration that showed no insecticidal activity against mosquito larvae and similarly the lowest concentartion of an insecticide that showed 100 % insecticidal activity against mosquito larvae were determined. Deltamethrin, chlorpyrifos and acetamiprid showed no mortality of larvae at the lowest tested concentration, 0.001 ppm. However, the remaining three insecticides i.e., cypermethrin, lambda cyhalothrin and dichlorvos showed mortality at the lowest concentration (0.001 ppm). Therefore the 0.001 ppm solutions of these three insecticides were further diluted several times by factor of 2 and then the dilutions were tested for larvicidal activity so as to reach their highest concentrations that cause no mortality of larvae. Thus the activity concentration ranges (concentration ranges for lowest to 100 % mortality) of the insecticides determined during range finding bioassay were as under: deltamethrin 0.002 ppm to 0.5 ppm, cypermethrin 0.001 ppm to 0.015 ppm, lambda cyhalothrin 0.000031 to 0.008, chlorpyrifos 0.002 ppm to 0.0625 ppm, dichlorvos 0.001 to 0.03 and acetamiprid 0.002 to 0.25 ppm.

Definitive test

The aim of this experiment was to determine and compare the LC_{50} and LC_{90} values of six different agricultural insecticides against the 4th instar larvae of *Cx quinquefasciatus*. The concentration ranges of the insecticides determined during range finding bioassay were used in the definitive test. During this test, guidence was taken from the WHO guidelines (WHO, 2005) for laboratory and field testing of mosquito larvicides. Twenty 4th instar larvae of *Cx quinquefasciatus* were exposed to various concentartions of each insecticide within the concentartion range determined during range finding bioassay. Twenty 4th instar larave of *Cx quinquefasciatus* were also exposed in control containers containing non-chlorinated tap water. The experiment was run in triplicate. The detail of conatiners, testing solution volume and method of introduction of mosquito larave to the containers was the same as described above during range finding bioassay. The mortality of larvae was checked after 24 hours of exposure period. The percentage mortalities in insecticide solutions were corrected by applying the Abbott's formula (Abbott, 1925) when 5 % or more than 5 % mortality in control was observed (WHO, 2005). Following is the Abbott's formula:

$$\text{Corrected mortality} = \frac{\text{Mortality in treatment} - \text{Mortality in control}}{100 - \text{Mortality in control}} X\,100$$

Analysis of data

Data was presented as mean with standard error. The percentage mortality data were subjected to log probit analysis (Finny, 1971) for calculating LC_{50} and LC90 values, using SPSS 16 software. The LC50 values of different extracts were compared by 95 % confidence limits overlap method (Wheeler et al. 2006) for determining significance difference.

RESULTS

Activity concentration ranges

Table 1 show the highest concentrations of different agricultural insecticides that showed no insecticidal activity against *Cx. quinquefasciatus* 4th larvae. The highest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid that showed no insecticidal activity against *Culex quinquefasciatus* 4th instar larvae were 0.001, 0.0005, 0.000015, 0.001, 0.0005 and 0.001 ppm, respectively.

Table1: Highest concentrations of different agricultural insecticides that showed no insecticidal activity against *Cx. quinquefasciatus* 4th instar larvae.

Insecticides	NIAC (ppm)
Deltamethrin	0.001
Cypermethrin	0.0005
Lambda cyhalothrin	0.000015
Chlorpyrifos	0.001
Dichlorvos	0.0005
Acetamiprid	0.001

NIAC. –no insecticidal activity concentration

Table 2 show the lowest concentrations of different agricultural insecticides that were capable of insecticidal activity against *Cx. quinquefasciatus* 4th larvae. The lowest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid that were capable of insecticidal activity against *Culex quinquefasciatus* 4th instar larvae were 0.002, 0.001, 0.000031, 0.002, 0.001 and 0.002 ppm, respectively. Table 3 show the lowest concentrations of different agricultural insecticides that showed 100 % insecticidal activity against *Cx. quinquefasciatus* 4th larvae. The lowest concentrations of deltamethrin, cypermethrin, lambda cyhalothrin, chlorpyrifos, dichlorvos and acetamiprid that showed 100 % insecticidal activity against *Culex quinquefasciatus* 4th instar larvae were 0.5, 0.015, 0.008, 0.0625, 0.031 and 0.25 ppm, respectively.

Table2: Lowest Insecticidal activity concentrations of different agricultural insecticides against *Cx. quinquefasciatus* 4th instar larvae

Insecticides	LIAC (ppm)
Deltamethrin	0.002
Cypermethrin	0.001
Lambda cyhalothrin	0.000031
Chlorpyrifos	0.002
Dichlorvos	0.001
Acetamiprid	0.002

LIAC. - lowest insecticidal activity concentration

Table 3: Lowest concentrations of different agricultural insecticides that showed 100 % insecticidal activity against *Cx. quinquefasciatus* 4th instar larvae

Insecticides	100IAC (ppm)
Deltamethrin	0.5
Cypermethrin	0.015
Lambda cyhalothrin	0.008
Chlorpyrifos	0.0625
Dichlorvos	0.031
Acetamiprid	0.25

100IAC. - 100 % insecticidal activity concentration

Thus, the activity ranges (concentration ranges for lowest to 100 % insecticidal activity) of the insecticides determined during the range finding bioassay were as under: deltamethrin 0.002 ppm to 0.5 ppm, cypermethrin 0.001 ppm to 0.015 ppm, lambdacyhalothrin 0.000031to 0.008, chlorpyrifos 0.002 ppm to 0.0625 ppm, dichlorvos 0.001 to 0.03 and acetamiprid 0.002 to 0.25 ppm.

Definitive Test

The concentration ranges of the insecticides determined during range finding bioassay were used in the definitive test for determining the LC_{50} and LC_{90} values against the 4th instar larvae of *Cx quinquefasciatus*. The LC_{50} and LC90 values of all insecticides are shown in Table 4. The LC_{50} and LC_{90} values of all the tested insecticides were compared by 95 % confidence overlap method. The significantly lowest (at P<0.05 significance level) LC_{50} and LC_{90} values were observed for lambdacyhalothrin (LC_{50}=0.001 ppm, LC90= 0.006 ppm) when compared to the LC_{50} and LC_{90} values of all the remaining insecticides. The LC_{50} and LC_{90} values of cypermethrin (LC_{50}=0.005 ppm, LC_{90}=0.017 ppm) and dichlorvos (LC_{50}=0.006 ppm, LC_{90}=0.027 ppm) were significantly lower (at P<0.05 significance level) when compared with the LC_{50} and LC_{90} values of the remaining insecticides i.e., chlorpyrifos, acetamiprid and deltamethrin.

Table 4: 24-hour LC_{50} and LC_{90} values of different agricultural insecticides against *Cx. quinquefasciatus*

Insecticides	LC_{50} (ppm) with CL	LC_{90} (ppm) with CL
Deltamethrin	0.047 (0.038- 0.06) d	0.501 (0.338- 0.826 d
Cypermethrin	0.005 (0.005 -0.006) b	0-017 (0.014- 0.04) b
Lambda cyhalothrin	0.001 (0.0009- 0.001) a	0.006 (0.004- 0.009)a
Chlorpyrifos	0.016 (0.013- 0.019c	0.047 (0.036- 0.068) c
Dichlorvos	0.006 (0.005-0.007) b	0.027 (0.02- 0.039) b
Acetamiprid	0.04 (0.031 - 0.054) d	0.384 (0.241- 0.72)d

CL.-95% confidence limits. Means values with similar letters are not different significantly on the basis of confidence limit overlapping

DISCUSSION

During this study a pyrethroid, lambda cyhalothrin, was found most effective against the 4th instar larvae of *Cx. quinquefasciatus*. The LC_{50} and LC_{90} values of lambda cyhalothrin against *Culex quinquefasciatus* 4th instar larvae were 0.001 and 0.006 ppm, respectively. Pyrethroids are highly toxic for aquatic insects (Mian and Mulla, 1992). Disruption of ionic balance due to inhibition of ATPase enzymes (responsible for movement of ions against concentration gradients in aquatic insects) is the main toxic effect of pyrethroids (Coats et al. 1989).

During the study of organophosphates, dichlorvos was found more effective. The LC_{50} and LC_{90} of values dichlorvos against *Culex quinquefasciatus* 4th instar larvae were 0.006 and 0.027 ppm, respectively. Organophosphate insecticides cause toxicity through inhibition of acetyl cholinesterase, which is responsible for the degradation of the excitatory neurotransmitter, acetylcholine, thereby terminating transmission of nerve impulses at cholinergic synapses. Inhibition of this enzyme prolongs the residence time of acetylcholine at synapses resulting in hyper-excitation and eventual death (Fukuto, 1990).

During the study of neonicotinoid, the concentration of acetamiprid that caused lowest mortality of *Culex quinquefasciatus* 4th instar larvae was 0.002 ppm. The lowest concentration of acetamiprid that caused 100 % mortality (< 5 %) of *Culex quinquefasciatus* 4th instar larvae was 0.25 ppm. Neonicotinoid acts as agonists on nicotinic acetylcholine receptors (nAChRs) opening cation channels (Casida and Durkin 2013). At the same time, voltage-gated calcium channels are also involved (Jepson et al. 2006). This agonistic action results in continuous excitation of neuronal membrane, production of discharge that lead to paralysis and exhaustion of cell energy. This leads to the death of insects. Thus, the channel opening of nAChRs induced by the binding of neonicotinoids to receptors leads to insecticidal activity (Nishiwaki et al. 2003).

The LC_{50} and LC_{90} values of all the tested insecticides were compared by 95 % confidence overlap method. The significantly lowest (at $P<0.05$ significance level) LC_{50} and LC_{90} values were observed for lambdacyhalothrin (LC_{50}=0.001 ppm, LC90= 0.006 ppm). After lambda cyhalothrin, cypermethrin (LC_{50}=0.005 ppm, LC_{90}=0.017 ppm) and dichlorvos (LC_{50}=0.006 ppm, LC_{90}=0.027 ppm) were found more effective larvicidal against *Culex quinquefasciatus* 4th instar larvae.

Several synthetic chemical insecticides have been tested against the larvae of various species of disease transmitting mosquitoes. For example, Kalyanasundaran et al. (2003), tested the insecticidal efficacy of an organophosphate insecticide, Reldan (chlorpyrifos-methyl) and compared its insecticidal activity with Dursban (chlorphyrifos-ethyl) against the larvae of *Anopheles fluviatilis*, *Aedes aegypti*, *Anopheles stephensi* and *Annopheles culicifacies* in laboratory conditions and against the larvae of *Culex quinquefasciatus* in fields. Dursban and Reldan showed maximum larvicidal activity against the larvae of *Anopheles fluviatilis* with LC_{50} values of 5.90×10^{-7} and 1.07×10^{-9} ppm, respectively. In case of *Cx. quinquefasciatus*, Reldan showed nine times higher larvicidal activity when compared to Dursban. Bansal and Singh (2006), compared the larvicidal activity of three synthetic pyrethroids, cypermethrin, lambdacyhalothrin and cyfluthrin against the 3rd or early 4th instar larvae of *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Larvae of *Cx. quinquefasciatus* were found most susceptible followed by *Ae. aegypti* and *An. stephensi* to all the three pyrethroid insecticides. Sarar et al. (2011), conducted laboratory and field studies for testing the efficiency of temephos, *Bacillus thuringiensis* and pyriproxyfen against the immature stages of *Culex* species. Lowest LC_{50} value was observed for pyriproxyfen (0.00079 ppm) and temephos (0.0059 ppm) under laboratory conditions. Temephos was also found most effective in field conditions followed by *Bacillus thuringiensis*. However, the effectiveness of the insecticides decreased with the passage of time (at the 5th week). Nkya et al. (2014), exposed the larvae of *Anopheles gambiae* susceptible strain to sub-lethal concentrations of mixture of pesticides, herbicides and fungicides. Their subsequent tolerances were measured to deltamethrin, DDT, and bendiocarb. There occurred increase in larval tolerance to deltamethrin, DDT and bendiocarb following exposure to mixture of insecticides.

CONCLUSION

From the findings of this research, it is concluded that lambda cyhalothrin is highly effective larvicide against *Cx quinquefasciatus*. Such information could be helpful in effective control of mosquito borne diseases through integrated pest management.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

II and AMY designed and conducted the experiments and also wrote the manuscript. MA, AR, DN and TUH participated in the experiments and data analysis. All authors read and approved the final version.

REFERENCES

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. J Am Mosq Control Assoc 3, 302–303.

Amer A and Mehlhorn H, 2006b. Persistency of larvicidal effects of plant oil extracts under different storage conditions. Parasitol Res 99: 473–477.

Amer A, Mehlhorn H, 2006a. Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). Parasitol Res 99:466–472.

Andreadis TG, 2012. The contribution of Culex pipiens complex mosquitoes to transmission and persistence of West Nile virus in North America. J Am Mosq Control Assoc, 28 (4 Suppl): 137–151.

Bansal SK and Singh KV, 2006. Laboratory evaluation for comparative insecticidal activity of some synthetic pyrethroids against vector mosquitoes in arid region. J Environ Biol 27: 251-255.

Beg MA, Naqvi A, Zamanand V and Hussain R, 2001. Tropical Pulmonary Eosinophilia and Filariasis in Pakistan. Southeast Asian J Trop Med Public Health 32(1): 73-75.

Benelli G, 2015. Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: A systematic review. Parasitol Res 114: 3201–3212.

Chouaïbou MS, Fodjo BK, Fokou G, Allassane OF, Koudou BG. David JP, Antonio-nkondjio C, Ranson H and Bonfoh B, 2016. Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte d'Ivoire. Malar J 15(1): 426.

Coats JR, Symonik DM, Bradbury SP, Dyer SD, Timson LK and Atchison GJ, 1989. Toxicology of synthetic pyrethroids in aquatic organisms: An overview. Environ Toxicol Chem 8(8): 671–679.

EPA-Pakistan Environmental Protection Agency, 997. Sectorial guidelines for environmental reports–Major chemical and manufacturing plants.

Finney DJ, 1971. Probit analysis. Cambridge University Press, London, pp 68–78.

Fukuto TR, 1990. Mechanism of Action of Organophosphorus and Carbamate Insecticides. Environ. Health Perspect 87: 245-254.

Glad A and Crampton LH, 2015. Local prevalence and transmission of avian malaria in the Alakai Plateau of Kauai, Hawaii, U.S.A. J. Vector Ecol 40(2): 221-229.

Gullan PJ and Cranston PS, 2005.The Insects: An Outline of Entomology, 3rd Edition, Blackwell Publishing Ltd.

Hribar LJ, Vlach JJ, Demay DJ, Stark LM, Stoner RL and Godsey MS. et al, 2003. Mosquitoes infected with West Nile virus in the Florida Keys, Monroe County, Florida, USA. J Med Entomol 40 (3): 361–363.

Ilahi I and Suleman M, 2013. Species Composition and Relative Abundance of Mosqutoes in Swat, Pakistan. International Journal of Innovation and Applied Studies 2(4): 454-463.

Inam UR, 2000. Labor migration from the macro-environment and agriculture marketing in Mingora. Annual Report of Environmental Protection Society, Mingora, Swat, NWFP, Pakistan. 16-27.

Jepson JEC, Brown LA and Sattelle DB, 2006. The actions of the neonicotinoid imidacloprid on cholinergic neurons of Drosophila melanogaster. Invert Neurosci 6: 33–40.

Kalyanasundaran M, Jambulingam P, Sahu SS, Doss PS, Amalraj DD and Das PK, 2003. Efficacy of two organophosphorous insecticides, Reldan and Dursban against the larvae of *Culex quinquefasciatus*. Indian J Med Res 117: 25-29.

Khan AM, Dutta P, Sarmah CK, Baruah NK, Das S and Pathak AK, 2015. Prevalence of lymphatic filariasis in a tea garden worker population of Dibrugarh (Assam), India after six rounds of mass drug administration. J Vector Borne Dis 52 (4): 314–320.

Khan HA1, Akram W, Shehzad K and Shaalan E.A, 2011. First report of field evolved resistance to agrochemicals in dengue mosquito, Aedes albopictus (Diptera: Culicidae), from Pakistan. Parasit Vectors 22(4): 146.

Kramer LD, Styer LM and Ebel GD, 2008. A global perspective on the epidemiology of West Nile virus. Annu Rev of Entomol 53: 61–81.

Mian L and Mulla M, 1992. Effects of pyrethroid insecticides on non-target invertebrates in aquatic ecosystems. J Agric Entomol 9: 73–98.

Muturi J, Shililu J, Jacob B, Githure J, Gu W and Novak R, 2006. Mosquito species diversity and abundance in relation to land use in a riceland agro-ecosystem in Mwea Kenya. J Vector Ecol 31: 129-137.

Nafees M, Jan MR and Hizbullah K, 2008.

Pesticide use in Swat Valley, Pakistan: Exploring remodel measures to mitigate environmental and socio-economic impacts. Mt Res Dev 28 (3-4): 201-204.

Nishiwaki H, Nakagawa Y, Kuwamura M, Sato K, Akamatsu M, Matsuda K, Komai K and Miyagawa H, 2003. Correlations of the electrophysiological activity of neonicotinoids with their binding and insecticidal activities. Pest Manag Sci 59: 1023–1030.

Nkya TE, Mosha FW, Magesaa, SM and Kisinza WN, 2014. Increased tolerance of *Anopheles gambiae* S.S. to chemical insecticides after exposure to agrochemical mixture. Tanzan J Health Res 16 (4): 1-6.

Rajasekariah GR, Parab PB, Chandrashekar R, Deshpande L and Subrahmanyan D, 1991. Pattern of Wuchereria bancrofti microfilaraemia in young and adolescent school children in Bessein, India, an endemic area for lymphatic filariasis. Ann Trop Med Parasitol 85: 663 – 665.

Ramaiah K D, Das PK, Pani SP, Vanamail P and Pani KD, 2003. The impact of six rounds of single-dose mass administration of diethylcarbamazine or invermectin on the transmission of Wuchereria bancrofti by Culex quinquefasciatus and its implications for lymphatic filariasis elimination programs. Trop Med Int Health. 8 (12): 1082-1092.

Rutledge CR, Day JF, Lord CC, Stark LM and Tabachnick WJ, 2003. West Nile virus infection rates in Culex nigripalpus (Diptera: Culicidae) do not reflect transmission rates in Florida. J Med Entomol 40 (3): 253–58.

Sang R, Kioko E, Lutomiah J, Warigia M, Ochieng C and O'Guinn M, 2010. Rift Valley fever virus epidemic in Kenya, 2006/2007: the entomologic investigations. Am J Trop Med Hyg 83 (2): 28–37.

Sarar AS, Abobakr Y and Hussein HI, 2011. Laboratory and field evaluation of some chemical and biological larvicides against Culex spp. (Diptera: Culicidae) immature stages. Int J Agric Biol 13 (1): 115-119.

Semmler M, Abdel-Ghaffar F, Al-Rasheid K and Mehlhorn, H, 2009. Nature helps: From research to products against blood-sucking arthropods. Parasitol Res 105: 1483–1487.

Siegfried BD and Scharf ME, 2001. Mechanisms of Organophosphate Resistance in Insects. In: Ishaaya I. (eds) Biochemical Sites of Insecticide Action and Resistance. Springer, Berlin, Heidelberg.

Simonsen PE, 2009. Filariases. In: Cook GC, Zumla AI, editors. Manson's tropical diseases. 22. London: Saunders Elsevier. pp. 1477–1513.

Thanispong K, Sathantriphop S and Chareonviriyaphap T, 2008. Insecticide resistance of *Aedes aegypti* and *Culex quinquefasciatus* in Thailand. J Pestic Sci 33(4): 351–356.

Valdimir T, Valery R and Lorenzo T, 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. Environ Health Perspec 110 (2): 125-128.

Wheeler MW, Park RM and Bailer AJ, 2006. Comparing median lethal concentrationvalues using confidence interval overlap or ratio tests. Environ Toxicol Chem 25: 1441–1444.

WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. World Health Organization Communicable Disease Control, Prevention and Eradication WHO Pesticide Evaluation Scheme.

WHO. 2002. Malaria Entomology and Vector Control. Learners guide. World Health Organization, Geneva.

Wolfe MS, Aslamkhan M, 1969. Filariasis survey in Karachi. Trans R Soc Trop Med Hyg 63: 147.

Yadouléton A, Badirou K, Agbanrin R, Jöst H, Attolou R, Srinivasan R, Padonou G and Akogbéto M, 2015. Insecticide resistance status in *Culex quinquefasciatus* in Benin. Parasite & Vector 8: 17.

Yadouleton AW, Asidi A., Djouaka RF, Braïma J, Agossou CD, Akogbeto MC, 2009. Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. Malar J 8: 103.

Zacharia JT, 2011. Ecological Effects of Pesticides, Pesticides in the Modern World - Risks and Benefits, Dr. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-458-0.