MONITORING OF RESISTANCE STATUS IN FIELD POPULATION OF *CULEX QUINQUEFASCIATUS* (DIPTERA, CULICIDAE) IN DISTRICT HARIPUR

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Abstract

Mosquitoes belong to order Diptera, sub order Nematocera and family Culicidae. *Culexquinquefasciatus* Say, is a medically important mosquito and major pest species with aworldwide distribution. Indiscriminate use of pesticides causes environmental constraints such ashealth problem, disturbance of natural enemies, eco-system and resistance development in insectpests. Therefore, the present study was planned to minimize the excessive use of pesticides bythe alternative novel chemistry insecticides. Susceptible (%) and resistance (%) status of 3 rdinstar larvae of *Culexquinquefasciatus* against conventional chemicals (Lamda-cyhalothrin,Permathrin, Deltamathrin) and new chemicals (Spinosad, Ivermectin, Emamectin) wasdetermined by using their different concentrations rates viz., 100ppm,

125ppm & 150ppm afterdifferent exposure time (24hr, 48hr & 72hr). Generally, exposure time did not affect thesusceptibility and resistant % of *Culexquinquefasciatus*. Change in concentration directly affectsthe susceptibility and the resistance in case of both conventional and new chemistry chemicals.Lamda-cyhalothrine showing high value of LD_{50} (1.80) while spinosad showing low value ofLD_{50} (0.03). The Value of LD_{90} is high in Deltamethrin and spinosad is lowest value in alltreatments.

Keywords-*Culexquinquefasciatus*, Health problem, Pesticides, Resistance.

I. Introduction

The mosquitoes belong to Diptera order and known to be a group of tiny but considered as a major insect pest of medical importance (Rahman and Howlader 2018). They are considered as a key factor in transmitting many important pathogens, microbes and parasites from medical point of view including viruses, bacteria, protozoans, &nematodes and considerably the chief reasonfor inducing lethaldiseases including malaria, dengue, and Chikungunya (Becker *et al*., 2010). Furthermore, they also play a vital role in causing the health problems for humans in severalregions of the world. The vital genera of mosquito consist of *Anopheles, Aedes* and *Culex* out of which *Culex* is considered to be the one of the most crucial genus act as a transmitter in the formation of many harmful diseases in humans, birds, and other animals including West Nile virus, Japanese encephalitis, or St. Louis encephalitis, filariasis, and avian malaria (Rahman and Howlader 2018). In addition, they are also involving in causing irritation while biting through sucking blood. They are considering as irritating mosquitoes in urban as well as rural areas especially countries under development (Rahman and Howlader 2018).

The first case was recognized as West Nile virusin Harris County, TX in 2002 and is known to be the most dangerous mosquito that caused disease with *Cx.quinquefasciatus* (Stark *et al*., 2017). The viruses of West Nile cause considerablya very serious problem in the United States. Infection caused by WNV can result in a very big burden of infected person and the case of neuroinvasive WNV infection may result in a loss of around $624 to $439,945 in prolonged treatment& the patients (Staple *et al*., 2014)as viral attack may remain up to eight years post infection (Murray *et al*., 2013).Activemonitoring& mosquito can exposethe occurrence several weeks before animal transmission, which can leadto efforts as precaution of infection in humans(Healy *et al*., 2015).

The developmental cycle consists of about two weeks in hot weather for most of the mosquito species. The eggsare laid in rafts by female mosquito(about 300) on the surface of the water.Spill, pools, ditches, cans, water buckets, plastic bottles, and tanks for water storage serve as a suitable medium for egg laying in standing fresh water. Smallcigar-shaped and a dark browneggs stick to each other via adhesion forces. The eggs are not very tightly attached, and can be separated very easily.Presence of water is pre-requisite for eggs hatching. Larvae keep its position and predominantly have vertical orientation in water just becauseof movements of their bristly mouthparts. Larvae twitch their bodies in back and forth motion through the surface of water to swim (Carzoli, 2017; Mike, 2008).

Theinsectis totally immersed in water during larval stage and uses molecules oforganic matter and microorganism as find.After various instars, it develops in to a pupa. During pupae stage, there is no feeding and it has comma-shaped appearance. Pupa can swim by the motion of rapid jerking to avoid their potential predators. It is very important for it to remain in touch with the surface to carry out breathing. The pupa ruptures after 24–48 hours and the adult arises from the sheded exoskeleton.

Insecticides such as organochlorines, organophosphates, carbamates, andpyrethroids are considered to be key agents as control in worldwide programs of vector control (Low *et al*., 2013). Over-dependence and large scale useof insecticides have created resistance against insecticides(World Health Organization [WHO] 2006). As a matter of fact, insecticide resistance is not a new situation and is a terrible problem all around the world. Several cases have been reported from different regions of the world about the occurrence of resistance in*Cx. quinquefasciatus* to a number of insecticide classes (Sathantriphop*et al*., 2006; Kasai*et al*., 2007).

There are several control strategies for mosquitoes includingadulticiding with ultra-low volume (ULV), fogging, thermal fogging, surface residual spray, or household insecticide products specially designed against adult mosquitoes (Yap *et al*., 2000b). Generallya number of cases have been reported from many urban areas regarding the high level of resistance against organochlorine and organophosphate.Larvicidal strategy is alsoused worldwide against*Cx.quinquefasciatus*(Chavasse and Yap, 1997).

There are a number of concerns, hazards and harmful effects produced by the use ofbroad rangeconventional insecticides against mosquitoes that forces to use new chemistry insecticide that are efficient as well as safer to use, and less toxic to environment as compared to conventional insecticide (Korrat*et al*., 2012). However, new chemistry insecticides are much more specific and behave as specialists in pest control management program of particular pests (Bhatti *et al*., 2013). In order to enhance the production of crops with multiple pest scenario, a mixture of more than one insecticide are used having distinct chemical groups are used (Bhatti *et al.*, 2013; Ahmad *et al*., 2009). Insecticides mixtures in different compositions are assumed to increase the toxicity level in a cynergistic fashion against the target pests. There is a need to develop better strategies to mitigate these challenges and implement management strategies of insecticide resistance to develop innovatory and novel vector control tools. Current studies of monitoring resistance status of field population of *Cx.quinquefasciatus* at district Haripur was planned for helping in the development of effective control tools and assess the resistance level of the prevailing mosquito populations against their insecticides.

II. Objectives

1) What will be the toxic effect of conventional and new chemistry insecticides against wrigglers?
2) To monitor resistance in wrigglers of *Cx.quinquefasciatus*?
3) To findout the effective insecticide against *Cx. quinquefasciatus.*
4) To determine the LC_{50} and LC_{90} values of tested insecticides.

III. Material and methods

1. Experimental Materials:

This research work was conducted in Medical Entomology Laboratory at Nuclear Institute for Food & Agriculture (NIFA) Peshawar during 2019. Completely Randomized Design (CRD) having 10 replications was the planned design to conduct the experiment.

2. Collection and Rearing of Mosquito Larvae:

Collection of mosquito larvae was done from four different locations i.e. Khanpur, Havelian, Refugee camp and University area at District Haripur. Iron dipper was used by six random dips from each breeding sites includes (standing water, Irrigation channel, road sites etc). The field collected larvae were brought into laboratory for identification to species level. The culture was kept in larval trays at 26±1^{o}C and 75±5% RH inthe laboratory. Continuously larvae were fed with larval diet (IAEA) at 1, 2 and 3% concentrations for larval development. After pupations, the pupae were collected and transferred into adults rearing cages. After 2 days, adults were emerged and werefed with 10 % glucose solution and females fed on albino mice (as a source of blood) for egg laying. Usually third instars larvae of *Culex* mosquito was used for the experiment.

Laboratory Rearing and Protocols

Lab Conditions	Temperature 26±1^{o}C, relative humidity 75±5%
Larval Rearing.	Provided 3% with fish diet as per body requirement after hatching larvae according to their size and development rate.
Adult Rearing.	Solution of Sucrose and 2g sodium benzoate was used to rear adult.
Blood feeding	Blood was fed after five days of post emergence.
Schedule	Bovine blood was fed by using membrane to adults on Monday morning or at Friday afternoon for about 1 hour.
Egg collection	Egg cups were placed in adult cages after 2 days of t blood feeding. If Friday blood fed was done, then egg cups were placed in cages the same day after blood fed.
Egg Hatching	Rinse egg from blot paper to the water surface in the middle of a suspend wire, provided with pinch of diet to the medium for hatching larvae.

3. Insecticides used for Resistance Bioassays and effectiveness:

The following insecticides were used in the experiment.

1. Lamda-cyhalothrin.
2. Permethrin
3. Deltamethrin

New chemistry insecticides

1. Spinosad
2. Ivermectin
3. Emamectin benzoate

3.1. Development of susceptible strain of Cx.quinquefasciatus

The susceptible laboratory strain of *Cx.quinquefasciatus* was developed in the Entomology Laboratory for Comparison tests. For this purpose, collection was made from area of comparatively less selection pressure and Male pupae were selected from the early pupae coharts and were crossed with the female pupae of the late puape cohorts and subjected for few consective generations.

3.2. Evaluation of different insecticides

The following insecticides were used against *Cx.quinquefasciatus* larvae. They were used in three replications per treatment, Lamda-cyhalothrin, Permethrin, Deltamethrinand new chemistry insecticides Spinosad, IvermectinandEmamectin Benzoate.

4. Bioassays:

4.1. Susceptibility (%) and Resistance (%)

The Susceptibility (%) and Resistance (%)of conventional and new chemistry insecticides were evaluated at various concentrations 0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 100, 125 and 150 ppm) against theCulex mosquito in accordance with the guidelines of World Health Organization 2004. Batches of 50 third instars larvae of Cx.quinquefasciatus wereput in a small plastic disposable transparent cupshaving 100 ml distilled water and put in the netted area in the Laboratory room at 26±1^{o}C, 75±5% relative humidity. For the control group, the mosquito larvae were exposed to onlyplaindistilled water without addition of chemicals. Each tested concentration wasrepeated ten times. The insecticides were monitored carefully counting the susceptible and resistant ratio after 24, 48 and 72 hours.Susceptibility (%) and Resistance (%)were calculated by using the Abbot formula 1925.

Percent Susceptibility (%)= Number of susceptible larvae x100 / Number of larvae introduces

Percent Resistance (%) = Number of resistant larvae x100 / Number of larvae introduces

5.Tools

1. Hemotek device that maintained the temperature of the blood
2. Backpack aspirator
3. Dropper
4. Adults Mass rearing cages
5. Larval trays
6. Petri dishes small/ large size
7. Volumetric Flasks/ Beakers 250 ml
8. Micro Pipette (20-100ul)
9. Digital Balance
10. Ph. meter / Thermometer

6. Statistical Analysis

The experiments were conducted in Completely Randomized Design (CRD).The mean values and SD was calculated from ten replications. The susceptibility and resistance values were corrected by Abbott's formula and the lethal concentration values of LC50 and LC90 were calculated by using probability analysis program polo plus (version 1.5).

IV. RESULTS AND DISCUSSION

LAMDA-CYHALOTHRIN:

The analysis of variance for the application of old chemical (lamda-cyhalothrin) against the susceptibility (%) and resistance (%) level of*Cx.quinquefasciatus* have been presented in Appendix (1-2). Susceptibility (%) and Resistance (%) statusof 3rd instars larvae of *Cx.quinquefasciatus* in field population were directly affected bythe application of lamda-cyhalothrinat different concentrations rates as (0.125ppm, 0.25ppm, 0.5ppm, 1ppm, 2ppm 3ppm, 100ppm, 125ppm & 150ppm) at after different exposure of time (24hr, 48hr & 72hr) and shows significant difference with respect to different concentrations and time exposure.

Mean values regarding susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus* against lamda-cyhalothrin are presented in Table (1).Complete susceptibility (%) level is shown by lamda-cyhalothrin at 100ppm followed by 125ppm and 150ppm of concentration at 24 to 72 hours. The susceptibility (%) of *Cx.quinquefasciatus* against 2ppm and 3ppm concentrations of lamda-cyhalothrin were 83.33% to 86.67%, respectively at different exposure of time, which has been followed by 1ppm of concentration that is 80.00 % while the susceptibility (%) of *Cx.quinquefasciatus* against control is 0.00 %. C*x.quinquefasciatus* shows 100 % resistant against control which has been followed by the application of0.5, 0.25ppm and0.125ppm concentration of lamda-cyhalothrin that is33.33% to 36.67%, at 100ppm, 125ppm & 150ppm of concentration lamda-cyhalothrin shows the least resistance % that is 0.00 %. In overall results, it was found that variation of concentrations directly affects by the application of lamda-cyhalothrin on *Cx.quinquefasciatus* rather than time exposure. These results are in line with the findings of (Arivoli*et al*., 2011; Vasquez *et al*., 2009; Mohan *et al*., 2006). Estimation of susceptibility (%) and resistance (%) lamda-cyhalothrin shows that there are the more affected conventional insecticides in terms of insecticides against *Cx.quinquefasciatus* (Asidi*et al*., 2005).

Table 1: Mean values of susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus*against different concentrations of lamda-cyhalothrin at interval of time exposure

Concentration (ppm)	Time Exposure(hr.)	Susceptibility (%)	Resistance (%)
0.125	24	63.333 b	36.667 bc
	48	63.333 b	36.667 bc
	72	63.333 b	36.667 bc
0.25	24	63.333 b	36.667 bc
	48	66.667 b	36.667 bc
	72	66.667 b	36.667 bc
0.5	24	66.667 b	33.333 bc
	48	66.667 b	33.333 bc
	72	70.000 b	33.333 bc
1.0	24	80.00 ab	20.00 cd

	48	80.00 ab	20.00 cd
	72	80.00 ab	20.00 cd
2.0	24	83.333 ab	16.667 cd
	48	83.333 ab	16.667 cd
	72	83.333 ab	16.667 cd
3.0	24	83.333 ab	13.333 cd
	48	86.667 ab	13.33 cd
	72	86.667 ab	13,33 cd
100	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
125	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
150	24	100 a	0.00 d
	48	100 a	0.00 d
	72	100 a	0.00 d
Control	24	0.00 c	100 a
	48	0.00 c	100 a
	72	0.00 c	100 a
Mean		74.556	26.00
CV		20.14	58.61

Susceptibility Critical Value for Comparison 14.16

ResistanceCritical Value for Comparison 7.75

PERMATHRIN:

The analysis of variance for the application of conventional chemical (permathrin) against the susceptibility (%) and resistance (%) level of*Cx.quinquefasciatus* have been presented in Appendix (3-4). Susceptibility (%) and Resistance (%) status of 3rdinstars larvae of *Culex* in field population were directly affected by the application of permethrin at different concentrations rates as (100ppm, 125ppm & 150ppm) after different exposure of time (24hr, 48hr & 72hr) and shows significant difference with respect to concentrations and time.

Mean values regarding susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus*against Permathrinare presented in Table (2). Higher susceptibility (%) level isshown by permathrin at 150ppm concentration which is 76.67%, whereas, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150 ppm concentration. The susceptibility (%) of *Culex* against 125ppm concentration ofpermathrin was 73.33% which has been followed by 100ppm of concentration which is 70.00 % while the susceptibility (%) of *Cx.quinquefasciatus*against control is 0.00 %*Cx.quinquefasciatus* shows 100 % resistance against control which has been followed by the application of 100ppm concentration of permathrin that is 30.00 %, at 125 ppm concentration its resistance is 26.67% while at 150ppm concentration *Cx.quinquefasciatus* shows the least 23.33% resistance with respect to all the other concentrations of the application of permathrin. In overall results, It was found that variation of concentrations directly affects the application of permathrin on *Cx.quinquefasciatus* rather than time exposure. These results are in line with the findings of (Corbel *et al*., 2007; Sombon*et al*., 2003; Rao *et al*., 1995). Estimation of susceptibility (%) and resistance (%) of insect pests play a significant role in any vector control program in addition to the knowledge of this status assists significantly in devising a long term sustainable control vector population (Brogdon *et al*., 1998).

Table 2: Mean values of susceptibility (%) and ResisStance (%) of *Cx.quinquefasciatus* against different concentrations of permathrin at interval of time exposure

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	70.00 a	30.00 b
	48	70.00 a	30.00 b
	72	70.00 a	30.00 b
125	24	73.33 a	26.67 b
	48	73.33 a	26.67 b
	72	73.33 a	26.67 b
150	24	76.67 a	23.33 b
	48	76.67 a	23.33 b
	72	76.67 a	23.33 b
Control	24	0.000 b	100 a
	48	0.000 b	100 a
	72	0.000 b	100 a
Mean		54.16	45.83
CV		19.293	39.819

Susceptibility Critical Value for Comparison 22.98
ResistanceCritical Value for Comparison 19.90

DELTAMATHRIN:

The analysis of variance for the application of conventional chemical (deltamathrin) against the susceptibility (%) and resistance (%) level of *Culex* have been presented in Appendix (5-6).Susceptibility (%) and Resistant (%) statusof 3rd instars larvae of*Culex* in field population were directly affected by the application of deltamathrinat different concentrations rates as (100ppm, 125ppm & 150ppm) after different exposure of time (24hr, 48hr & 72hr) and shows significant difference with respect to concentrations and time.

Mean values regarding susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus* against deltamathrin are presented in Table (3). Higher susceptibility (%) level were shown by deltamathrin at 150ppm of concentration which is 93.33%, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150ppm concentration. The susceptibility (%) of *Cx.quinquefasciatus* against 125ppm concentration of deltamathrin were same as found in 150ppm concentration that's is 93.33% which has been followed by 100ppm of concentration which is 60.00 % during time duration from 24 to 48hrs while at 72hrs its susceptibility was reduced upto 56.00 %. The susceptibility (%) of *Cx.quinquefasciatus* against control is 0.00 %. *Culex* shows 100 % resistance against control which has been followed by the application of 100ppm concentration of deltamathrin that is 36.67 at 24 to 48hrs while the resistance % was increased upto 40.00% during 72hrs, at 125 ppm concentration its resistance is 13.33 % at 150ppm concentration.*Culex*shows the least 6.67 % resistance with respect to all the other concentrations of the application of deltamathrin. In overall results, found that variation of concentrations directly affects the application of deltamathrin on *Culex.*These results are in line with the findings of (Guessan*et al*., 2010; Mosha*et al*., 2008; Raghavandra*et al*., 2011). Estimation of susceptibility (%) and resistance (%) shows inverse relation with each other as the concentration of deltamathrin increases the susceptibility percent increases and vice versa to the resistance %.

Table 3: Mean values of susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus*against different concentrations of deltamathrin at interval of time exposure

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	56.00 a	40.00 b
	48	60.00 a	36.67 b
	72	60.00 a	36.67 b
125	24	93.33 a	13.33 b
	48	93.33 a	13.33 b
	72	93.33 a	13.33 b
150	24	93.33 a	6.67 b
	48	93.33 a	6.67 b
	72	93.33 a	6.67 b
Control	24	0.000 b	100.0 a
	48	0.000 b	100.0 a
	72	0.000 b	100.0 a
Mean		61.38	39.44
C V		41.08	66.94

Susceptibility Critical Value for Comparison 24.53

ResistanceCritical Value for Comparison 21.25

SPINOSAD:

The analysis of variance for the application of new chemical (spinosad) against the susceptibility (%) and resistance (%) level of *Cx.quinquefasciatus* have been presented in Appendix (7-8). Susceptibility (%) and Resistance (%) statusof 3rd instars larvae of *Culex* in field population were directly affected by the application of spinosadat different concentrations rates as (100ppm, 125ppm & 150ppm) after different exposure of time (24hr, 48hr & 72hr) and shows significant difference with respect to concentrations and time.

Mean values regarding susceptibility (%) and Resistance (%) of *Culex* against spinosadare present in Table (4). Higher susceptibility (%) level was shown by spinosad at 150ppm of concentration which is 66.67%, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 100ppm concentration. The susceptibility (%) of *Cx.quinquefasciatus* against 125ppm concentration of spinosad were 67.67% at 24hr while its susceptibility was change up to 60.00% 48 and 72hrs of time interval. which is been followed by 100ppm of concentration that is 59.33 % while the susceptibility (%) of *Cx.quinquefasciatus* against control is 0.00 %. *Cx.quinquefasciatus* shows 100% resistant against control which has been followed by the application of 100ppm concentration of spinosad that is 40.67%, at 125ppm concentration its resistance is 34.33 % to 33.33 % with the change of time interval while at 150ppm concentration *Culex*shows the least 33.33% resistance with respect to all the other concentrations of the application of spinosad. In overall results, found that variation of concentrations directly affects the application of spinosad on *Culex* rather than time exposure. These results are in line with the findings of (Su and Cheng, 2014; Jiang and Mulla, 2009; Liu *et al*., 2014). Estimation of susceptibility (%) and resistance (%) of insect pests play a significant role in any vector control program in addition to the knowledge of this status assists significantly in devising a long term sustainable control vector population (Brogdon *et al*., 1998).

Table 4: Mean values of susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus* against different concentrations of spinosad at interval of time exposure.

Concentration (ppm)	Time Exposure(hr.)	Susceptibility (%)	Resistance (%)
100	24	59.33 a	40.67
	48	59.33 a	40.67 b
	72	59.33 a	40.67 b
125	24	66.67 a	34.33 b
	48	66.00 a	34.00 b
	72	66.00 a	33.33 b
150	24	66.67 a	33.33 b
	48	66.67 a	33.33 b
	72	66.67 a	33.33 b
Control	24	0.00 b	100.0 a
	48	0.00 b	100.0 a
	72	0.00 b	100.0 a
Mean		48.056	51.972
C V		55.27	51.11

Susceptibility Critical Value for Comparison 25.84
ResistanceCritical Value for Comparison 22.37

EMAMECTIN:

The analysis of variance for the application of new chemical (emamectin) against the susceptibility (%) and resistance (%) level of *Culex* have been presented in Appendix (9-10). susceptibility (%) and resistant (%) statusof 3rd instars larvae of *Cx.quinquefasciatus* in field population were directly affected bythe application of emamectinat different concentrations rates as (100ppm, 125ppm & 150ppm) after different exposure of time (24hr, 48hr & 72hr) and shows significant difference with respect to concentrations and time.

Mean values regarding susceptibility (%) and Resistance (%) of *Culex* against emamectin are presented in Table (5). Higher susceptibility (%) level was shown by emamectin at 150ppm of concentration which is67.67 %, change in time from 24 to 72 hours did not show any variation in susceptibility (%) at 150 ppm concentration. The susceptibility (%) of *Cx.quinquefasciatus* against 125ppm concentration of emamectin were 66.67% at 24hrs while it's were lower down up to 60.00% during 48 &72hrs of exposure time, which has been followed by 100ppm of concentration that is 62.67% while the susceptibility (%) of *Culex* against control is 0.00%. *Cx.quinquefasciatus* shows 100 % resistance against control which has been followed by the application of 100ppm concentration of emamectin that is 37.33 %, at 125ppm concentration its resistant is 33.33 to 34.33% during the exposure of time from 24hrs to 72hrs while at 150ppm concentration *Culex*shows the least 32.33% resistance with respect to all other concentrations of the application of emamectin.

In overall results, it was found that variation of concentrations directly affects the application of emamectin on *Culex*rather than time exposure. These results are in line with the findings of (Shah *et al*., 2016; Zahran*et al*., 2013; Buss *et al*., 2002). Estimation of susceptibility (%) and resistance (%) of emamectin shows that variation in concentration and time exposure did not directly affect the growth of insect.

Table 5: Mean values of susceptibility (%) and Resistance (%) of *Cx.quinquefasciatus* against different concentrations of ememectin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	62.67 a	37.33 b
	48	62.67 a	37.33 b
	72	62.67 a	37.33 b
125	24	66.67 a	33.33 b
	48	66.00 a	34.00 b
	72	66.00 a	34.33 b
150	24	67.66 a	32.33 b
	48	67.66 a	32.33 b
	72	67.66 a	32.33 b
Control	24	0.00 b	100 a
	48	0.00 b	100 a
	72	0.00 b	100 a
Mean		49.139	54.40
C V		56.34	54.40

Susceptibility Critical Value for Comparison 25.84

ResistanceCritical Value for Comparison 22.37

IVERMECTIN:

The analysis of variance for the application of new chemistry chemical (ivermectin) against the susceptibility (%) and resistance (%) level of *Culex*have been presented in Appendix (11-12). Susceptibility (%) and resistance (%) statusof 3rd instars larvae of *Culex* in field populationwere not affected by the application of ivermectinat different concentrations rates as (100ppm, 125ppm & 150ppm) and after different exposure of time (24hr, 48hr & 72hr) and shows non-significant difference with respect to concentrations and time.

Mean values regarding susceptibility (%) and Resistantce(%) of *Culex* against ivermectin are presented in Table (6). Susceptibility % of *Cx.quinquefasciatus*aginst all the concentrations (100, 125 & 150ppm) and at different time exposure (24, 48 & 72hrs) were 0.00% while resistance % of *Cx.quinquefasciatus*against all the concentrations (100, 125 & 150ppm) and at different time exposure (24, 48 & 72hrs) were 100 %.

Table 6: Mean values of susceptibility (%) and Resistant (%) of *Cx.quinquefasciatus*against different concentrations of ivermectin at interval of time exposure.

Concentration (ppm)	Time Exposure (hr.)	Susceptibility (%)	Resistance (%)
100	24	0.00	100
	48	0.00	100

	72	0.00	100
125	24	0.00	100
	48	0.00	100
	72	0.00	100
150	24	0.00	100
	48	0.00	100
	72	0.00	100
Control	24	0.00	100
	48	0.00	100
	72	0.00	100
Mean		0.00	100

Susceptibility Critical Value for Comparison 00.00

ResistanceCritical Value for Comparison 00.00

LETHAL DOSE CONCENTRATION (ppm):

Efficacy of both conventional (Lamda-cyhalothrin, Permathrin andDeltamathrin) and new chemistry chemicals (Spinosad, Ivermectin andEmamectin) were observed against 3rd instar *Culex* and LD_{50} and LD_{90} values observed at 12hours interval with the application of different concentrations of these chemicals and observe their affects. Showing lethal dose concentration (ppm) for different treatments. Lamda-cyhalothrin showing high value of LD_{50} (1.80) while spinosad showing low value of LD_{50} (0.03). Similarly lambda-cyhalothrin shows maximum percentage of 95%Cl (3.884) while spinosad shows minimum of 95%Cl (0.69). Value of LD_{90} is high in Deltamethrin and spinosad is lowest value in all treatments. Theslop and Chi square (x2) value of lambda-cyhalothrin is highest (14.787) in table while Emamectine show shows lowest value (0.01). (Ahmad, 2008, 2009; Ahmad *et al*., 2009).

Table 7: Lethal dose concentration (ppm):

Treatments	Lethal dose concentration (ppm)					
	LD_{50}	**95% CL**	**LD_{90}**	**95% CL**	**Slope**	**$\chi 2$**
Lambda cyclothrin	1.80	3.882	0.022	0.119	0.28	14.787
Deltamethrin	0.08	0.73	9.77	0.13	0.43	0.05
Permethrin	0.08	2.58	0.00	0.06	0.44	3.80
Emamectin	0.49	0.99	0.09	0.78	0.16	0.01
Ivermectin	0.09	0.12	0.06	0.11	0.23	2.39
Spinosad	0.03	0.09	0.00	0.00	0.16	0.132

V. CONCLUSION AND RECOMMENDATIONS

Susceptibility (%) and resistance (%) status of 3rd instars larvae of *Culex*in field population were analyzed against conventional chemicals (Lamda-cyhalothrin, Permathrin, Deltamathrin) and new chemistry chemicals (Spinosad, Ivermectin, Emamectin)by using their different concentrations rates as (100ppm, 125ppm & 150ppm) after different exposures of time (24hr, 48hr & 72hr) mostly did not affect the susceptibility (%) and resistant(%)of *Culex* While change in concentration directly affectsthe susceptibility and resistance percent in both conventional and new chemistry chemicals. Not even a single concentration of ivermectin effects *Culex,* it shows 0.00% susceptibility and 100% resistant. Lamda-cyhalothrin from conventional chemicals and ememectin from new chemicals shows the best results against*Culex*. So in future the effect in combination of both these chemicals should be analyzed to get even better results. For the tested both conventional and new chemicals follows the same pattern lambda-cyhalothrinshowing high value of LD_{50} (1.80) while spinosad showing low value of LD_{50} (0.03). Similarly lambda-cyhalothrinshows maximum percentage of 95%Cl (3.884) while spinosad shows minimum of 95%Cl (0.69). Value of LD_{90} is high in Deltamethrin and spinosad is lowest value in all treatments. Theslop and Chi square (x2) value of Lamda-cyhalothrinis highest (14.787) in table while Emamectine show shows lowest value (0.01).

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The preferred spelling of the word "acknowledgment" in American English is without an "e" after the "g." Use the singular heading even if you have many acknowledgments.

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