

## Insecticide Resistance in Sand Flies (*Phlebotomus Papatasi*) against Bifenthrin and Cypermethrin in Chakwal, Pakistan

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### Abstract:

*Sand flies are the most important vector after mosquitoes carrying different pathogens to human beings. In different parts of the world sand flies are responsible for causing the Leishmaniases in human beings. Different insecticides are used for the control of sandflies. A study was conducted to test the insecticide resistance level in sand flies collected from five different localities of the city against two synthetic pyrethroids i.e. bifenthrin and cypermethrin. Results showed that sand flies (Phlebotomus papatasi) collected from the 7*

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*Marla Scheme and Christian colony were resistant against bifenthrin and cypermethrin (F value 13.3178 while the P value is 0.0000). While sand flies (*Phlebotomus papatasi*) collected from Madina town, Ashraf town and Odharwal were totally susceptible against the applied insecticides.*

**Key words:** Insecticide resistance, bifenthrin and cypermethrin, Sand flies, Chakwal

## **Introduction**

Leishmaniasis is the second most important and emerging protozoal disease after the malaria in term of number of people affected (WHO, 2010). Leishmaniasis is caused by the vector belongs to the genus *Leishmania*. It is the 88<sup>th</sup> most important public health problem around the world in relation to mortalities and morbidities. It is also important in relation serious economic loss and effects very badly the socio economic development in many countries (WHO, 2006).

Sand flies are the most important vector of leishmaniasis. *Phlebotomine* is the only one proved insect that is considered the vector of leishmaniasis which is transmitted by the infected female that previously feed on the infested mammals. Sand flies transmit the pathogens by following ways (a) congenital transmission (Eltoum et al., 1992) (b) venereal transmission (Symmers, 1960) (c) infection by the blood transfusion (Bruce-Chwatt, 1972) and (d) needle transmission among the user of drugs (Alvar, Aparicio, & Aseffa, 2008). But some scientists reported that Leishmaniasis is also transmitted by the bites of insects like fleas and ticks but it is not supported by the experiments (R. Killick-Kendrick, 1999). Leishmaniasis is the most important endemic disease in over 70 countries of the world and causes about 1500000 cases of disease around the

world 1(WHO, 1998). This is due to large number of traveling of peoples around the world and disturbance in the equilibrium ecological conditions of the world.

Sand flies also transmit different pathogens in humans like *Bartonella* spp, different viral pathogens are transmitted by the sand flies known as sandfly fever vesicular stomatitis, summer meningitis and chandipura virus encephalitis (Depaquit, Grandadam, Fouque, Andry, & Peyrefitte, 2010). Significant resurgence over the last decades has been occurring to vector born diseases including malaria, dengue and leishmaniasis. In most cases the appearance of new diseases and resurgence of old diseases occur due to ecological and climatic changes that have favoured increased the density of diseases. Different irrigation, dam construction, deforestation and urbanization all these factors helped to increase the vector population densities. Travel of human beings from one place to another is the easy method of spread of these infectious disease causing vectors (Colwell, Dantas-Torres, & Otranto, 2011).

In Pakistan near about 37 species of sand flies have been discovered (Kakarsulemakhel, 2004b). *Phlebotomus papatasi* is the predominant sand fly specie in Pakistan. The climatic conditions of the Afghanistan and some parts of Western border of Pakistan have less or more same so in these areas *P. papatasi* is the most important specie (R. Killick-Kendrick, M, Killick-Kendrick, & Y.Tang, 1994). In Pakistan sporadically outbreaks of the Leishmaniasis occur throughout the year but sometime more frequent outbreaks are also occurs. Over the last few decades leishmaniases outbreaks occurs in large numbers (AM, RA, S, & Y., 2003). Endemic form of disease occurs once in Baluchistan while it is highly prevalent in Sindh province. It is also prevalent in some parts of KPK and Punjab (Soomro et al., 2009).

## **Materials and Method**

Experiment was conducted at University College of Agriculture, University of Sargodha Punjab, Pakistan. Sand flies (*Phlebotomus papatasi*) were collected from five different places of the city Chakwal (Madina Town, Ashraf town, Odharwal, and 7marla scheme). About 100 sand flies were collected from each location of the city so the total numbers of sand flies collected from five locations were about 500 hundred. Collected sand flies were placed in five different boxes tagging of these boxes was done by the name of location from where collection was done. Rearing of sand flies in lab was done for five generations to get the susceptible generation. Blood feeding of sand flies was done white rats reared in the laboratory. When the laboratory reared strain is ready for the experiment then another collection was done from the same places. All the strains are kept separate in small boxes and tagging was done. Paper bio assayed method was used to test the insecticide resistance in sand flies.

## **Bioassay Tests**

Two synthetic pyrethroids were used in the experiment (Bifenthrin and cypermethrin) to determine the insecticide resistance in sand flies population collected from five different locations of the city chakwal (Madina Town, Ashraf town, Christian colony, Odharwal, 7marla scheme). Both insecticides were selected from one class synthetic pyrethroid because it is highly used in Pakistan against the household insect pests. Optimum conditions of about 26°C temperature and 70 - 80% relative humidity were maintained for the experiment. Stock solutions of 0.75% of both Bifenthrin and Cypermethrin were prepared for the application. Three treatments namely bifenthrin, cypermethrin and one control were used and each

treatment was replicated thrice. In each replication 15 sand flies (*Phlebotomus papatasi*) were released in the petri dish having paper treated with insecticides. Papers were treated with selected insecticide with the help of plastic pipet and when the paper was dried then it was placed in the petri dishes. Data was collected after 10mint, 20, 30, 40, 50 and 60 mints and noted the total number of dead and surviving sand flies. After the 60 mints surviving sandflies were transferred into clean boxes and placed the cotton plug soaked in 30% sucrose solution.

## Results

Results obtained from the experiment (Insecticide resistance in sand flies (*Phlebotomus*) against bifenthrin and cypermethrin in Chakwal was shown in table 1. WHO standard method was used for the identification of resistant strain. According to this any strain having mortality less than 80% was considered as indicator of resistance. Sand flies Collected from the Madina town shows high susceptibility against Bifenthrin ( $13.667 \pm 0.333$ ) and Cypermethrin ( $13.667 \pm 0.333$ ). It showed 91% mortality against Bifenthrin while in case of Cypermethrin it again showed the 91% mortality. While in case of sand flies collected from the Odharwal showed susceptibility against the both bifenthrin and Cypermethrin. But in this case number of killed sand flies was low against the bifenthrin so it showed the 88% mortality on the other hand Cypermethrin showed the highest mortality it is effective upto 93% of the sand flies. While the sand flies collected from Christian colony and 7 Marla scheme were showed resistance against both bifenthrin and cypermethrin. Sand flies collected from both these locations showed 50-55% mortalities against both bifenthrin and cypermethrin. Percent mortalities of the sand flies were calculated by using the Abbot's formula i.e.

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Corrected % =  $(1 - \frac{n \text{ in T after treatment}}{n \text{ in T after treatment}}) * 100$

Where: n= insect population, T= treated, Co= control

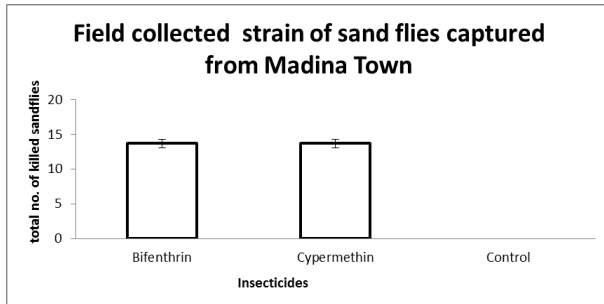
**Table 1: shows the number and percentage of mortality sand flies (*Phlebotomus papatasi*) from five different localities of Chakwal, Punjab, Pakistan exposed to two synthetic pyrethroids.**

Location	Insecticide tested	No of tested (replicated)	Mortality (%) of 3 replicates after 1 hours (Field collected strain)	Mortality (%) of 3 replicates after 1 hours (lab reared strain)
Madina town	Bifenthrin	45(3)	91	100
	Cypermethrin	45(3)	91	100
	Control	45(3)	0.0444	6.666
Odharwal	Bifenthrin	45(3)	88	100
	Cypermethrin	45(3)	93	100
	Control	45(3)	0.0444	0
Ashraf Town	Bifenthrin	45(3)	86	100
	Cypermethrin	45(3)	93	97.7
	Control	45(3)	0.1555	0
Christian Colony	Bifenthrin	45(3)	53	95
	Cypermethrin	45(3)	53	100
	Control	45(3)	0.022	13.33
7 Marla scheme	Bifenthrin	45(3)	51	97.777
	Cypermethrin	45(3)	55	97.777
	Control	45(3)	0.00	33.333

**Table 2: Percent mortality of Sand flies calculated by using the Abbot's formula**

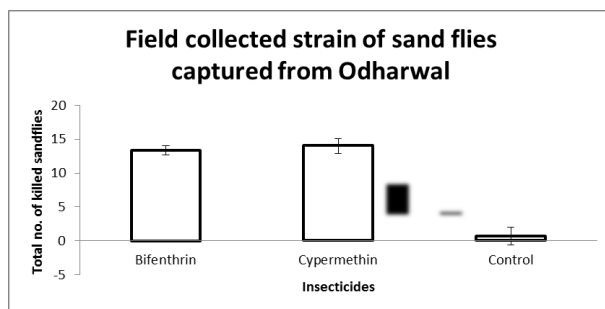
Sr.no.	Locality	Percent mortality (%) In field collected strain		Percent mortality (%) in laboratory reared strain	
		Bifenthrin	Cypermethrin	Bifenthrin	Cypermethrin
1	Madina Town	86.67	86.67	100	100
2	Odharwal	86.67	92.86	100	100
3	Ashraf town	75	91.67	93.33	100
4	Christian Colony	50	50%	100	100
5	7marla scheme	46.67	60	100	100

## 1. Insecticide resistance in sand flies captured from Madina Town



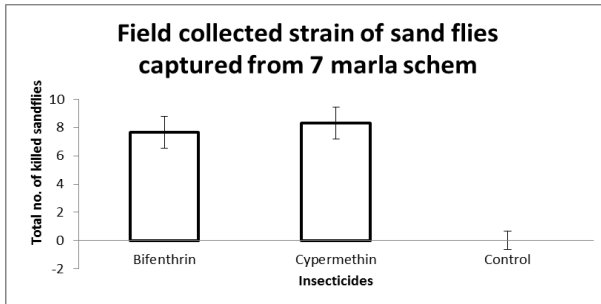
Result showed that the sand flies collected from the Madina town were highly susceptible against the both selected synthetic pyrethroid insecticides (Bifenthrin and Cypermethrin). Both bifenthrin and cypermethrin were highly effective against the sand fly strain these insecticides killed about 13.66667 sandflies from 15 released in the treated box so they produces about 91 % results.

## 2. Insecticide resistance in sand flies captured from Odharwal



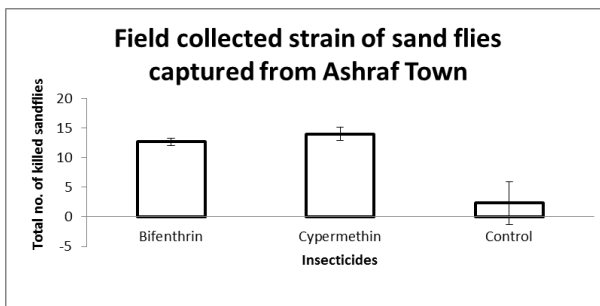
Sand flies collected from the Odharwal were moderately susceptible against the bifenthrin while in case of Cypermethrin sand flies are again highly susceptible to Cypermethrin and it kills about 14 flies from the 15 which were released in the treated dishes.

### 3. Insecticide resistance in sand flies captured from 7 Marla scheme



Sand flies collected from the 7 marla schem were found resistant against the both bifenthrin and Cypermethrin. Both the insecticides kill about 7.66667 and 8.333333 which was about 51% and 55% of the total released sand flies into the petri dishes.

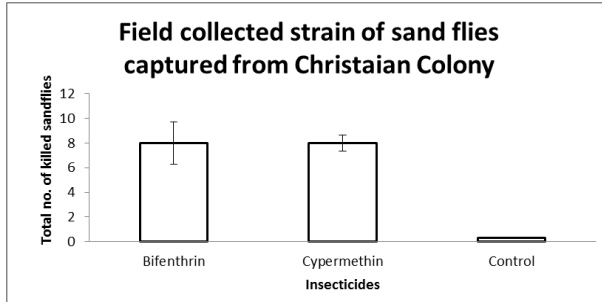
### 4. Insecticide resistance in sand flies captured from Ashraf Town



Sand flies of the Ashraf town showed variable results against synthetic pyrethroids. In case of Cypermethrin it produces about 93% results were obtained which indicated that sand flies are highly susceptible against the Cypermethrin.

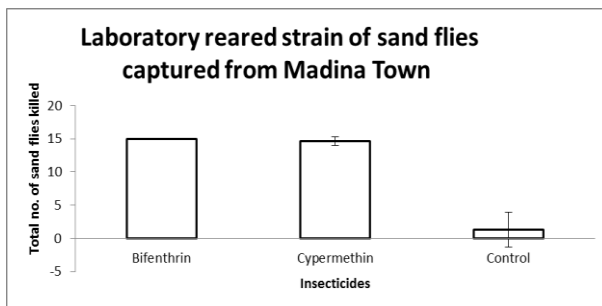


## 5. Insecticide resistance in sand flies captured from Christaian Colony



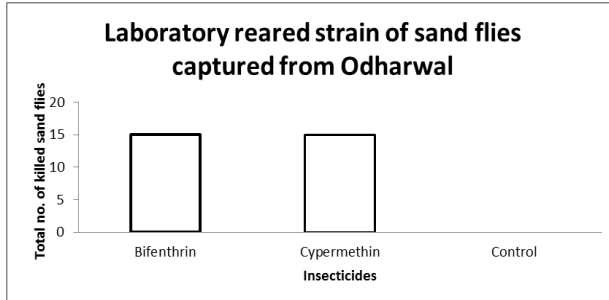
Last one graph showed that sand flies collected from the Christian colony showed high resistance against both insecticides i.e. Cypermethrin and bifenthrin. Both the insecticides produces about 53% results.

## 6. Insecticide resistance in sand flies captured from Madina Town



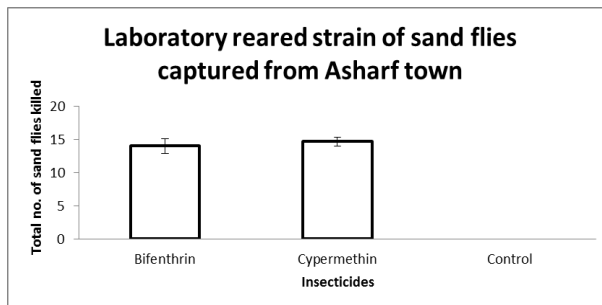
Sand flies captured from Madina town and reared up to 5th generation to become susceptible. After that insecticide application was done in 6th generation. Result showed that the susceptible strain of Madina town were highly susceptible to Bifenthrin (100%) followed cypermethrin (93%) and control (2.3%).

## 7. Insecticide resistance in sand flies captured from Odharwal



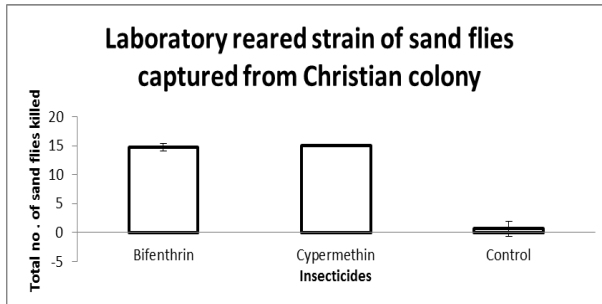
Sand flies captured from Odharwal town and reared up to 5th generation to become susceptible. After that insecticide application was done in 6th generation. Result showed that the susceptible strain of Odharwal town was highly susceptible to Bifenthrin (100%) and cypermethrin (100%) as compare to control (2.3%).

## 8. Insecticide resistance in sand flies captured from Ashraf Town



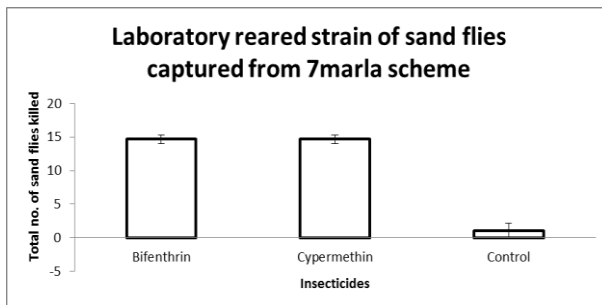
Sand flies captured from Ashraf town and reared up to 5 the generation to become susceptible. After that insecticide application was done in 6 the generation. Result showed that the laboratory reared strain of Ashraf town was highly susceptible to Bifenthrin (100%) and cypermethrin (97%) as compare to control (0%).

## 9. Insecticide resistance in sand flies captured from Christaian Colony



Sand flies captured from Christian colony and reared up to 5th generation to become susceptible. After that insecticide application was done in 6th generation. Result showed that the susceptible strain of Christian colony was highly susceptible to cypermethrin (100%), Bifenthrin (95%) as compared to control (33%).

## 10. Insecticide resistance in sand flies captured from 7 Marla scheme



Sand flies captured from 7 Marla scheme and reared up to 5th generation to become susceptible. After that insecticide application was done in 6th generation. Results showed that the susceptible strain of 7 Marla scheme highly susceptible to cypermethrin (97%), Bifenthrin (97%) and as compare to control (1%).

## **Discussion**

Insecticide resistance in different vectors is an important public health problem mainly in tropical and sub tropical regions of the world. Several studies indicated that sand flies developed resistance against different insecticides especially against the DDT and to some extent other insecticides like organophosphates and synthetic pyrethroids (AK, K, & SJ, 1987). So it is very important in relation to control of sand flies and leishmaniasis to develop a baseline data and to measure the insecticide resistance and susceptibility level in sand flies, important vector of *Leishmania*. There were no studies were conducted previously to determine the insecticide susceptibility level in sand flies in Pakistan. So it is important to carry out this study to develop the baseline for further study and control of sand flies in Pakistan.

Results obtained from the experiment collected after 10, 20, 30, 40, 50 and 60mints showed that *Phlebotomus* population collected from the Madina town, Odharwal and Ashraf town showed the high level of insecticide susceptibility against both Cypermethrin and Bifenthrin. While the population collected from the Christian colony and 7 Marla scheme showed the high level of insecticide resistance in sand flies.

The three populations were collected from the area of city where no insecticides are applied for the mosquito control and no agricultural farms are present. On the other hand Chakwal is a Barani area so here minimum insecticides were applied for the agricultural purpose. The populations collected from the Madina town Odharwal and Ashraf town were highly susceptible against both Cypermethrin and Bifenthrin because the use of insecticides in these areas was very low. While the population collected from the Christian colony were highly susceptible against these both insecticides. It is highly resistant because TMA department regularly applied insecticide to

control the mosquito population in the colony. While in case of 7 Marla scheme it is about 5 km away from the city and it is surrounded by the fields of vegetables and other crops. So here in 7 Marla scheme insecticide was applied to control the agricultural pest and it was also applied to minimize the population of mosquitoes for the control of malaria.

Sand flies capture from Madina town and reared up to 5<sup>th</sup> generation to become susceptible. After that different insecticide application was done in 6<sup>th</sup> generation. Result showed that the susceptible strain of Madina town were highly susceptible to Bifenthrin followed cypermethrin and control. The susceptible strain of Odharwal town was highly susceptible to Bifenthrin and cypermethrin as compare to control. The susceptible strain of Ashraf town was highly susceptible to Bifenthrin and cypermethrin as compare to control. The susceptible strain of Christian colony highly susceptible to cypermethrin Bifenthrin and as compare to control. Result showed that the susceptible strain of 7 Marla scheme highly susceptible to cypermethrin Bifenthrin and as compare to control.

Synthetic insecticides were used against the vector control programmes around the world. Highly and regular use of insecticide Leeds the development of insecticide resistance in these vectors (Nauen, 2007). So the development of insecticide resistance has become the important issue around the world in all kinds of vectors (Hemingway & Ranson, 2000). Susceptible and resistance status play pivotal role in any vector control programme and it is also helpful to control vector population in particular area.

The use of DDT and Malathion in Pakistan was began in the last three decades in Pakistan against household insect pests like mosquitoes(Pakistan, 2003). DDT and malathion was replaced by the Deltamethrin, fenthion and temephos these all

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insecticides are synthetic pyrethroids and they were used against the mosquitoes in Pakistan.

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