Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9 (3): 91-97

Received: 01-03-2021
Accepted: 03-04-2021

Muhammad Mohsin
Health Department, Punjab, Faisalabad, Pakistan

Dr. Jamila Shafi
Poultry Disease Diagnostic Lab, Samundri, Pakistan

Samina Iqbal Naz
Health Department, Punjab, Faisalabad, Pakistan

Saba Aslam
Agriculture University, Faisalabad, Pakistan

Shahzad Hassan
Health Department, Punjab Faisalabad, Pakistan

Corresponding Author:
Muhammad Mohsin
Health Department, Punjab, Faisalabad, Pakistan

Variability in susceptibility status of malaria vectors and other *Anopheles* species against different insecticides in district Faisalabad, Central Punjab, Pakistan

Muhammad Mohsin, Dr. Jamila Shafi, Samina Iqbal Naz, Saba Aslam and Shahzad Hassan

Abstract
A study on susceptibility status of *Anopheles* species of mosquitoes was conducted in district Faisalabad, Pakistan. Toxicity of six different insecticides such as 4% DDT, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 0.05% Permethrin, 5% Malathion and 0.1% Bandiocarb was studied and tested against six *Anopheles* species, *An. stephensi, An. culicifacies, An. subpictus, An. annularus, An. fluviaitlis* and *An. pulcherimus* under field and laboratory condition at 27-29ºC and 95-97% humidity. All *Anopheles* species have become resistant to 4% DDT with a maximum 77.27% mortality. *An. stephensi, An. culicifacies* and *An. subpictus*, have also become resistant to Deltamethrin 0.05%, lambdacyhalothrin 0.05% with mortality range of 33.33% to 93.33%. *An. annularis and An. fluviaitilis* exhibited mortality range of 92.69% to 100%. *An. pulcherimus* were found susceptible to Deltamethrin and lambdacyhalothrin with 100% mortality under lab condition. *An. stephensi* exhibited resistance to malathion but *An. culicifacies and An. subpictus* requires further verifications with 90.00% to 96.36% mortality rate, while *An. annularus* and *An. pulcherimus* were susceptible to malathion but again *An. fluviaitlis* requires verification against malathion under laboratory condition. *An. stephensi* requires verification in field condition but it was susceptible under lab condition against bandiocarb. While the remaining tested species were susceptible to bandiocarb under both laboratory and field conditions with 100% mortalities. Resistance development in *Anopheles* species and malaria vectors against DDT and pyrethroids group is a matter of concern, which can influence the efforts done for malarial control. However, bandiocarb appears to be the best alternative to pyrethroids.

Keywords: resistance, susceptible, species, *An. stephensi, An. culicifacies, An. subpictus*, insecticides

Introduction
Malaria is the world's most important vector borne parasitic infectious disease. It is estimated that in 2019 a cumulative 229 million malaria cases have been reported globally and 409, 000 malarial patient deaths occurred from which about 274, 000 (67%) were children aged under 5 years (WHO 2019) [4]. Between 2016 and 2017, estimated 216 Million to 219 Million cases of malaria has been reported around the globe in which 445, 000 and 435, 000 deaths has been occurred respectively (WHO 2017) [2].

Pakistan has 98% total contribution in regional malarial statistics and has been included in the list of seven countries of the WHO Eastern Mediterranean Region. Among total population of Pakistan, 98% (205 million) suffering from varying risk of malaria while 60% which is about 123 million population is facing a very high risk of the malaria (WHO 2018) [3]. In Pakistan about 3.5 million presumed and confirmed malaria cases report annually (Directorate of malaria control 2020).

In Pakistan malaria usually occurs irregularly and major transmission period is post monsoon i.e. from August to November. Main vector species involve in the transmission of malaria are *An. culicifacies* and *An. stephensi*, the two parasites which account for malaria in Pakistan are *Plasmodium vivax* and *falciparum* (Directorate of malaria control). There is substantial drug resistance (chloroquine and Fansidar resistance) prevalent throughout the country, which implicate the importance for vector control to combat malaria in Pakistan (Directorate of malaria control). In Punjab, Pakistan, in 2013 annual parasite incidence was 0.1 whereas in district Faisalabad in Punjab annual parasite incidence reported was 0.03, although it is a low,

but it confirms the presence of malarial parasite in Faisalabad, which could adopt alarming situation if vector density in this area get high (Directorate of Malaria Control). Out of 24 reported *Anopheles* species in Pakistan (Aslam 1971) [6], only two *An. stephensi* and *An. culicifacies* are known primary malaria vectors (Rathor *et al*. 1996) [7].

To control the disease, vector control is the key option. So the most effective insecticide used worldwide for the control of mosquitoes is Pyrethroids (Jahan *et al*. 2013; WHO 2011) [9, 13]. From 2000- 2009, 394 tons of organophosphates and 154 tons of pyrethroids have been applied annually, against mosquito vectors (World Health Organization 2011) [9]. In Pakistan, Dichlorodiphenyltrichloroethane (DDT) was used for mosquito control in the province of Punjab from 1961 to 1975 (two cycles/year of 1–2 g/m2), which consequently produced strong DDT resistance in malaria vectors. In 1975 DDT was partially replaced by b-hexachlorocyclohexane (BHC), to which resistance developed rapidly. In 1976 malathion (two cycles/year, 1 g/m2) was introduced for malaria vector control (Rathor *et al*. 1980) [10].

Since last 15 years pyrethroids like Deltamethrin 1.5% EC, Permethrin 2.5% EC, Deltamethrin 5% WP and Lambdacyhalotrin 1.5% are being used in vector control programs (Directorate General Health Punjab).

The development of vector resistance to currently being used pyrethroids can lead to uncontrollable epidemics by vector-borne diseases. Only in 1985, the first large-scale field survey to map insecticide resistance status in 11 randomly selected districts including Faisalabad was carried out in the Punjab (Rathor *et al*. 1985) [12]. After that, since last 25 years, very little work has been done to monitor the insecticide resistance status of anopheline mosquitoes in Pakistan. This lack of information on the resistance status of vector mosquitoes can have serious technical and financial consequences, especially when pyrethroids are being used extensively for agricultural and household purposes.

So the knowledge of vector susceptibility to pesticides and insight into changing trends of resistance and expected operational implications provide the evidence base to form effective national policy and pesticide use strategies for vector- borne disease and pest control programs. Therefore, insecticide resistance monitoring must be an integral part of disease vector and public health pest control programs.

Methods

Study site and mosquito collection

Faisalabad is situated in Punjab province of (Latitude: 31°25.0002′ N, Longitude: 73°4.9998′ E) Pakistan. It has developed into the third most populated city after Karachi and Lahore. Faisalabad District covers total area of 58.56 km^2 (22.61 sq mi) while the area controlled by the Faisalabad Development Authority (FDA) is 1, 280 km^2 (490 sq mi) (Urban Management Initiatives in Pakistan). Faisalabad has grown to a major industrial and distribution center because of its geographical location in the region and connecting roads, rails, and air transportation (Ghulam *et al*. 2009) [14]. The climate of the district can see extremes, with a summer maximum temperature of 50 °C (122 °F), and a winter temperature of −2 °C (28 °F). The summer season typically ranges from April to October with the hottest temperatures occurring in May, June and July. Winter season usually begins in November and continues until March with the coldest temperatures occurring in December, January and February. Average annual rainfall is approximately 384.683 mm (15.145 in), and highly seasonal, with nearly half of all precipitation occurring in July and August (World Heritage Encyclopedia)

In the current study, adult *Anopheles* species of mosquitoes were collected using mouth aspirator and CDC sweeper from six different localities in district Faisalabad, like Chak 209 RB (209 RB), Dara Ameer Bakhsh (AM), Dara Ahmad Saeed (AH), 3Chak (3CH), 58 Chak (58 CH) and Chabhal (CHB). Global Positioning System (GPS) was used to take proper location of mosquito collection site.

WHO bioassay testing procedure

In the current study, wild adult *Anopheles* species (field collected) of mosquitoes were exposed for 1-h to the discriminating doses of WHO provided papers impregnated with 4% DDT, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 5% Malathion and 0.1% Bandiocarb (WHO 2013) [17]. Tests were performed on wild female in the field (same environment) where females were collected. Mortalities were observed after the 24-hour holding period and results were recorded on the susceptibility test data form. Those wild females (less available) which were not tested in field and some which survived from exposure to discriminating dose of insecticide were isolated in laboratory and then tests were applied on their F1 progeny under lab condition at 27.2c temperature and 95% humidity. The identification and separation of adults female *Anopheles* was done using identification keys (WHO 2013) [17].

Data interpretation and analysis

Following WHO recommended interpretations was followed in data interpretation

- 98-100% mortality indicate susceptibility;
- 90-97% mortality suggest the possibility of resistance that requires verification;
- Mortality rates < 90%, indicates resistance;
- Normally, no mortalities were observed in the controls, but where 5–20% mortalities were observed, Abbott's formula was applied to correct the percentage of mortalities (WHO 2013) [17].

$$\text{Abbott formula} = \frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}}$$

ANOVA (Analysis of Variance) test was applied to find out, if there is significant difference in mosquito mortalities after 1 hour, after 24 h and in total% mortalities among the species against different insecticides.

Results

Susceptibility status of wild *Anopheles* female

Susceptibility tests applied on three species, *An. stephensi*, *An. culicifacies* and *An. subpictus* at different localities of district Faisalabad showed that all the species were resistant to 4% DDT at all tested localities. In *Anopheles stephensi* minimum mortalility (13.33%) at Chabhal (CHB) and maximum mortality (42.85%) was observed at 58 Chak. In *Anopheles culicifacies* maximum mortality was only 36.66% at Dara Ameer Bakhsh (AM) and in *An. subpictus* maximum mortality (35.00%) against 4% DDT was recorded at (58 CH) (Table 1).

Table 1: Summary of results on susceptibility/resistance of female *Anopheles* species against 0.05% Deltamethrin, 0.05% Lambda-cyhalothrin, 0.75% Permethrin, 4% DDT, 5% Malathion and 0.1% Bandiocarb at different localities of district Faisalabad

		DDT			Deltamethrin			Lambdacyhalothrin			Malathion			Bandiocarb		
Species	**Localities**	**No. of females tested**	**Corrected mortality %**	**Status****	**No. of females tested**	**Corrected mortality %**	**Status****	**No. of females tested**	**Corrected mortality %**	**Status****	**No. of females tested**	**Corrected mortality %**	**Status****	**No. of females tested**	**Corrected mortality %**	**Status****
Ae. stephensi	209 RB	48	17.50	R	60	56.66	R	52	56.66	R	80	57.50	R	70	76.66	R
	AM	80	22.50	R	103	61.29	R	90	61.42	R	95	31.66	R	90	71.71	R
	AH	34	16.66	R	44	62.50	R	45	44.00	R	64	66.66	R	70	90.00	?
	3CH	40	20.00	R	57	33.33	R	50	70.00	R	70	46.66	R	74	93.33	?
	58CH	58	42.85	R	48	93.33	?	42	59.09	R	64	92.35	?	68	95.71	?
	CHB	35	13.33	R	50	53.30	R	50	76.66	R	54	75.00	R	70	96.33	?
Ae. culicifacies	209 RB	24	29.16	R	40	65.00	R	50	70.00	R	62	96.36	?	62	100.0	S
	AM	38	36.66	R	44	45.83	R	44	91.66	?	60	90.00	?	66	100.0	S
	AH							40	90.00	?	56	90.00	?			
	3CH							36	43.75	R				58	100.0	S
	CHB	28	11.11	R	38	93.33	?	38	72.22	R	61	95.23	?			
Ae. subpictus	209 RB	34	33.33	R	40	70.00	R	42	63.63	R	76	94.61	?	58	100.0	S
	AM	28	27.77	R	42	54.54	R	46	94.61	?	70	90.00	?	66	100.0	S
	AH													62	100.0	S
	58CH	30	35.00	R												

* = Progeny of those wild females that were not exposed in the field. ** = Progeny of those wild females which were resistant to susceptible doses. R = Resistant; S = Susceptible? = Verification required/resistance possible to be confirmed

Against 0.05% Deltamethren, *An. stephensi* showed resistance at all localities with less than 62.50% mortality except at 58 chak (93.33% mortality) where further confirmation is required. In *Anopheles Culicifacies* maximum mortality (93.33%) was observed at Chabhal (CHB) which require further verification, whereas at chak 209 RB (65.0% mortality) and at Dara Ameer Bakhsh (AM) (45.83% mortality) it has become resistant. *An. subpictus* was also found resistant with maximum 70.00% mortality at Chak 209 RB against 0.05% Deltamethren (Table 1).

Susceptibility status of *An. stephensi* showed resistance to 0.05% Lambdacyhalothrin at all examind localities with maximum 76.66% mortality. *An. culicifacies* exhibited resistance against 0.05% Lambdacyhalothrine with less than 72.23% mortality at Chak 209 RB, 3Chak and (CHB), whereas verification was required (91.66% mortality) at AH and at DAM (90% mortality). *An. subpictus* showed resentence (63.63%) at chak 209 RB and require verification (94.61%) at DAM to 0.05% Lambdacyhalothrin (Table 1).

Susceptibility status of *An. stephensi* was found resistant against 5% Malathion at all observed localities with less than 75% mortality except at 58 chak where further confirmation is needed with 92.35% mortality. In case of *Anopheles culicifacies* and *An. subpictus* verifications were required at all examined localities with mortalities range from 90% to 96.36% mortality (Table 1).

Against 0.1% Bandiocarb, susceptibility status of *An. stephensi* requires verification at four localities like AH, 3 Chak, 58 CH and CHB with a range from 90% to 96.33% mortalities, whereas specie is resistant to 0.1% Bandiocarb at chak 209 RB (76.66% mortality) and at Dara Ameer Bakhsh (AM) (71.71% mortality). *An. culicifacies* and *An. subpictus* were found susceptible to 0.1% Bandiocarb with 100% mortalities at all localities where test was applied (Table 1).

According to statistical analysis, there is significant difference in% mortalities of *Anopheles* species to different insecticides 4% DDT, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 5% Malathion and 0.1% Bandiocarb [$F_{(4, 114)} = 35.713$, $P < 0.000$]. Bandiocarb causes 100% mortality at most locations against wild *Anopheles* species.

Susceptibility status of F1 progeny

Current study in Table II shows that Susceptibility tests applied on F1 progeny of six species *An. stephensi*, *An. culicifacies*, *An. subpictus*, *An. pulcherimus*, *An. annularis* and *An. fluviatilis* showed resistance against 4% DDT with maximum 77.27% mortality.

Susceptibility status of F1 progeny of (resistant) survived and wild *An. stephensi* against 0.05% Deltamethrin at AM, 3 Chak and AH and against 5% Lambdacyhalothren at 209 RB and CHB was found resistant with less than 68% mortality. F1 progeny of survived species of *An. stephensi* against 5% Malathion at AM was also found resistant while F1 of wild *An. stephensi* requires verification at 58CH with 96% mortality.

Progeny of wild *An. stephensi* against 0.1% Bandiocarb at 58 CH requires verification whereas progeny of (resistant) survived *An. stephensi* CHB were found susceptible (Table II).

F1 progeny of wild *An. culicifacies* requires verification at all tested localities against Deltamethrin and Lambdacyhalothrin with a range of 91% to 97.98% mortalities except from two localities like 3CH where resistance (64.70%) was observed against Deltamethrin and at 209RB where resitance (74.50%) was observed against Lambdacyhalothrin.

F1 progeny of wild *An. culicifacies* was found susceptible at all observed localities against 0.1% Bandiocarb and 5% Malathion, while progeny of survived *An. culicifacies* requires verification at AM (90% mortality).

F1 progeny of wild *An. pulcherimus* was found susceptible with 100% mortality at two localities 209RB and AM against Deltamethrin, Lambdacyhalothrin, 5% Malathion and 0.1% Bandiocarb.

F1 progeny of wild *An. annularis* was susceptible at 209RB against Deltamethrin and Lambdacyhalothrin whereas requires verification at AM against both insecticides. Susceptibility status of *An. annularis* against 0.1% Bandiocarb and 5% Malathion was found susceptible with 100% mortality at all collected localities. F1 progeny of wild *An. fluviatilis* only collected from 209 RB require verification against Deltamethrin, Lambdacyhalothrin and 5% Malathion with mortalties range from 92.30% to 96.92%, while found susceptible against 0.1% Bandiocarb with 100% mortality. Bandiocarb was also found most effective in F1 progeney of *Anpheles* species with comparison to all other tested insecticides. Later on malthion, deltametrin and

lamdacyhalothrin also showed 100% efficacy against F1 progeny but not at all places and against all species.

Discussion

This study demonstrates the phenotypic resistance of *Anopheles* mosquitoes through susceptibility test against various insecticides. In the operational perspective of insecticide resistance monitoring, susceptibility tests are a cost-effective tool for the evaluation of susceptibility in vector populations (WHO 2012) [17]. Resistance has become common phenomena which is detected in more than 500 insect species worldwide among which more than 50 *Anopheles* species, which are responsible for the transmission of malaria sporozoite to humans (Hemingway J and Ranson H, 2000) [18]. Since few years, malaria vectors have developed resistance against main chemical classes (i.e. pyrethroids, DDT, carbamates and organophosphates) used in public health. The occurrence of cross-resistance and multiple resistance pose a serious threat in achieving the specified targets for malaria control (WHO 2012) [17]. The use of insecticides for agricultural purposes and more recently for public health has played pivotal step in the selection of resistance in malaria vectors (Ranson *et al*. 2011; Temu *et al*. 2012) [19, 20].

During early study on insecticide susceptibility in 1983 at Pakistan, DDT was reported resistant in *An. stephensi*, *An. culicifacies*, *An. subpictus* and *An. annularis* but was observed susceptible in *An. pulcherimus* due to their exophilic nature (Rathor 1983) [21], but now in current study not only all these species but, *An. fluviatilis* and *An. pulcherimus* have also become resistant against DDT.

In Pakistan, *An. subpictus*, in Mirpur (Sindh) and kasur (Punjab) (Hammad *et al*. 2015; Naeem *et al*. 2015) [22, 23] and *An. stephensi* in District Bahawalpur and Muzafergarh were also observed resistant against diagnostic dose of 4% DDT (Mehmood *et al*. 2013; Rana *et al.* 2014) [24, 26]. In the southern districts of the Punjab, Pakistan both *An. stephensi* and *An. culicifacies* also remained resistant to DDT (Rathor 2012) [27] however at Goth Bhoorji (sindh) Pakistan 4% DDT showed 100% percentage mortality (Hammad *et al*. 2015) [22]. In similar study in Iran which is neighboring country of Pakistan, *An. stephensi has also developed* resistance against DDT (Gorouhi *et al*. 2015; Fathian *et al*. 2015; Hanafi et al. 2012) [29, 30, 31]. In contrast with India, most of the studies unmasks the resistance against DDT in most of the malaria vectors like *An. culicifacies, An. stephensi* and moreover in *An. fluviatilis, An. minimus* and *An. annularis* (Kumar et al. 2014) [32]. In Assam India, *An. annularis* (Dhiman *et al*. 2016) [33], In Odisha State (Sahu *et al*. 2015) [36] and in another four states *An. culicifacies* was found resistant to DDT (Raghavendra *et al*. 2014) [34]. However in Mangalore city of South India India *An. stephensi* was susceptible to DDT with 98.1% mortality (Tiwari *et al*. 2010) [35]. This all may be because of extensive use of DDT in past decades or through cross resistance with pyrethroids. But resistance to DDT in current study may not be unconnected with the historical use of this insecticide in vector control activities in Pakistan (Rathor 1983) [21].

In current study the development of resistance in *An. stephensi*, *An. subpictus* and *An. culicifacies* against pyrethroids like deltamethrin and lambdacyhalothrin is matter of concern. This may be due to high use of these molecules in interventions against mosquito's vector control and agricultural spraying since last two decades (Malaria directorate). Similarities in chemical structure between DDT and pyrethroid insecticides also have led to widespread concern that cross-resistance between them might limit the usefulness of the pyrethroid (WHO 2012) [17].

Previous study in Pakistan at Mirpur (sindh), village Goth Bhoorji (Sindh) and kasur (Punjab) reveals, *Anopheles subpictus* reported resistance against Lambda-cyhalothrin, Permethrin and Deltamethrin (Hammad *et al*. 2015; Naeem *et al*. 2015) [22, 23]. Similarly at Bahawalpur and four other southern districts of the Punjab, Pakistan, lambda-cyhalothrin, and deltamethrin noticed resistance in *An. stephensi* (Mehmood *et al*. 2013; Rathor 2012) [24, 27] and *An. culicifacies* (Rathor 2012; Rana *et al*. 2014) [26, 27].

Similar study in other countries of the world like In Iran *An. stephensi and An. culicifacies* reported resistant or developing resistance to lambdacyhalothrin and deltamethrin (Gorouhi *et al*. 2015; Hanafi *et al*. 2012) [29, 31]. Similarly in India, *An. culicifacies* and *An. stephensi* is also developing resistance against synthetic pyrethroid an even An. culicifacies have also roported resistance to deltamethrin (Tiwari *et al*. 2010; Kumar *et al*. 2014; Sahu *et al*. 2015; Raghavendra *et al*. 2014) [32, 34-36]. Moreover, *An. fluviatilis*, *An. minimus* and *An. annularis* reported susceptible to deltamethrin and lambda-cyhalothrin (Dhiman *et al*. 2016; Kumar 2014) [32, 33].

Against malathion, in district Faisalabad *An. stephensi* was reported resistant was tolerant in *An. culicifacies* and *An. subpictus*, (Rathore 1983) [21]. But in current study at district Faisalabad *An. stephensi* showed same resistance behaviour to malathion, whereas *An. culicifacies, An. subpictus, An. pulcherimus, An. annularis* and *An. fluviatilis,* showed suceptile behaiour even in F1 generation. In previous study in Pakistan at district Bahawalpur, Muazfargarh and other southern districts of the Punjab province, both *An. stephensi* and *An. culicifacies* also remained resistant to malathion (Mehmood *et al*. 2013; Rathor 2012; Rana *et al*. 2014) [24, 26, 27], but at Mirpur and village Goth Bhoorji (Sindh) and kasur (punjab) *Anopheles subpictus* found susceptible to 5% Malathion (Hammad *et al*. 2015) [22]. Same study conducted in different areas of India reveals, *An. culicifacies* and *An. stephensi* are resistant to malathion (Kumar *et al*., 2014 ; Sahu *et al*., 2015; Raghavendra *et al*. 2014) [34, 36], Moreover, *An. fluviatilis*, *An. minimus* and *An. annularis* are susceptible to malathion (Tiwari *et al*. 2010; Kumar *et al*. 2014, Dhiman *et al*. 2016) [32-35]. In Iran tolerance in *An. stephensi* to malathion is reportedn (Hanafi *et al*. 2012) [31].

In current study only 0.1% Bandiocarb took superiority in effectiveness on wild and F1 progeny of *An. culicifacies, An. subpictus, An. stephensi, An. annularus, An. fluviaitlis* and *An. pulcherimus*. This is because bendiocarb is rarely used in Pakistan (Malaria directorate). In contrast at Iran tolerance in *Anophles* specie to bendiocarb reported (Hanafi et al. 2012) [31] but in central Africa also 100% susceptibility rate to bendiocarb was recorded (Olé Sangba *et al*. 2016) [39].

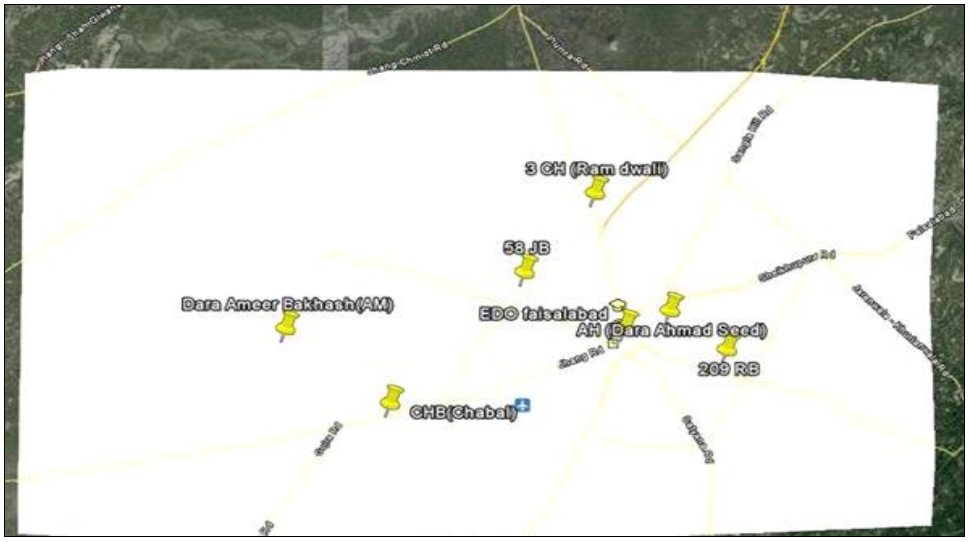

Fig 1: Geo-tagging of *Anopheles* species adult collection sites

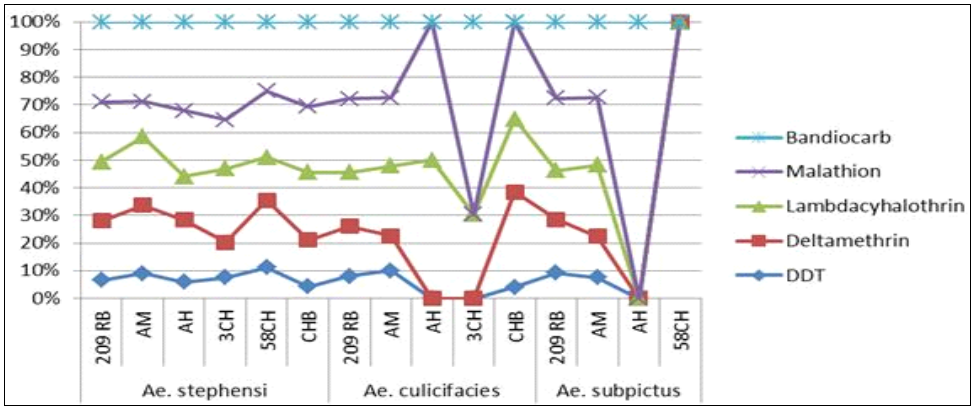

Fig 2: Efficacy of insecticides against wild *Anopheles* species

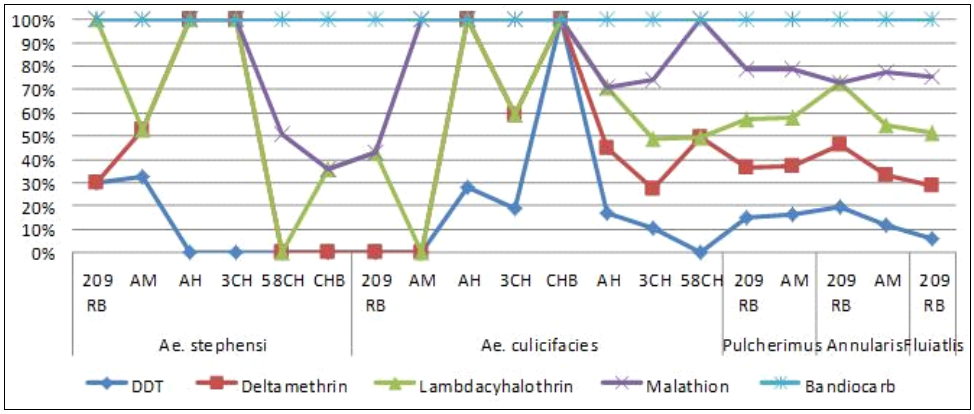

Fig 2: Efficacy of insecticides against F1 progeny (lab condition) of *Anopheles* species

Conclusion

This kind of development of resistance or incipient resistance in *Anopheles* species and malaria vectors in areas of China, Afghanistan, central Africa, across western Kenya, Iran, India and now in Pakistan against DDT, pyrethroids and organophostae (Dai *et al*. 2015; Ahmad *et al*. 2016; Olé Sangba *et al*. 2016; Christine *et al*. 2015; Hanafi et al. 2012; Raghavendra *et al*. 2014) [31, 34, 37-39] is a matter of concern, which, can influence the efforts made for malaria control worldwide. In Pakistan in our study bendiocarb was found most effective insecticide and malthion at 2nd one option. However, repetitive use of malathion and bandiocarb may substantiate as best alternative of pyrethroids against *Anopheles* species.

Author's contribution

Muhammad Mohsin and Samina Iqbal Naz conceived the idea, built the conceptual study framework. Muhammad Mohsin, Samina Iqbal Naz & Aliya Jabeen conducted the survey. Muhammad Mohsin and Samina Iqbal Naz performed the screening and gathered the primary data. Muhammad Mohsin and Samina Iqbal Naz analyzed the data. Muhammad Mohsin and Aliya Jabeen wrote the primary draft and assessed related reviews. Muhammad Mohsin and Samina Iqbal Naz provided additional evidence and insights. All authors read and approved the final version of the manuscript.

Acknowledgment
Thanks to Health Services Academy especially to Dr. HR Rathore for providing the enabling environment.

References

1. World Health Organization. World Malaria Report 2016, Global Malaria Programme 2016. www.who.int/malaria
2. World Health Organization. World Malaria Report 2017, Global Malaria Programme 2017. www.who.int/malaria
3. World Health Organization. World Malaria Report 2018, Global Malaria Programme 2018. www.who.int/malaria
4. World Health Organization. World Malaria Report 2019, Global Malaria Programme 2019. www.who.int/malaria
5. Directorate of Malaria Control. Strategic plan malaria control program Pakistan 2020.
6. Aslam K. The mosquitoes of Pakistan: A check list. Mosq Syst Newsletter 1971;3:147-159.
7. Rathor HR. The role of vectors in emerging and reemerging diseases in Eastern Mediterranean Region. Eastern Mediterr Hlth J 1996;2:61-67.
8. Jahan Shahid N. Evaluation of resistance against Deltamethrin and Cypermethrin in Dengue vector from Lahore, Pakistan. J Anim Pl Sci 2013;23:1321-1326.
9. WHO. Global insecticide use for vector-borne disease control a 10 year assessment (2000-2009). World Health Organization 2011. https://en.wikipedia.org/wiki/Lahore, accessed 16th May 2016.
10. Rathor HR. Status of insecticide resistance in anopheline mosquitoes of Punjab Province, Pakistan. Southeast Asian. J Trop Med Publ Hlth 1980;11:332-40.
11. Punjab direcroate of dengue control DG Health office Lahore 1980.
12. Rathore HR, Toqir G, Rashid S, Mujtaba SM, Nasir SM. Insecticide resistance in *Anopheline* mosquitoes of Punjab Province, Pakistan. Pakistan J Zool 1985;17:35-49.
13. Urban Management Initiatives in Pakistan (PDF) Pakistan research repository of the development plan on city growth (Report) (Pakistan Higher Education Commission) 2016, P84-85.
14. Ghulam M. Regional profile, Faisalabad (PDF) (Report). University of Agriculture, Faisalabad 2009.
15. World Heritage Encyclopedia. Geology study of Pakistan: study area and sampling 2009. http://www.worldheritage.org/articles/eng/Faisalabad.
16. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes 2013.
17. WHO. Global plan for insecticide resistance management in malaria vectors. Geneva: World Health Organization 2012.
18. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 2000;45:371-391.
19. Ranson HN, Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African Anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 2011;27:91-98.
20. Temu EA, Maxwell C, Munyekenye G, Howard AFV, Munga S, Avicor SW *et al*. Pyrethroid resistance in *Anopheles gambiae*, in Bomi County, Liberia, compromises malaria vector control. Plos One 2012;7:1371.
21. Rathor HR. Preliminary survey of insecticide susceptibility in *anopheline* populatin of selected index localities Faisalabad district Pakistan J Med Res 1983;22.
22. Hammad M, Adil B, Uzair MM, Ali A, Shumaila M, Arqam BZ. Susceptibility/resistance of selected insecticides in *Anopheles* mosquitoes of district Mirpur Khas, Sindh, Pakistan J Entomol Zool Stud 2015;3:321-325.
23. Naeem H, Kamran A, Muhammad I, Habib R, Muhammad O, Haroon A. Insecticide resistance status of *anopheles subpictus* from district Kasur, Punjab, Pakistan. Sci Int 2014;26:1205-1208.
24. Mehmood K, Rathor HR, Khan IA, Hassan SA, Faridi TA. Insecticide Susceptibility/resistance status of *Anopheles* mosquitoes in District Bahawalpur, Punjab
25. Pakistan: an entomological survey. Pak J Pub Health 2013;3:44-45.
26. Rana SM, Khan EA, Yaqoob A, Latif AA, Abbasi MM. Susceptibility and irritability of adult forms of main malaria vectors against insecticides used in the indoor residual sprays in Muzaffargarh District, Pakistan: a field survey. J Med Entomol 2014;51:387-91.
27. Rathor HR, Ghazala N, Imtinan AK. Pesticide susceptibility status of *Anopheles* Mosquitoes in four flood-affected districts of South Punjab, Pakistan. Vector Borne Zoo Dis 2012;12.
28. Hammad M, Nazeer A, Imtiaz AK, Bismillah S, Ashraf K, Murad A *et al*. Studies on the efficacy of selected insecticides against *Anopheles* mosquitoes of village Goth Bhoorji (Sindh) Pakistan; J Ent Zool Stud 2015;3:169-173.
29. Gorouhi M, Hassan V, Mohammad A, Ahmad R, Ahmad A, Hossein M *et al*. Current susceptibility status of *Anopheles stephensi* (Diptera: Culicidae) to different imagicides in a malarious area, Southeastern Iran. J Arthropod Borne Dis 2015.
30. Fathian M, Vatandoost H, Moosa SH, Raeisi A, Yaghoobi MR, Oshaghi MA *et al*. Susceptibility of Culicidae Mosquitoes to some insecticides recommended by WHO in a malaria endemic area of southeastern Iran. J Arthropod Borne Dis 2014;9:22-34.
31. Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Haghdoost AA, Shahi M, Sedaghat MM *et al*. Entomological and epidemiological attributes for malaria transmission and implementation of vector control in southern Iran. Acta Trop 2012;121:85-92.
32. Kumar R, Gaurav K, Pradeep KM. Insecticide susceptibility status of malaria vectors in India: A review International Journal of Mosquito Res 2014;1:5-9.
33. Dhiman S, Kavita Y, Bipul R, Diganta G, Hazarika S, Varun T. Evaluation of insecticide susceptibility and malaria vector potential of *Anopheles annularis* and *Anopheles vagus* in Assam, India. PLoS One 2016, 11.
34. Raghavendra K1, Barik TK, Sharma SK, Das MK, Dua VK, Pandey A *et al*. A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India. J Vector Borne Dis 2014, 51.
35. Tiwari S, Ghosh SK, Ojha VP, Dash AP, Raghavendra K. Reduced susceptibility to selected synthetic pyrethroids in urban malaria vector *Anopheles stephensi*: a case study in Mangalore city, South India. Malar 2010;J9:179.
36. Sahu SK, Gunasekaran T, Vijayakumar, Jambulingam P. Triple insecticide resistance in *Anopheles culicifacies*: a

practical impediment for malaria control in Odisha State, India. J Med Res 2015;142:59-63.
37. Ahmad M, Buhler C, Pignatelli P, Ranson H, Nahzat SM, Naseem M *et al*. Status of insecticide resistance in high-risk malaria provinces in Afghanistan. Malar J 2016;15:98.
38. Dai Y, Huang X, Cheng P, Liu L, Wang H, Wang H *et al*. Development of insecticide resistance in malaria vector *Anopheles sinensis* populations from Shandong province in China. Malar 2015;J62.
39. Olé S, Deketramete ML, Wango TI, Kazanji SP, Akogbeto M, Ndiath MO. Insecticide resistance status of the *Anopheles funestus* population in Central African Republic: a challenge in the war. Parasit Vectors 2016;9:230.
40. Christine L, Wanjala JP, Mbugi EO, Maxwell G, Yaw A, Afrane E *et al*. Pyrethroid and DDT resistance and organophosphate susceptibility among *Anopheles* spp. Mosquitoes, Western Kenya. J Emerg Infect D 2015;21:234-256.