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Nazif Ullah Khan
(1). Department of Medical Entomology and Disease Vector Control, Health Services Academy Islamabad, Faculty of Medicine Quaid-i-Azam University Islamabad, 44000 Pakistan.
(2). Department of Biology Government Post Graduate College Miran Shah North Waziristan Agency, FATA, 28200 Pakistan.

Shams Ullah Khan
Department of Biochimstry Quaid-i-Azam University Islamabad, 44000 Pakistan.

Ansarullah Khan
Department of Medical Entomology and Disease Vector Control, Health Services Academy Islamabad, Faculty of Medicine Quaid-i-Azam University Islamabad, 44000 Pakistan.

Inayat ur Rehman
Department of Medical Entomology and Disease Vector Control, Health Services Academy Islamabad, Faculty of Medicine Quaid-i-Azam University Islamabad, 44000 Pakistan.

Salahuddin Khan
Department of Medical Entomology and Disease Vector Control, Health Services Academy Islamabad, Faculty of Medicine Quaid-i-Azam University Islamabad, 44000 Pakistan.

Shahid Ullah Khan
Khyber Medical College Peshawar, 25000 Khyber Pakhtunkhwa, Pakistan

Correspondence
Nazif Ullah Khan
(1). Department of Medical Entomology and Disease Vector Control, Health Services Academy Islamabad, Faculty of Medicine Quaid-i-Azam University Islamabad, 44000 Pakistan.
(2). Department of Biology Government Post Graduate College Miran Shah North Waziristan Agency, FATA, 28200 Pakistan.

Susceptibility status of Dengue vector (*Aedes aegypti*) against different insecticides in district Mansehra, Khyber Pakhtunkhwa, Pakistan

Nazif Ullah Khan, Shams Ullah Khan, Ansarullah Khan, Inayat ur Rehman, Salahuddin Khan and Shahid Ullah Khan

Abstract
Aedes aegypti is the primary and *Aedes albopictus* is the secondary vector of dengue virus and is of Public health concern. The primary strategy to control the spread of dengue is to control the disease vector density which mainly depends upon the use of chemical insecticides but unfortunately the control intervention facing operational challenges regarding insecticides resistance in dengue vector and other vector borne diseases. But no data was available on susceptibility of dengue vector of Mansehra. The aim of this study was to assess the susceptibility level of *Aedes aegypti* to different insecticides recommended by World Health Organization.
During this study *Aedes aegypti* larvae were collected from five localities of district Mansehra and after rearing in the insectary the resistance/susceptibility status of F1 generation was determined by using World Health Organization (WHO) test kit, utilizing papers impregnated with DDT 4%, Malathion 5%, Bendiocarb 0.1%, Lambdacialotrina 0.05% and Deltamethrin 0.05%.
The test results showed that the *Aedes aegypti* population of district Mansehra is resistant to DDT, Malathion, Lambdacialotrina and Deltamethrin but Bendiocarb is placed under the verification required status.
The results of this study concludes that if appropriate resistance management strategies are applied in this area then the high level of resistance can be prevent or delayed. This study provides important evidence base data for strategic planning for vector control in district Mansehra.

Keywords: *Aedes,* Insecticides resistance, DDT, Malathion, Bendiocarb, Lambdacyhlothrin, Deltamethrin

1. Introduction
Dengue Fever and dengue haemorrhagic fever is a vector borne disease of public health concern in tropical, subtropical and temperate regions of the world. *Aedes aegypti* is the principal vector for dengue and Yellow fever, and a secondary vector of chikungunya fever virus [1-3]. It is reported by WHO that during the past fifty years thirty fold increase has occurred in the incidence of dengue cases worldwide. Dengue is prevalent in more than 110 countries, especially in South Asia and Southeast Asia, and 2.5 billion people are living in dengue endemic areas of the World [4]. It is estimated that 50-100 million infections occur each year, including 500,000 hospitalizations for dengue haemorrhagic fever, mainly among children, with the case fatality rate more than 5% in some areas [5-7]. Despite of poor surveillance and no official reporting dengue cases were also reported to World Health Organization from Eastern Mediterranean and African regions. The first outbreak of dengue has been well documented in the EMR possibly as early as 1799 from Egypt [8]. But in Pakistan the incidence of dengue has increased episodically since the first recognized outbreak in 1994 in Karachi, Sindh Province [9]. Since 1994, the expansion of dengue cases with the increasing frequency and severity has been reported from all around Pakistan, but now the situation of dengue is alarming across all the country. During 2013 WHO disease early warning system (DEWS) reported dengue cases majority of which were from Khyber Pakhtunkhwa followed by Sindh, Punjab and Baluchistan [10].
In the absence of specific antiviral drug and vaccine against dengue virus the reduction of population density of disease vector is the key to success to any vector control program to prevent the spread and transmission of the disease. This strategy is mainly dependent on the use of chemical insecticides of different classes particularly Pyrethroid group of insecticides

having low human and mammalian toxicity [11]. During prevention/control programs the target vector are mainly controlled by targeting it at larval stage in the breeding sites by the use of larvicides and the adult infective stages by the use of thermal fogging, ultra-low volume sprays and insecticides impregnated nets and other materials [12]. During outbreak situations for the control of dengue both the larvae and adult stages can be targeted through the use of space sprays which is the routine control procedure for adult *Aedes spp*. vector. Development and spread of resistance against different insecticides used for the control of vector species in dengue endemic countries is the main problem which threaten the control programmes [13, 14].

In early 1969 DDT was the first insecticide used as indoor residual spraying for malaria control program in Pakistan. Then in 1972 Benzene hexachloride (BHC) was also used to control malaria vector till 1983. In 1976 Organophosphates particularly Malathion were also used by Malaria Control Program to achieve better results and continued till 1996. DDT was banned due to the emergence of resistance during 1979 in malaria vectors [15]. And from late 1980 synthetic Pyrethroid was introduce and still is used as IRS, insecticides treated bed nets and fogging to control malaria, dengue and other vector borne disease as well as Agriculture pests [16 17].

But now Dengue and Malaria endemic countries are facing problem of Pyrethroid's resistance due to its irrational and extensive use and has been reported from many countries of the world [18]. Insecticides resistance in disease vectors especially dengue vector is poorly documented in Pakistan and very limited published data are available from Punjab province. *Aedes aegypti* collected from slum area of Misri Shah, Lahore and tested for resistance to deltamethrin and cypermethrin. It is reported that *Aedes aegypti* were resistant to deltamethrin and cypermethrin, but the larvae were ten times more resistant to Bti than susceptible species of *Aedes aegypti* [19, 20]. Adult *Aedes aegypti* were collected form Government College Lahore (GC) and Government Islamia College for Women (GICW) Lahore and tested for resistance to Bifenthrin 10% EC and reported that the GICW strain was resistant and GC strain was susceptible to the tested insecticide [21]. Similarly *Aedes albopictus* collected from agricultural areas of Punjab; Lahore, Sargodha and Faisalabad tested for resistance and revealed that high level of resistance to Organophosphates, moderate level of resistance to Pyrethroids and Carbamates were present in *Aedes albopictus* [22].

To the best of our knowledge no data was available regarding insecticides susceptibility of *Aedes aegypti* in district Mansehra, Khyber Pakhtunkhwa. The aim of this study was to investigate the level of resistance of *Aedes aegypti* to five different insecticides including the Pyrethroid group of insecticides currently in use for vector control. The data generated will be helpful for effective vector control strategies in future.

2. Materials and Method

2.1 Study area: We conducted a field survey during March 2015 to September 2015 with the aim to determine the susceptibility level of *Aedes aegypti* population collected from district Mansehra (34.33^0 N, 73.2^0 E) with an altitude of 1067 meter from sea level in province Khyber Pakhtunkhwa. The total area consists of 4579 square kilometer with a total population of 1,700,000. Mansehra is a mountainous green area with natural vegetation, Kunhar (Nain Sukh) and Siran are the main rivers having rich mosquitoes Fauna during summer. The climate in summer is warm but the average temperature of Mansehra is 18.5 °C with an average rainfall of 1445mm annually.

2.2 Collection and Rearing of *Aedes aegypti*: *Aedes aegypti* larvae or pupae were collected from breeding sites such as tires, fountain, water storage tanks etc. in five selected areas of district Mansehra; Bahali, Phulra, Baffa, Battal and Jabori. *Aedes aegypti* larvae were collected from urban sites of heavy use of insecticides for the control of disease vector by the public health especially Malaria vector control authorities and in agriculture sector for pest control in rural sites and from sites of less insecticide usage. The field collected larvae were transferred to the insectary and after post emergence the adult female *Aedes aegypti* were allowed to blood feed on mice to become gravid and allow to ovulation and reared under standard condition of temperature and humidity in the Insectary of department of Medical Entomology and disease vector control, Health Services Academy, Islamabad.

2.3 Bioassay: After rearing adult *Aedes aegypti* 2-3 days old F1 unfed mosquitoes were used for susceptibility and exposed to five insecticides (DDT 4%, Malathion 5%, Bendiocarb 0.1%, Lambdacyhlothrin 0.05%, and Deltamethrin 0.05%) from all four classes of insecticides for one hour using the standard WHO test kit method [23]. *Aedes aegypti* identification was made morphologically through pectoral key [24]. All susceptibility tests were conducted at 25 ± 2°C and 70-80% relative humidity in Insectary of Health Services Academy Islamabad. For each insecticide in each tube 20-25 adult female *Aedes aegypti* were exposed in four exposure tubes and two control tubes having susceptible mosquitoes exposed to corresponding impregnated with Reisella oil (DDT control), Olive oil (Malathion and Bendiocarb control) and Silicone oil (Lambdacyhlothrin and Deltamethrin control). The tested population was exposed to the insecticides for one hour in the exposure tubes and after exposure the live mosquitoes were transferred to the holding tubes and maintained with a pad of moist cotton soaked with 10% sucrose solution for 24 hours [23].

Then after one hour and 24 hours of exposure the quantity of dead and surviving mosquitoes were counted and observed percentage mortalities were calculated. If the control mortality was between 5% to 20% the observed percentage mortalities were corrected through Abbott's formula by following the standard WHO guidelines [25]. If the control mortality was above the 20% tests were discorded and repeated again. If the control mortality was below 5% it was ignored and no correction were made.

2.4 Data Analysis: The resistance status of mosquito's samples was determined according to WHO guidelines. The test results were summarized in three classes of resistance and susceptibility defined by WHO. The mosquito's populations were considered as susceptible when the mortality ranges between 98-100% level, shows resistance and require further verification if mortality ranges between 90-97% and were considered resistant if the mortality was less than 90% [23]. Data were analyzed through SPSS 20 by applying Chi square test to check the heterogeneity of the sample population.

3. Results

This is the first study to assess the resistance and susceptibility status of *Aedes aegypti* to insecticides in

Mansehra Khyber Pakhtunkhwa province in recent times. *Aedes aegypti* were exposed to the diagnostic doses of DDT, Malathion, Bendiocarb, Lambdacyhlothrin and Deltamethrin. This shows a range of mortalities in all five localities of District Mansehra. *Aedes aegypti* was resistant to DDT, Malathion, Lambdacyhlothrin and Deltamethrin but the Bendiocarb is placed under the verification required status.

As described in Table 1, results of test done on *Aedes aegypti* population collected from all the five locations of Mansehra were highly resistant to the diagnostic dose of DDT. The mortalities of the tested population of *Aedes aegypti* ranged between 33.75% and 43.75% with an average mortality of 38.95% against DDT at all five localities.

Similarly resistance were also detected according to WHO guidelines in female *Aedes aegypti* collected from all five localities to the diagnostic dose of Malathion 5% but the level of resistance was less then DDT. The mortalities rates were ranges in between 79.97% and 87.50% with an average mortality of 83.24% from all the study sites. But it was noted that the mortalities of *Aedes aegypti* against Bendiocarb 0.1% were in the range of 90.38% to 96.42% in all five localities with an average mortality of 93.82%. According to WHO standard guidelines there is the possibility of resistance but still it needs further confirmation.

Susceptibility status of *Aedes aegypti* were also investigated in this study against Lambdacyhlothrin 0.05% and Deltamethrin 0.05% most widely used in Public health for vector control and in Agriculture for pest control. The test result shows that the mortality level of adult *Aedes aegypti* against Lambdacyhlothrin 0.05% in the range of 77.63% to 87.83% with an average mortality of 81.22% at all five collected sites. According to standard WHO results interpretation the tested population was resistant to Lambdacyhlothrin 0.05%. Similarly the *Aedes aegypti* collected from all five localities were also resistant to Deltamethrin 0.05%. The mortalities of *Aedes aegypti* to Deltamethrin were in the range of 75.3% to 86.11% with an average mortality of 82.58%. The results indicate that insecticides resistance is present in all the study areas. The results of this study are summarized in Table. 1 with their Chi square values of all the five localities.

Table 1: Summary of results of susceptibility/resistance tests done on *Ae. aegypti* with 4% DDT, 5%Malathion, 0.1% Bendiocarb, 0.05% Lambda-cyhalothrin and 0.05% Deltamethrin in all five localities of district Mansehra.

District	Localities	DDT			Malathion			Bendiocarb			Lambdacyhlothrin			Deltamethrin		
		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
Mansehra	BAH	80	43.75	R	80	85.50	R	80	92.10	?	80	87.83	R	80	86.11	R
	PHU	80	34.21	R	80	82.50	R	81	95.06	?	83	80.72	R	84	85.83	R
	BAF	80	41.25	R	80	87.50	R	84	96.42	?	88	81.56	R	81	75.30	R
	BAT	80	33.75	R	83	80.72	R	83	90.36	?	85	78.37	R	87	80.64	R
	JAB	83	41.78	R	81	79.97	R	82	95.18	?	80	77.63	R	80	85.00	R
	Total	403			404			410			416			412		
Chi square		P=0.534, df=4, χ^2= 3.143			P=0.770, df=4, χ^2= 1.812			P=0.382, df=4, χ^2= 4.185			P=0.487, df=4, χ^2= 3.437			P=0.211, df=4, χ^2= 5.849		

S= Susceptible if 98-100% observed mortality

? = 90-97% observed mortality suggests the possibility of resistance that needs to be further confirmed.

R= Resistant if < 90% observed mortality

R1-R5= Replicates, C1-C2= Control

Level of Significance p<0.05; non-significance *p*>0.05, df= Degree of freedom, χ^2= Chi Square Value

BAH= Bahali; PHU=Phulra; BAF=Baffa; BAT,=Battal and JAB=Jabori.

4. Discussion

The purpose of this study was to assess the baseline susceptibility status of *Aedes aegypti* primary vector of dengue virus against different insecticides representing the major classes of insecticides i.e. Organochlorine, Organophosphate, Carbamates and Pyrethroid in Mansehra, Khyber Pakhtunkhwa. These chemicals were used in the past as indoor residual spraying by the malaria control program of Pakistan but many insecticides of Pyrethroid group are still in use to suppress the disease vectors including Malaria and Dengue vectors as IRS, LLINs and thermal/cold fogging. *Aedes aegypti* have highly anthropophilic, with markedly endophilic and endophagic behaviors and these characteristics prove *Aedes aegypti* as an effective disease vector. Dengue transmission is controlled through vector control strategies mainly by use of chemical insecticides due to the absence of proper vaccine and therapeutic medicines [26, 27]. But the regular and extensive use of chemical insecticides for a prolonged time to control disease vectors in public health as well as in Agriculture settings leads a problem to the development of resistance in disease vector in many endemic countries [13, 14, 28].

Adult bioassay of *Aedes aegypti* against DDT 4% result showed that the test mosquitoes were resistant in all five localities with percentage mortality range from 33.75% to 45.78%. DDT resistance might have been developed over time due to the prolonged and irrational use in vector control programs of public health as well as in agriculture. DDT resistance in *Aedes aegypti* population is wide spread and is reported by many researchers worldwide. High DDT

resistance was reported in both *Aedes aegypti* and *Aedes albopictus* collected from six districts i.e. Jaffna, Kandy, Kurunegala, Gampaha, Ratnapura and Puttalam of Srilanka [29]. *Aedes aegypti* were collected from urban areas of India to check the susceptibility level against Temephos, Fenthion, Malathion and DDT and reported low level of resistance to DDT. Resistances to DDT in the population of adult *Aedes aegypti* collected form the houses of dengue infected patients in Bangkok, Thailand were also reported. But in Pakistan and India Malaria vectors have already been reported by many researchers to be resistant to DDT and Malathion used as IRS for a long time in the country [15, 30, 31, 32]. The tested *Aedes aegypti* were also resistant to 5% Malathion in all union council with percentage mortalities ranged from 79.97% to 87.50%. The result of this study is comparable to a study from Yemen on field collected *Aedes aegypti* population and reported that the *Aedes aegypti* population were resistant to Malathion 5% and Fenitrothion 1% having mortalities of 64% and 29% respectively [33]. Malathion resistance in *Aedes aegypti* population of Hainan Province China was also reported but a moderate level of resistance were reported from Jaffna in both the primary and secondary vectors of dengue and were susceptible in Kandy, Kurunegala, Gampaha, Ratnapura and Puttalam of Srilanka to Malathion and Propoxur. Similarly moderate level of resistance was also reported to Malathion form Batticaloa in eastern Srilanka [29, 34, 35]. Resistance of *Ae. aegypti* to Malathion in the study area may be due to the high selection pressure of Malathion because this insecticide has been used widely and irrationally by the vector control program and in agriculture sector to control agricultural pest in past. According to a report the use of DDT and Malathion in Pakistan were banned for vector control program in public health and are replaced by Pyrethroid group of insecticides which are still used both in agriculture and public health [15]. Based on previous and present study DDT and Malathion should not be reintroduced for public health as well as agriculture purposes because of the resistance of the vector and pest population of Pakistan.

This study also evaluates the resistance and susceptibility status of field collected *Aedes aegypti* from all the study sites of Mansehra against Carbamates i.e. Bendiocarb 0.1% and the test results reveals that the percentage mortalities were in the range of 90.36% to 96.42%. According to the WHO interpretation the mosquito population of *Aedes aegypti* was moderately resistant and falls in the category of further verification. But a study from Penang, Kuala Lumpur, Johor Bharu, Kota Bharu of Malaysia reported a high level of resistance to DDT, Pyrethroid and Bendiocarb. Similarly in another study from Shah Alam, Selangor, Malaysia also reported Bendiocarb resistance [36, 37]. A similar study conducted on *Aedes aegypti* and *Aedes albopictus* in Andaman and Nicobar Island, India and reported that both species were resistant to Carbamates and Bendiocarb 0.1% [38]. Bendiocarb resistance were also documented from Colombia and Venezuela and it was reported that the dengue vectors were resistant to 0.1% Bendiocarb [39, 40], but the studies from Sudan and Costa Rica it was reported that *Ae. aegypti* were susceptible to 0.1% Bendiocarb [41, 42]. This low and high level of resistance may be due to the high or low selection pressure of Bendiocarb in the mentioned areas.

Aedes aegypti populations were also resistant to Pyrethroid group of insecticides at all five study sites of district Mansehra. The mortalities of *Ae. aegypti* were in the range of 77.63% to 87.83% against Lambdacyhlothrin 0.05% and 75.30% to 86.83% against Deltamethrin 0.05%. Pyrethroid group of insecticides are widely used to control disease vectors in Public health especially by the malaria control program as well as in pest control in agriculture all around Pakistan. Pyrethroid group of insecticides are used to control the dengue vector in the form of thermal fogging, IRS, ultralow volume sprays, impregnated bed nets and other materials. Due to the extensive and irrational use of this group of insecticides has increased the selection pressure on the vector species of dengue, malaria and other disease vectors and due to this mosquitoes vector has developed resistance against this insecticides. Pyrethroid resistance also may be due to the cross resistance with DDT. There is also a potential threat of cross resistance between Organophosphate and Pyrethroid to vector control program [43]. The results of our study of Pyrethroid resistance are further strengthening by the other studies from the region and all over the world [18]. Pyrethroid resistance in dengue vector was well documented by the studies from Yemen [33], Singapore [44], Thailand [45], French Guiana [14], Paraguay [46] and less resistance was also reported from Mexico [47]. But it was reported that malaria vectors were resistance to synthetic pyrethroids. *Anopheles subpictus* were reported to be resistance to permethrin and deltamethrin from district Kasur, Punjab and similarly the *Anopheles* mosquitoes were resistant to permethrin, Lambdacyhlothrin and deltamethrin from Mirpur Khas, Sindh of Pakistan [48, 49]. As the national disease control program has no alternate insecticide for effective vectors control or for insecticides resistance management [50]. However with proper resistance management strategies especially integrated vector management (IVM) approach this high level of resistance in disease vector species against the main insecticides groups especially Pyrethroids can be delayed or prevented. The results of this study provide baseline information of insecticides resistance in dengue vector and recommend the proper insecticides resistance monitoring and timely entomological investigation in all around the country.

4. Conclusion

We concluded in the light of the present study that the adult *Ae. aegypti* population were resistant to all tested insecticides in district Mansehra. The results of present study confirm the need for regular and proper monitoring of resistance and susceptibility status of *Ae. aegypti* in Mansehra as one of the best and important approach of integrated vector management for the effective disease vector control in the area. But unfortunately the surveillance and monitoring system of insecticides resistance of disease vector is very poor in Pakistan.

In order to prevent or slow down resistance to the presently used insecticides for vector control program in Public health, the development and implementation of new strategies for vector control in particular the integrated vector management is very important.

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6. References

1. Kasai S, Komagata O, Itokawa K, Shono TLCNg, Kobayashi M, Tomita T. Mechanisms of Pyrethroid Resistance in the Dengue Mosquito Vector, *Aedes aegypti*: Target Site Insensitivity, Penetration, and Metabolism. PLoS Negl *Trop Dis.* 2014; 8(6):e2948.
2. Kumar K, Sharma AK, Sarkar M, Chauhan A, Sharma R. Surveillance of *Aedes aegypti* (L.) Mosquitoes in Mumbai International Seaport (India) to Monitor Potential Global Health Risks. J Insct. 2014; ID 951015 http://dx.doi.org/10.1155/2014/951015.
3. Sarkar A, Taraphdar D, Chatterjee S. Molecular Typing of Dengue Virus Circulating in Kolkata, India in 2010. J Trop Med. 2012; 10:1155.
4. Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. Nat Med. 2004; 10:S98-S109.
5. World Health Organisation. Global strategy for dengue prevention and control 2012-2020. Geneva, Switzerland. 2012, 1-34.
6. Gubler DJ. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle? Comp. Immunol. Microbiol. Infect. Dis. 2004; 27:319-330.
7. Guzman GM, Kouri G. Dengue: an update. Lancet Infect. Dis. 2002; 2:33-42.
8. World Health Organization. Epidemiology, Burden of Disease and Transmission. Regional Office for the Eastern Mediterranean (WHO/EMRO.). Division of Communicable Disease Control. Newsletter. l2005. 2009, 7-8.
9. Din S, Akram W, Khan HAA, Hussain A, Hafeez F. Citrus waste-derived essential oils: Alternative larvicides for dengue fever mosquito, *Aedes albopictus* (Skuse) (Culicidae: Diptera). Pak J Zool. 2011; 43:367-372.
10. World Health Organization. Weekly Epidemiological Monitor. Eastern Mediterranean Regional Office (EMRO), Cairo, Egypt. 2013; 6(37).
11. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human diseases. Annul Rev Entomol. 2000; 45:371-391.
12. Hemingway J, Hawfes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochem Mol Biol. 2004; 34:653-665.
13. Kamgang B, Marcombe S, Chandre F, Nchoutpouen E, Nwane P, Etang J *et al*. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa. Parasit Vectors. 2011; 4:79.
14. Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R. Multiple insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. *Mem Inst Oswaldo Cruz*, Rio de Janeiro. 2011; 106(3):346-352.
15. Rathor HR, Toqir G, Rashid S, Mujtaba SM, Nasir SM. Insecticide resistance in *Anopheline* mosquitoes of Punjab province, Pakistan. Pak. J. Zool 1986; 17(1):35-50.
16. Khooharo AA, Rajab AM, Mallah MU. An Empirical analysis of Pesticides Marketing in Pakisstan. Pak. Eco. Socl. Rev. 2008; 46(1):57-74.
17. Malik TM. Pyrethroids – the harder weapon. Pakistan Agriculture. 1986, 23.
18. Vontas J, Kioulos E, Pavlidi N, Morou E, della Torre A, Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. Pestic Biochem Physiol. 2012; 104:126-131.
19. Jahan N, Shahid A. Evaluation of resistance against Deltamethrin and Cypermethrin in dengue vector from Lahore, Pakistan. J of Animal & Plant Sciences. 2013; 23(5):1321-1326.
20. Jahan N, Shahid A. Evaluation of Resistance Against *Bacillus thuringiensis israelensis* WDG in Dengue Vector from Lahore, Pakistan. Pak. J Zool. 2012; 44(4):945-949.
21. Jahan N, Sadiq A. Evaluation of resistance against Bifenthrin in dengue vector from Lahore, Pakistan. Biologia (Pakistan). 2012; 58(1,2):13-19.
22. Khan HAA, Akram W, Shehzad K, Shaalan EA. First report of field evolved resistance to agrochemicals in dengue mosquito, *Aedes albopictus* (Diptera: Culicidae), from Pakistan. Parasite and Vectors. 2011; 4:146-157.
23. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, Switzerland. 2013; http://www.who.int/malaria
24. Huang YM. The mosquitoes of Polynesia with a pictorial key to some species associated with filariasis and/or dengue fever. Mosq. Syst. 1977; 9:289-322.
25. Abbott SW. A method of computing the effectiveness of an insecticide. J Econ Entomol. 1925; 18:265-267.
26. Scott TW, Morrison AC. Vector dynamics and transmission of dengue virus: implications for dengue surveillance and prevention strategies: vector dynamics and dengue prevention. Curr Top Microbiol Immunol. 2010; 338:115-128.
27. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control: new edition Geneva. 2009; 147.
28. Singh RK, Dhiman RC, Mittal PK, Dua VK. Susceptibility status of dengue vectors against various insecticides in Koderma (Jharkhand), India. Vector Borne Dis. 2011; 48:116-118.
29. Karunaratne SHPP, Weeraratne TC, Perera MDB, Surendran SN. Insecticide resistance and, efficacy of space spraying and larviciding in the control of dengue vectors *Aedes aegypti* and *Aedes albopictus* in Sri Lanka. Pest Biochem Physiol. 2013; 107(1):98-105.
30. Ticker SN, Mendki MJ, Sharma AK, Sukumaran D, Veer V, Parkash S *et al*. Resistance Status of the Malaria Vector Mosquitoes, *Anopheles stephensi* and *Anopheles subpictus* Towards Adulticides and Larvicides in Arid and Semi-Arid Areas of India. J Insect. Sci. 2011; 11:85
31. Ticker SN, Mendki MJ, Chandel K, Parashar BD, Prakash S. Susceptibility if immature stages of *Aedes (Stegomyia) aegypti*; vector of dengue and chikungunya to insecticides from India. Parasitology Research. 2008; 102(5):907-913.
32. Komalamisra N, Srisawat R, Phanbhuwong T, Oatwaree S. Inscticides susceptibility of dengue vector, *Aedes aegypti* (L.) in Metropolitan Bangkok. Southeast Asian J Trop Med Public Health. 2011; 429(4):814-23.
33. Alhag SKN. Susceptibility status of dengue fever vector *Aedes aegypti*, (L.) in Republic of Yemen. Biomedical Communication Biosci. Biotech. Res. Comm. 2013; 6(2):138-141.
34. Dharshini S, Vinobaba M, Jude PJ, Karunaratne SHPP, SN. Prevalence and insecticide susceptibility of dengue vectors in the district of Batticaloa in eastern Sri Lanka.

Trop. Med. Health. 2011; 39(2):47-52.
35. Cui F, Raymond M, Qiao CL. Insecticides resistance in vector mosquitoes in China. Pest Mang. Sci. 2006; 62(11):1013-1022.
36. Ishak IH, Jaal Z, Ranson H, Wondji CS. Contrasting patterns of insecticide resistance and knockdown resistance (*kdr*) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. Parasites & Vectors. 2015; 8:181-194.
37. Rong LS, Ann AT, Ahmad NW, Lim LH, Azirun MS. Southeast Asian J Trop Med Public Health. 2012; 43(1):34-47.
38. Sivan A, Shriram AN, Sunish IP, Vidhya PT. Studies on insecticide susceptibility of *Aedes aegypti* (Linn) and *Aedes albopictus* (Skuse) vectors of dengue and chikungunya in Andaman and Nicobar Islands, India. Parasitol Res. 2015; DOI 10.1007/s00436-015-4717-3.
39. Ocampo CB, Salazar-Terreros MJ, Mina NJ, McAllister J, Brogdon W. Insecticides resistance status of *Aedes aegypti* in 10 Localities in Colombia. Acta Tropica. 2011; 118(1):37-44.
40. Mazarri MB, Georghiou GP. Characterization of Resistance to Organophosphate, Carbamate and Pyrethroid Insecticides in Field Populations of *Aedes aegypti* from Venezuela. Journal of the American Mosquito Control Association. 1995; 1l(3):315-322.
41. Bisset JA, Marín R, Rodríguez M, Severson DW, Ricardo Y, French L *et al* Insecticide resistance in two *Aedes aegypti* (Diptera: Culicidae) strains from Costa Rica. J Med. Entomol. 2013; 50(2):352-61.
42. Husham AQ, Abdulmajid MA, Brair M. Status Susceptibility of dengue vector; *Aedes aegypti* to different group of Insecticides in Port Sudan city-Red Sea state. Sudan Journal of Public Health. 2010; 5(4):199-202.
43. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Mol Biol. 2000; 9:491-497.
44. Koou SY, Chong CS**,** Vythilingam I, Lee CL, Ng LC. Insecticide resistance and its underlying mechanisms in field populations of *Aedes aegypti* adults (Diptera: Culicidae) in Singapore. Parasites & Vectors. 2014; 7:471.
45. Somboon P, Prapanthadara L, Suwonkerd W. Insecticide susceptibility tests of Anopheles minimuss. *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* in northern Thailand. Southeast Asian. J Trop. Med. Publ. Hlth. 2003; 34:87-93.
46. Bona ACD, Piccoli CF, Leandro ADS, Kafka R, Twerdochilib AT, Navarro-Silva MA. A single Genetic profile and molecular resistance of *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) in Foz do Iguaçu (Brazil), at the border with Argentina and Paraguay. Zoologia. 2012; 29(6):540-548.
47. Lopez B, Ponce G, Gonzalez JA, Gutierrez SM, Villanueva OK, Gonzalez G *et al* Black,4th, Flores AE. Susceptibility to chlorpyrifos in Pyrethroid resistant populations of *Aedes aegypti* (Diptera: Culicidae) from Mexico. J Med. Entomol. 2014; 51(3):644-649.
48. Hammad M, Bhatti A, Mukhtar MU, Arslan A, Mushtaq S, Zaki AB. Susceptibility/resistance of selected insecticides in *Anopheles* mosquitoes of district Mirpur Khas, Sindh, Pakistan. Journal of Entomology and Zoology Studies. 2015; 3(6): 321-325.
49. Naeem H, Ashraf K, Rashid MI, Rehman HU, Oneeb M, Akbar H. Insecticide resistance status of *Anopheles subpictus* from district Kasur, Punjab, Pakistan. Sci. Int (Lahore). 2014; 26(3):1205-1208.
50. Dash AP, Raghavendra K, Pillai MKK. Combating Resistance to Insecticides in Malaria Control- Gains Made in India. Bayer Environmental Science Journal. 2006; 18:30-37.