Bendiocarb 0.1% and Malathion 5% Resistance in *Anopheles stephensi* in Selected Areas of District Peshawar, Pakistan

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Abstract

Anopheles stephensi is a major species in Indian sub-continent that causes malaria. For its controlling wide spread of different insecticide is used, which has caused mutation in the specie. The mutation led to insecticide resistant in *Anopheles stephensi*. This study reveals the current resistance status of *Anopheles stephensi* against Bendiocarb and Malathion insecticide. For this, about 700 mosquito larvae collected from Hazarkhwani, Jamil Chowk, Surizai and Shalam. Of which 350 died, rest were reared in laboratory. Out of which approximately 250 were identified as *Anopheles* on adult emergence, 124 were female that were subjected to insecticide exposure for their susceptibility. Results showed that *Anopheles stephensi* were resistant to Bendiocarb with mortality rate 85.23%, this was followed by Malathion which was also found less effective against *Anopheles stephensi* with mean mortality rate 83.88%. These insecticides were deemed Confirmed Resistant as their fatality rates were below the WHO-recommended mortality threshold (<90%). This study revealed that Bendiocarb 0.1% and Malathion 5% were found ineffective against *Anopheles stephensi*, malarial-vector. Several beneficial outcomes of this work might include enhanced monitoring programs, vector control tactics, and public health protection against vector-borne illnesses. The examination of *Anopheles stephensi* resistance status to various insecticides provides insight into the need to analyze the impact of insecticide resistance in various vector control programs and to monitor it in impacted regions.

Keywords

Medicinal Plants Phytochemical, Antimicrobial, Anthraquinones, *Anopheles stephensi*

1. Introduction

1.1 Mosquito

Mosquitoes are actual flies that are members of the Culicidae family. The largest group of arthropods in the world are mosquitoes. The extent of deaths from illnesses spread by mosquitoes makes them the deadliest animal in the world [1]. Numerous species of these serve as vectors for delivering lethal diseases to humans [2]. The two most significant are malaria, which is spread by *Anopheles* mosquitoes; the yellow fever virus, which caused devastating epidemics in the New World in the 18th and 19th centuries, is spread by mosquitoes of the genus *Aedes*; and filarial elephantiasis, which is spread by nematodes (Wuchereria, Brugia). Additionally, livestock and wild animal diseases are also spread by mosquitoes. For instance, horses in the United States are afflicted by many equine encephalitis viruses. [3]. The loss of native bird species in Hawaii is mostly due to introduced insects and avian malaria [4].

1.2 Taxonomy

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

The family name Culicidae, derived from Culex, the Latin name for “gnat,” is a member of one of the main stocks of Nematocera, the infraorder Culicomorpha. It consists of two superfamilies, all of the piercing/sucking nematocerans, both predators and blood-feeding biters are included in it [5]. Among all the culicomorphs, the long proboscis of mosquitoes is distinctive. It shows a long and intimate relationship between mosquitoes and vertebrate animals and is thought to be the most specialized biting mouthpart among Nematocera [6]. The Culicidae family of mosquitoes currently has 3,547 species documented [7]. Tropical rainforests, where faunas are more varied but less thoroughly studied than in temperate regions, are likely home to the greatest number that has yet to be identified [8]. There are 41 genera of mosquitoes, 38 of which are in the subfamily Culicidae [9]. Because of their enormous medical and veterinary

value to humans, there is a very thorough description of the current fauna, which includes 3,547 species of mosquitoes [10].

1.3 Origin

Being an ancient group, mosquitoes are thought to have originated in South America, some 217 million years ago [11] on a large landmass known as Gondwana that had not yet broken apart [12]. Unfortunately, there is a rather sparse fossil record with only 23 species known [13].

1.4 Morphology

Adult mosquitoes are thin with long legs and narrow wings that are stretched out. Their bodies have small scales, bristles, and fine hairs that give each species its unique patterns and colors. [14].

Body comprising of three parts

1. Head 2. Thorax 3. Abdomen (as given in figure 1)

1.4.1 Head

A mosquito's head is made up of pieces for its mouth, antennae, and complex eyes. The compound eye contains 350–900 ommatidial lenses [15]. The mosquito proboscis is noticeable and typically sexually dimorphic, and the antennae are long and filamentous [16]. A fascicle for female penetration is formed by the mouthparts, which include the labium, maxillae, hypopharynx, mandibles, and labrum [17].

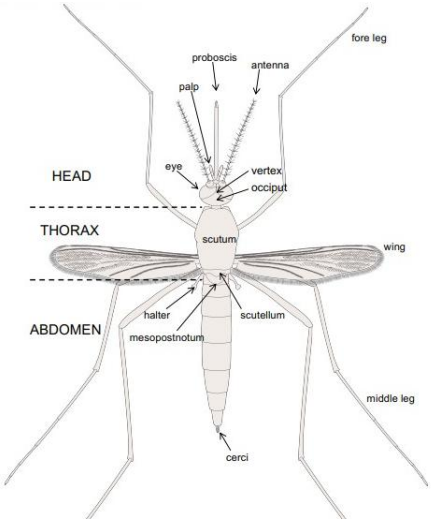

Figure 1. Dorsal view of Mosquito [18].

1.4.2 Thorax

The mosquito thorax is a rigid, muscle-filled locomotor unit with lateral spiracles, slender legs, and tarsi [19]. Its wings are narrow, vein-patterned, and bear scales. The halters help to detect changes in orientation and movement in environment during flight [20].

1.4.3 Abdomen

The abdomen, which is the back part of the body, helps with digestion, waste removal, and making babies. It has ten sections and includes parts called sternites and tergites. At the end of the abdomen are two thin, finger-like structures called cerci, which are used for laying eggs and mating as given in figure 2. [21].

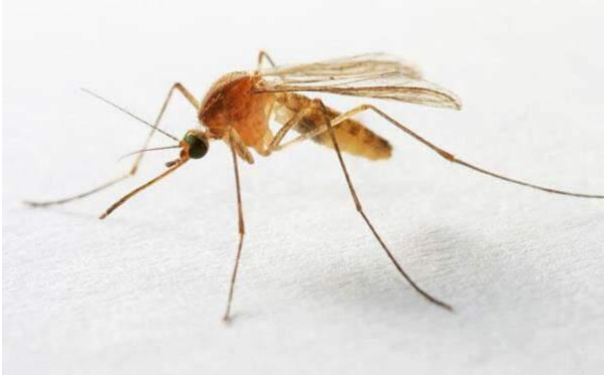

Figure 2. Adult mosquito at resting position [22].

1.5 Body Size

Mosquitoes are small insects, typically weighing between 3 and 10mm [23]. Variation in adult body size in field populations of various mosquito species suggests that body size is related to blood-feeding success [24].

1.6 Feeding

Female mosquitoes rarely start blood-feeding until at least one to three days following adult emergence, and in many cases, only after mating and sugar-feeding. All vertebrate classes—mammals, birds, reptiles, amphibians, and even amphibious fish, earthworms, and leeches—are among their hosts [25]. They have been known to consume other insects' hemolymph, but it's possible that this only happens after the insects have been exposed to vertebrate smells. Some species opportunistically target members of two or three vertebrate classes, whereas others feed nearly exclusively on animals in a single genus [26]. Both the mosquito's natural host choice and the hosts that are accessible to it during and after its activity determine host specificity.

1.7 Habitat

Some mosquitoes like to reside in forests, marshes, or thick grasses, others prefer to live close to people. Since their larvae and pupae live in water with little to no flow, all mosquitoes prefer water. Different mosquito species are drawn to different sorts of water.

1.7.1 Permanent Water Mosquito

These mosquitoes usually lay their eggs in permanent or semi-permanent water bodies. Some mosquitoes like clean pools, while others prefer ones that are rich in nutrients. Some mosquitoes lay their eggs near the edges of lakes and ponds, among plants in marshes and swamps, or in containers that collect water [27].

1.7.2 Floodwater Mosquito

These Mosquitoes lay eggs in moist soil or containers above water level. After drying out, eggs hatch when rainwater fills [28]. Floodwater habitats include temporary pools, ponds, floodplains, irrigated fields, and tree holes [29].

1.8 Host-finding Behavior

Mosquitoes use certain chemicals that are easy to smell to find animals they can bite. Some of the best-known chemicals that attract them are octenol, lactic acid, and carbon dioxide [30]. There are also several fatty acids made by the good bacteria on the skin that are very good at attracting Anopheles gambiae to human feet [31]. Mixtures of these fatty acids and other chemicals probably play a big role in attracting most types of mosquitoes [32]. If the mosquito finds the right mix of signals from the host, the female will try to land on the animal, often preferring parts like the head or legs. Once she lands, she goes through four steps in her feeding process: exploring, finding a blood vessel, drinking, and then leaving.

1.9 Site for Blood Feeding

Mosquitoes can feed from a variety of skin surfaces, including the wet skin of frogs and the scaly legs of reptiles and birds [33]. They can penetrate material if it is not thicker than the proboscis's length, they may pierce mucous, matted hair, light layers of feathers, and heavier materials like denim. [34].

1.10 Blood Sucking Behavior

Once a feeding spot is chosen, the bundle of stylets goes through the skin, with the labium helping to guide it and bending back without actually breaking through [35]. On each side of the stylet bundle, the maxillae and mandibles move quickly back and forth, taking turns to stab or pierce. As they do this, the maxillary teeth, which point backward, hold onto the tissue as the stylets go through the skin and the tissue just under the skin.

1.11 Locating Blood Vessels

In order to assist the mosquito, find a blood artery and encourage swallowing, sensilla on the labrum and in the cibarium appear to detect plasma and cellular components, including adenyl nucleotides like ATP [36]. When the female finds a vessel, she inserts the tip of her fascicle into the lumen and uses the pharyngeal and cibarium pumps to suck blood up via the feeding canal.

1.12 Salivation to Prevent Blood Clotting

Saliva flows from the tip of the hypopharynx when small arteriole or venule is detected. The antihemostatic enzyme apyrase, which is present in saliva, prevents platelets from aggregating and allows randomly ruptured arteries to freely flow into the surrounding tissue spaces [37]. The mosquito spends less time on the host overall and finds a vessel more easily as a result. Saliva also includes anticoagulants, which help locate vessels and swallow blood by keeping it from clotting [38].

1.13 Ingestion and Excretion

In one to four minutes, the mosquito can fully engorge due to the accumulation of blood in the midgut. At this point, the female starts drawing water out of the bloodmeal and could leave tiny pee drops on the host's skin [39].

1.14 Get Rid of Over Feeding

Upon receiving a signal from abdominal stretch receptors that there is enough blood in the midgut, the female pushes with her forelegs to remove her stylets and takes off. Prior to a significant percentage of the water and salt in the blood meal being eliminated in the urine, which usually occurs after one to two hours, she is too heavy to fly very far [40].

1.15 Life History

Mosquito life cycle has four distinct stages (egg, larvae, pupae and adult). Duration of each stage depends upon temperature and food resources. The holometabolous life cycle of mosquitoes is completed in two different environments, one aquatic, the other terrestrial. These stages are:

1.15.1 Egg Stage

Mosquito eggs are laid on water or solid substrates that may become inundated. Females in the *Anophelinae* and *Culicinae* subfamilies scatter eggs individually on the water surface (as shown in figure 3), while those in the *Aedini* tribe attach them individually on a substrate that may become inundated. Clumped eggs are laid in boat-like rafts by several *Culicinae* genera (e.g., *Culex, Culiseta, Coquillettidia, Uranotaenia*) [41]. The majority of the eggs, which are initially white, turn black in a matter of hours when the chorion tans [42]. In the summer, eggs typically finish their embryonic development in two to three days, but in cool climes, this can take up to a week or more [43].

Figure 3. *Aedes albopictus* eggs [44].

1.15.2 Larvae Stage

Mosquito larvae, also called wigglers or wrigglers, go through four growth stages called instars (as shown in figure 4). These stages look very similar, but each one is a bit bigger than the last. The larvae usually come out of eggs when the water is warm, and this happens after the eggs have been under water for some time. The activity of tiny living things in the water causes the oxygen level to go down, which helps the eggs hatch [45]. The larvae have features that are easy to see when they're placed on a slide [46]. In species that lay their eggs directly in water, like all Anophelinae and most groups in Culicinae, the larvae hatch not long after the eggs start developing.

Figure 4. Larvae [47].

1.15.3 Pupa Stage

Mosquito pupae, commonly known as tumblers, are comma-shaped, with the head and thorax fused to form a cephalothorax and the abdomen curled beneath it [48] (as shown in figure 5). The pupa receives oxygen at the water's surface by a pair of respiratory tubes, often known as air trumpets, that protrude from the dorsal mesothorax [49].

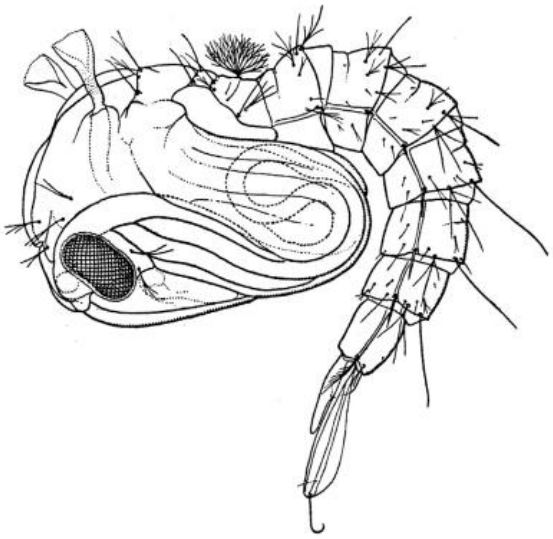

Figure 5. Mosquito pupa [50].

The pupa spends most of its time at the water surface, molting into a dark pharate adult within the pupal cuticle. The pupal stage typically lasts 2 days in warm water, longer at lower temperatures. Adult males emerge earlier due to shorter larval growth periods. As adult emergence approaches, the pupa remains stationary for 10-15 min. Entire process of emergence takes only a few minutes.

1.15.4 Adult Stage

In the last stage of adulthood, the abdomen is straightened, air is swallowed, and gas is pressed between the pupal and pharate cuticle [51]. The newly emerged adult is capable of short flights a few minutes later. Both sexes acquire sugar from plant nectar or honeydew during the first three to five days of adulthood, reach sexual maturity, and then mate [52].

1.16 Flight Range

Mosquitoes are excellent flyers, with an average flight range of 25m to 6km and a maximum of 50m to 50km. Their flight range varies greatly and is species-specific [53]. *Anopheles* mosquitoes can fly continuously for four hours at 1 to 2 km/h, traveling up to 12 km at night. Male mosquitoes can beat their wings 450 to 600 times per second. The maximum recorded flight range for *Anopheles sacharovi Favr* was 8.65 km. *Aedes vexans* flown 14 miles, while *Culex pipiens* flown 9 miles during the first day. *Anopheles sacharovi Favr* were found in Israel up to 13 km [54], and Adults of *Anopheles freeborni Aitken* in California up to 42km [55]. Flight capacity is influenced by topography, abiotic environmental conditions, and species physiology.

1.17 Development of Resistance to Insecticides

In addition to having global consequences for insect vector management, insecticide resistance is seen as a significant evolutionary phenomenon [56]. The mechanism by which insecticide resistance develops is intricate and is influenced by both direct and indirect variables, including genetic, physiological, behavioral, and ecological factors, as well as the amount and frequency of insecticide dosages. Insecticide resistance research is crucial for preventing the emergence and spread of resistance in vector populations. Insect acetylcholinesterase is the target site for carbamates and organophosphates [57]. The target location for pyrethroids and a class of organochlorines (DDT + its derivatives) is the neuronal membrane's Na+ channel regulating proteins [58] GABA receptors are responsible for the remaining organochlorines (cyclodienes) [59].

1.18 History of Insecticide Resistance in Mosquitoes

Resistance of *Anopheles* mosquitoes to insecticides, reported for the first time in Africa in the 1950s, concerns four main classes of insecticides used in public health for vector control purposes, namely pyrethroids, organochlorines, organophosphates and carbamates [60]. The first global strategy for malaria control was adopted the first time malarial control was adopted in 1955 at the start of the now notorious Global Malaria Eradication Program. This strategy called for the widespread and rapid application of dichlorodiphenyltrichloroethane (DDT) to interrupt the transmission of the disease in countries around the world, except for countries in sub-Saharan Africa, regardless of geography and epidemiology.

1.19 Major Classes of Insecticide

Insecticides play the most important role in controlling mosquito vectors of diseases worldwide. Although, six classes of insecticides viz. organochlorines, organophosphates, carbamates, pyrethroids, pyrroles, and phenyl pyrazoles are currently used in mosquito control programs worldwide.

1.20 How Resistance to an Insecticide Develops

In individual mosquito species, multiple mechanisms are involved in development of resistance to insecticide. Of which three major types of resistance mechanisms namely; metabolic resistance (changes in insect enzyme systems leading to rapid detoxification or sequestration of insecticides) [61], target- site resistance (alterations of the insecticide target sites preventing their binding to insecticides) [62] and cuticular resistance (reduced penetration of insecticides due to a thickening or change in chemical composition of the cuticle) have been described [63].

1.21 Mechanisms of Insecticide Resistance

1.21.1 Increased Detoxification by Metabolic Enzymes

The main targets of insecticides are receptors or enzymes of the nervous system: acetylcholinesterase (AChE), the voltage-dependent sodium channel (CNaVdp), and the receptor of c-aminobutyric acid. This involves three major metabolic detoxification enzymes. Metabolic enzyme genes have a greater plasticity than insecticide target site genes [64] and the increased metabolic capacity is usually achieved by increased activity of esterase (also known as carboxylesterases), glutathione S-transferases (GSTs) or monooxygenases (also known as oxidases or cytochrome P450s). The Esterase family of enzymes hydrolyses ester bonds, which are present in a wide range of insecticides [65]. The glutathione S-transferases detoxify and excrete endogenous and exogenous compound [66]. The Cytochrome P450s involves diverse physiological and biochemical activities, it performs detoxification or activate xenobiotic compounds [67]. Increased enzyme activity can be brought about by gene amplification, increased upregulation, coding sequence mutations or by a combination of these mechanisms. Basically, esterase can provide resistance to organophosphates, carbamates and pyrethroids which are rich with ester-bonds. GSTs can mediate resistance to organochlorines, organophosphates and pyrethroids, and P450s act against all classes of insecticides [68].

1.21.2 Target Site Insensitivity Through Alteration of Target Sites

Insects acquire target site insensitivity mainly through non- silent point mutations within structural genes [69]. However, only a limited number of changes can decrease insecticide sensitivity without disrupting the normal physiological functions of the target site [70]. Therefore, the number of possible amino acid substitutions is very limited. Hence, identical resistance- associated mutations are commonly found across highly diverged taxa. The classic leucine to phenylalanine mutation of voltage-gated sodium channel proteins, the target site of DDT and pyrethroids, is found in [71]. indicating an independent origin of the same mutation in two different species which are geographically isolated. Although, altered target sites do not mediate the same level of resistance to all the insecticides belong to a particular group. The degree to which the normal physiological function is impaired by the resistance mutation is reflected in the fitness of resistant individuals in the absence of insecticide selection. An increased production of an enzyme in the metabolic resistance may have a lower associated fitness cost than an alteration in the structural gene [72].

1.22 Literature Review

Insecticide Resistance

Mosquito-borne diseases are major health issues to mankind. Human interventions such as using chemical insecticide has greatly reduced their population in affected areas [73]. Resistance to insecticide in malarial vector (*Anopheles* mosquito) and West Nile Virus (*Culex pipiens*) has been reported more than 25 years ago in Africa, America and Europe [74]. Greece has been affected by outbreak of West Nile Virus in 2010, being an epicenter of economic and visited by over 16 million tourists a year, data was collected for insecticide resistance in mosquito population to ensure the successful application of vector control. High densities of *Aedes caspius*, a nuisance species, *Culex pipiens*, a known vector of WNV and *Anopheles hyrcanus* a potential vector of malaria being among the most prevalent species were reported. On testing they found *Culex pipiens* and *An. Hyrcanus* showed moderate resistant to Deltamethrin. In *Culex pipiens* low frequency insensitivity was reported against organophosphates and carbamates. This study also examined mechanisms of resistance [75].

In Mozambique, a high level of pyrethroid resistance has been observed for *Anopheles funestus*. By performing biochemical assays and quantitative PCR it was found that P450 gene upregulation results in pyrethroid resistance another product Glutathione-S-transferases also adds the efforts secondarily. Moreover, Resistance against carbamate, bendiocarb was also reported because of mutation in AChE gene along with action of esterase [76].

Insecticide resistance in *Aedes aegypti* is quite common in field population of US. In 2017, entomologist test the status of *Aedes agypti* in New Mexico. A study collected mosquito eggs, larvae, and adults from eight southern New Mexico cities to establish laboratory strains and perform resistance tests. Four insecticides were used: pyrethroids etofenprox, permethrin, deltamethrin, and chloropyrifos. DNA was extracted from individual mosquitoes using the DNeasy Blood & Tissue Kit. Results showed Alamogordo's mosquitoes were somewhat resistant to permethrin and deltamethrin, while

Las Cruces mosquitoes were resistant to permethrin, deltamethrin, and etofenprox. Roswell mosquitoes showed high resistance against permethrin, deltamethrin, and etofenprox [77]. Pyrethriods are considered to be preferably more effective for bed-nets. The insecticide used in LLIN and IRS are belonging to same Pyrethriods family and small amount of some other toxic chemicals [78]. But in rural areas of Sengel (Country of West Africa) first time long term LLINs efficacy was reported to be ineffective [79]. Moreover, in Benin no beneficial effect was reported by using LLINs and IRS in comparison to targeted LLIN [80].

1.23 Socio-Economic Impact of the Study

In the last few years, there was a wide spread of Malaria occurred in Peshawar. For controlling Malarial vector, *Anopheles stephensi* extensive insecticide was sprayed in the nearby places where many cases were reported. In order to know the susceptibility of *Anopheles stephensi* against Bendiocarb 0.1% and Malathion 0.5%. Furthermore, there is need to introduce an alternate method such IPM for controlling malarial vector rather than using chemical insecticide as it has impacts negatively on living creature and their environment.

1.24 Aims and objectives of study

The aims and objectives of this study were to:

- Investigate resistance in *Anopheles stephensi* in the study areas.
- Investigate the current status of *Anopheles stephensi* against Bendiocarb 0.1% and Malathion 5% in study areas.

2. Materials and Methods

2.1 Study Area

Peshawar is located in northern of Pakistan. It is situated in east of historic Khyber Pass. Peshawar's Latitude approximately 34.0151° N, Longitude approximately 71.5249° [81]. Peshawar is elevated approximately 1,200ft from sea level. Peshawar has hot semi-arid climate [82]. The summer season extended up to seven months (April-October), mean maximum temperature always greater than 30 Celsius while in winter it drops up to 10 degrees Celsius at night occasionally drops to 4-5 degree Celsius. This area has less rainfall than other parts of country.

2.2 Larval Sampling and Identification

Larval sampling was conducted in Shalam, Hazarkhwani, Suruzai and Jamil Chowk. Collections were made over 22 days (from September 19 to October 10, 2024). Before field work weather was dry and mosquito densities were approximately low. Mosquito larvae and pupae were collected mostly from standing water rich in vegetation. Sampling was done by using paddle, and were collected in beaker. The collected sample was then shifted to laboratory for identification and separation based on larval morphological characteristics. After identification and separation, *Anopheles* larvae were reared in separate container, and that of others in different containers. Each container was covered by gauze net to provide free air for respiration. Each collection of different areas was labelled according to location and date. Other larvae were reared just for identification of local fauna of each area.

The colony of the larvae was maintained at room temperature and prepared feed was given according to density of larvae and surface area of container. Pupal form was then shifted to another container with low water, in order to provide space for adult emergence and flight. Until enough mosquitoes were ready for test, feed is provided, a cotton soaked in sugar solution was placed over gauze net so that adult fed by sucking sugary solution.

2.3 Exposure to Insecticide

When enough adults were ready to run a test. Test bottles were taken in which insecticide paper is placed. Adults from container sucked via sucked by mouth aspirator and introduced to test bottle for bioassay of insecticide resistant. Reading of knockdown mosquitos were noted after each ten minutes upto one hour. After that another bottle jointed to this bottle for shifting of live mosquito. This second is not subjected to any insecticide, mosquito that remain alive after one-hour exposure to insecticide allowed to trapped in it for 24hr one end of this have sieve for free air and feed is provided from this part, this allows absorbed insecticide to show its action. After 24hr, presence of live mosquito confers that these are resistant to insecticide. These are then subjected to lower temperature to be killed.

2.4 Labelling the Tubes

Three tubes were taken, knockdown mosquitoes of each of the three tubes are inserted to it by camel brush with care so that their body parts are conserved. Each of these tubes were labelled namely let suppose we given it names A, B and C, based on time duration allowed for insecticide action and number of knockdown mosquitoes.

2.5 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.

3. Results

Susceptibility Status of *An. stephensi* to Different Insecticides

By using WHO test kit, the susceptibility status of *An. stephensi* was observed against, two classes of insecticides. Namely Bendiocarb 0.1%(Carbamate) and Malathion 5% (organophosphates). The results for each insecticide are presented in Table 1 to 4. The knock down rates within 1hr period for the two insecticides were recorded which are mentioned below in odd tables from 1 to 3, after 24 hours of exposure to insecticides the mortality rates were calculated and noted in the tables 2 and 4. Our results showed that *An. stephensi* collected from district Peshawar were highly resistant to Bendiocarb 0.1% with a mortality rate of 85 %, which was very low from the resistant mortality i.e. 90% recommended by WHO. This was followed by Malathion (5%). The mortality rates of these both insecticides were found to be less than that of recommended mortality (<90%) and were considered as Confirmed Resistant.

Three bioassay tests (triplet) were performed for each insecticide. Each test was actively observed and the knockdown readings were carefully recorded in two steps, after an hour and after 24 hours of the exposure time. Two tables, for the detail information of each bioassay test, were made. Where the 1 and 3 table showed the results of the test triplet while the 2 and 4 table showed the total number anopheles mosquitoes introduced along with their control and their corresponding mortality rates.

Table 1. showing bioassay test results for Bendiocarb 0.1%.

Exposure time duration (01hour) to an insecticide	No. of knockdown mosquitoes after an hour of exposure			No. of knockdown mosquitoes after 24 hours of test time			Resistant mosquito		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1st 10 minutes	1	0	1						
2nd 10 minutes	2	0	0						
3rd 10 minutes	0	3	1						
4th 10 minutes	1	0	1						
5th 10 minutes	1	0	1						
6th 10 minutes	2	2	1						
Total	7	3	5	10	15	12	3	3	4

Table 2. showing bioassay test results for Bandiocarp 0.1% in triplet & control.

Chemical Insecticide and its control	Tube	Number of introduced mosquitoes	Number of knockdown mosquitoes after an hour of exposure	Number of knockdown and resistant mosquitoes after 24 hours of the test time		Mortality after 24 hours of the exposure to insecticide
				No. dead	No. live	Mortality%
Anopheles mosquitoes exposed to DC of Bandiocarp 0.1%	1st	22	7	10	5	85%
	2nd	21	3	15	3	85.71%
	3rd	21	5	12	4	85%
insecticide control test	4th	20	00	00	20	00%

Test result interpretation: since the mortality rate for Bendiocarp insecticide, with discriminating concentration of 0.1 %, in each of the bioassay test (85.00%, 85.71% and 85.00%) was < 90 %. Thus, according to WHO criteria of bioassay test, the *Anopheles stephensi* population was found to be resistant to Bendiocarp 0.1 %. Above table showed bioassay test (triplet) for Bendiocarb and the knockdown mosquitoes (in *stephensi*) in two steps, after an hour (noted readings within 10 minutes of interval) and after 24 hours of the exposure time. Besides, the resistant and live mosquitoes, after 24 hours of the exposure, were observed and noted in right of table.

Table 3. showing bioassay test results for Malathion 5%.

Exposure time duration (01hour) to an insecticide	No. of knockdown mosquitoes after an hour of exposure			No. of knockdown mosquitoes after 24 hours of test time			Resistant mosquito		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1st 10 minutes	2	0	0						
2nd 10 minutes	1	2	1						
3rd 10 minutes	1	0	1						
4th 10 minutes	2	1	0						
5th 10 minutes	0	1	2						
6th 10 minutes	2	0	3						
Total	8	4	7	10	13	10	3	4	3

Table 4. showing bioassay test results for Malathion 5% in triplet & control.

Chemical Insecticide and its control	Tube	Number of introduced mosquitoes	Number of knockdown mosquitoes after an hour of exposure	Number of knockdown and resistant mosquitoes after 24 hours of the test time		Mortality after 24 hours of the exposure to insecticide
				No. dead	No. live	Mortality%
Anopheles mosquitoes exposed to DC of Malathion	1st	21	8	10	3	85.71%
	2nd	21	4	13	4	80.95%
	3rd	20	7	10	3	85%
insecticide control test	4th	20	00	00	20	00%

Test result interpretation: since the mortality rate for Malathion insecticide, with discriminating concentration of 5%, in each of the bioassay test (85.71%, 80.95% and 85.00%) was < 90 %. Thus, according to WHO criteria of bioassay test, the *Anopheles stephensi* population was found to be resistant to Malathion 5%. Above table showed bioassay test (triplet) for Malathion 5% and the knockdown mosquitoes (in *stephensi*) in two steps, after an hour (noted readings within 10 minutes of interval) and after 24 hours of the exposure time. Besides, the resistant and live mosquitoes, after 24 hours of the exposure, were observed and noted in right of table.

Table 5. showing insecticide susceptibility status of *An. stephensi* against Bendiocarb 0.1% and Malathion 5%.

Insecticides	Mosquitoes exposed		Mosquitoes died		Observed mortality (%)		Status
	T*	C*	T*	C*	T*	C*	
Bendiocarb 0.1%	64	20	52	00	81	00	**R**
Malathion 5%	62	20	52	00	83	00	**R**

Note. T* = Test, **C*** = Control, **R** = Confirmed Resistance (**M** <90%).

In table 5, the mortality rate of Bendiocarb 0.1% and Malathion 5% were 81 %, 83 % respectively which are <90% (WHO recommended) and are considered as Confirmed Resistant.

4. Discussion

Insecticides are the most important tool for controlling mosquito vectors of diseases worldwide. Mosquitoes are important disease vectors that can cause a number of serious diseases in humans. Mosquito-borne diseases are a major public health concern, particularly in tropical countries. Since 2000, the incidence of malaria has decreased by 50%, with the use of insecticides such as indoor residual spraying (IRS), insecticide-treated nets (ITNs), and long-lasting insecticide-treated bed nets (LLITS) responsible for 80% of the decrease [83].

Despite insecticide use nearly one million deaths and over 700 million infections worldwide annually by mosquito [84]. It is because mosquitoes are constantly developing resistance against insecticide. In 1955, Global Malaria Eradication program proposed by WHO Assembly which was officially reverted into Malarial Control in 1976. The reason behind this policy change was appearance of resistance against DDT in many groups of mosquito vector [85]. Developing resistance to insecticide is a complex process depends directly on genetic, physiological, behavioral and ecological factors and indirectly on the frequency of applications of insecticides and its volume used [86]. Resistance mostly develops due to two main processes that are detoxifying enzyme and target site insensitivity against insecticide [87]. In Aedes aegypti all four pesticide classes—pyrethroids, carbamates, organochlorines, and organophosphates—have evolved resistance [88]. In *Anopheles arabiensis* and *Anopheles funestus* higher level resistance was reported, this effect mediated by higher activity of catalase and glutathione peroxidase enzyme [89]. More than 500 insect species have evolved resistant to a pesticide [90]. Other sources estimate the number to be around 1000 species since 1945 [91].

This study demonstrated the phenotypic resistant of *Anopheles stephensi* to through susceptibility test against Bandiocarb 0.1% and Malathion 5%. By comparing the survival rates of mosquito strains or wild populations exposed to different pesticides in a lab setting (WHO cones or Tube test), the effect of insecticide resistance on vector control effectiveness is often assessed. Malaria vectors has developed resistance against various chemical classes (i.e. Carbamates, Organophosphate and pyrethriods etc.). Excess use of insecticides for crop protection leads to resistance acquiring in malarial vector. Due to low mortality rate to these two insecticides we found *Anopheles stephensi*, resistant to Carbamate and Organophosphate class.

Similar study was performed in Afghanistan and India where *Anopheles stephensi* and *Anopheles culicifacies* showed resistance to multiple classes of insecticides including organochlorines, organophosphates pyrethriod [92]. Multiple resistant also reported in Turkey against organochlorine, organophosphate and carbamates [93]. In Iran, the lowest

susceptibility was seen against propoxur and bendiocarb at 5% and 8.3% mortality, and resistance reported to Malathion [94]. *Anopheles* species in Thailand-Myanmar border and in Sri Lanka were multiple resistant to organophosphate [95]. This multiple resistance was reported in 14 malaria vector in Asia; these included: *An. stephensi, An. superpictus, An. culinary, An. annularis, An. minimus, An. hyrcanus, An. barbirostris, An. vagus, An. maculatus, An. jamessi, An. nivipes, An. philippinensis, An. umbrosus,* and *An. Sinensis*. In 1974, *Anopheles* were reported resistant to wide range of organophosphates and carbamates in Dubai [96]. In Cukurova (region in southern Turkey). *Anopheles* specie reported resistant to wide range of organophosphates and carbamates in 1984 [97]. In central America the cotton growing belt *Anopheles* sp. showed moderate to high resistance levels to a number of organophosphates and carbamates [98]. Some degree of resistance was observed in *Anopheles* sp. against carbamates [99]. *An. gambiae* populations from Seme, Kandi and Malanville were fully susceptible to bendiocarb [100].

Our results contrasts with the study performed in Punjab, where nine localities provided susceptibility result of six species of *Anopheles* to DDT, dieldrin, malathion, fenitrothion, fenthion and propoxur in 1980 [101]. In Faisalabad where anopheles found to be susceptible to Carbamate class [102]. No insecticide resistance was detected in *An. arabiensis* from nine of the study sites or in *An. gambiae s.s.* from Namialo, and low level resistance was found in *Anopheles*' species to carbamate propoxur detected in two southern sites, Catembe and Ressano Garcia (Mozambique) [103]. *An. gambiae* was susceptible to carbamate in Benin [104].

Bandiocarb being an efficient insecticide which has reduced *Anopheles* population greatly in past [105] but now it is reported to be as less or even ineffective against Malarial vector in various countries. In this bioassay test we have checked status of *Anopheles stephensi* against bendiocarb 0.1%. mortality rate of this test was 85%. Therefore, *Anopheles stephensi* was found resistant against bendiocarb 0.1% in Peshawar. In 2013, two sites (Bukora and Kivumu) suggested the existence of resistance in Rwanda and significant decrease of susceptibility of *An. gambiae s.l.* to bendiocarb was found in one site Bukora [106]. In Tanguieta, strong resistance in *Anopheles sp.* was reported against bendiocarb [107]. *Anopheles gambiae sl*. from Sèdjè-Dénou rice field population was resistant to bendiocarb (0.1%) with a mortality rate of 72.2% [108]. In 2010, *Anopheles* were found resistant to carbamate in Lagos, South-Western Nigeria [109].

In some countries' insecticide resistance test's results contradicts to our result. In Punjab, wild *Anopheles* were found susceptible at all observed localities against 0.1% Bendiocarb while *An. pulcherimus* was found susceptible with 100% mortality at two localities against Deltamethrin, Lambdacyhalothrin, 5% Malathion and 0.1% Bendiocarb [110]. In Rwanda *Anopheles* Mosquitoes were fully susceptible to bendiocarb in 92% of sites in 2011 [111]. In 2017, in Ila-Orangun, Southwest Nigeria *Anopheles* mosquitoes were highly susceptible to Bendiocarb, [112].

Malathion is an organophosphorus (OP) insecticide that is extensively used in public health sectors since the 1950s [113]. In view of the complications of cross resistance among organophosphates, it is a well-off interpretation that for malathion, strains exist which are resistant almost exclusively to this compound [114]. We found *Anopheles stephensi* resistant to Malathion 5% with mean mortality rate 83.88 %. In Punjab, *Anopheles stephensi* and *Anopheles culicifacies* testified resistant to DDT and malathion [115]. Similar results were also reported from other countries of the WHO Eastern Mediterranean Region (EMR), including Saudi Arabia, Iraq, and Afghanistan [116]. In Iran malathion was reported ineffective against *Anopheles* with mortality rate 50% [117]. In 2010, Côte d'Ivoire (Country of West Africa) *Anopheles stephensi* population would have been classified as confirmed resistant regardless of the age class used in WHO tests [118]. In Sudan malathion resistance reported in *An. arabiensis* [119].

Our results contradict with previous result in Faisalabad, where *Anopheles* found to be susceptible to malathion 5% in two localities with mortality rate 100% [120]. Mortality rate of *Anopheles pulcherrimus* to malathion reported as 98% in Sistan and Baluchistan Province, Iran [121]. Results from Chad and Zimbabwe showed that *An. arabiensis* was susceptible to Malathion [122]. In India, no record is reported for the development of resistance in *Anopheles* mosquitoes against malathion, with mortality rate 100% only a case was reported where susceptibility reduced to 97.6% [123]. In Turkey, malathion was reported to be an effective insecticide in 2007 but its mortality rate is below 97% [124]. In Ibeju-Lekki and Kosofe exposure to malathion resulted in over 80 % mortality in 30 minutes. While for 1hr exposure 100 % mortality of *An. gambiae s.s.* was shown [125].

5. Conclusion

This research revealed that both Bendiocarb 0.1% and Malathion 5% were found less effective against *Anopheles stephensi*, malaria-vector. This study can have several positive outcomes such as Improvement of vector control strategies, surveillance programs and protection of public health from vector borne-diseases. The assessment of resistance status against different insecticides in *Anopheles stephensi* sheds light on advance evaluation of insecticide resistance to be monitored in affected areas as well as its impact must analyze in various vector control programs.

References

[1] Shaukat, M. A., Ali, S., Saddiq, B., Hassan, M. W., Ahmad, A., & Kamran, M. (2019). Effective mechanisms to control mosquito borne diseases: a review. Am J Clin Neurol Neurosurg, 4(1),-30.

[2] Hillary, V. E., & Ceasar, S. A. (2021). Genome engineering in insects for the control of vector borne diseases. Progress in Molecular Biology and Translational Science, 179, 197-223.

[3] Ma, H. (2017). Investigation of the natural history of Equine Encephalitis Viruses with radiofrequency telemetry for detection of subclinical disease patterns (Doctoral dissertation, University of Pittsburgh).
[4] Liao, W., Atkinson, C. T., LaPointe, D. A., & Samuel, M. D. (2017). Mitigating future avian malaria threats to Hawaiian forest birds from climate change. PLoS One, 12(1), e0168880.
[5] Foster, W. A., & Walker, E. D. (2019). mosquitoes (Culicidae). In Medical and veterinary entomology (pp. 261-325). Academic press.
[6] Reeves, L. E., Holderman, C. J., Blosser, E. M., Gillett-Kaufman, J. L., Kawahara, A. Y., Kaufman, P. E., & Burkett-Cadena, N. D. (2018). Identification of Uranotaenia sapphirina as a specialist of annelids broadens known mosquito host use patterns. Communications biology, 1(1), 92
[7] Fu WenBo, F. W., & Chen Bin, C. B. (2018). Taxonomy and fauna of Culicidae: history and current knowledge.
[8] Le Goff, G., Boussès, P., Julienne, S., Brengues, C., Rahola, N., Rocamora, G., & Robert, V. (2012). The mosquitoes (Diptera: Culidae) of Seychelles: taxonomy, ecology, vectorial importance, and identification keys. Parasites & Vectors, 5, 1-16.
[9] Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., .. & Kaiser, A. (2010). Subfamily Culicinae. Mosquitoes and their control, 187-314.control, 187-314.
[10] Tyagi, B. K., Sarkar, M., Kandasamy, C., & Bhattacharya, S. (2025). Mosquitoes as Vectors, Pests, and Allergenics. In *Mosquitoes of India* (pp. 173-190). CRC Press.
[11] Braack, L., Gouveia de Almeida, A. P., Cornel, A. J., Swanepoel, R., & De Jager, C. (2018). Mosquito-borne arboviruses of African origin: review of key viruses and vectors. Parasites & vectors, 11, 1-26
[12] Trompette, R. (2000). Gondwana evolution; its assembly at around 600 Ma. Comptes Rendus de l'Académie des Sciences-Series IIA-Earth and Planetary Science, 330(5), 305-315.
[13] Benton, M. J., Dunhill, A. M., Lloyd, G. T., & Marx, F. G. (2011). Assessing the quality of the fossil record: insights from vertebrates.
[14] Cumming, J. M., Wood, D. M., Brown, B. V., & Borkent, A. (2009). Adult morphology and terminology. Manual of central american Diptera, 1, 9-50.
[15] Piper, R. (2021). What Insects Do, and Why. Princeton University Press.
[16] Elgar, M. A., Zhang, D., Wang, Q., Wittwer, B., Pham, H. T., Johnson, T. L., .. & Coquilleau, M. (2018). Focus: ecology and evolution: insect antennal morphology: the evolution of diverse solutions to odorant perception. The Yale journal of biology and medicine, 91(4), 457.
[17] Labandeira, C. C. (2019). The fossil record of insect mouthparts: innovation, functional convergence, and associations with other organisms. Insect mouthparts: form, function, development and performance, 567-671.
[18] Burkett-Cadena, N. D. (2013). Mosquitoes of the southeastern United States. University of Alabama Press.
[19] Hogue, C. L. (1993). Latin American insects and entomology. Univ of California Press.
[20] Reynolds, D. R., & Riley, J. R. (2002). Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. Computers and electronics in agriculture, 35(2-3), 271-307.
[21] Pont, A. C., & Meier, R. (2002). The sepsidae (Diptera) of Europe [electronic resource] (Vol. 37). Brill.s
[22] Gray, Elmer, and Ray Noblet. University of Georgia, Cooperative Extension Service and Department of Entomology. "Controlling Mosquitoes," 31 May 2016
[23] Jones, J. C. (1978). The feeding behavior of mosquitoes. Scientific American, 238(6), 138-150.
[24] Nasci, R. S. (1986). Relationship between adult mosquito (Diptera: Culicidae) body size and parity in field populations. Environmental Entomology, 15(4), 874-876.
[25] Davies, A. J., & Johnston, M. R. L. (2000). The biology of some intraerythrocytic parasites of fishes, amphibia and reptiles.
[26] Melgarejo-Colmenares, K., Cardo, M. V., & Vezzani, D. (2022). Blood feeding habits of mosquitoes: hardly a bite in South America. Parasitology Research, 121(7), 1829-1852.
[27] Rey, J. R., Walton, W. E., Wolfe, R. J., Connelly, R., O'Connell, S. M., Berg, J., .. & Laderman, A. D. (2012). North American wetlands and mosquito control. International journal of environmental research and public health, 9(12), 4537-4605.
[28] Silver, J. B. (2008). Mosquito ecology: field sampling methods. Dordrecht: Springer Netherlands.
[29] Dunnett, N., & Clayden, A. (2007). Rain gardens. Managing Water Sustainably in the Garden and Designed Landscape; Timber Press: Portland, OR, USA.
[30] Kline D. L., Bernier, U. R., & Hogsette, J. A. (2012). Efficacy of three attractant blends tested in combination with carbon dioxide against natural populations of mosquitoes and biting flies at the Lower Suwannee Wildlife Refuge. Journal of the American Mosquito Control Association, 28(2), 123-127.
[31] Omolo, M. O., Ndiege, I. O., & Hassanali, A. (2021). Semiochemical signatures associated with differential attraction of Anopheles gambiae to human feet. PLoS One, 16(12), e0260149.
[32] Braks, M. A. H., Anderson, R. A., & Knols, B. G. J. (1999). Infochemicals in mosquito host selection: human skin microflora and Plasmodium parasites. Parasitology today, 15(10), 409-413.
[33] Dutta, S. K., Nair, M. V., Mohapatra, P. P., & Mahapatra, A. K. (2009). Amphibians and Reptiles.
[34] Wucherer, T. (2021). Mosquitoes of Wisconsin: The Biology, Life History, Identification and Control.
[35] Krenn, H. W. (2019). Fluid-feeding mouthparts. Insect mouthparts: form, function, development and performance, 47-99.
[36] Milleron, R. S. (2002). Adaptive significance of polymorphism in maxadilan, a salivary protein from the sand fly vector of American visceral leishmaniasis (Doctoral dissertation, The University of Texas Medical Branch Graduate School of Biomedical Sciences).
[37] Limo, M. K. (1985). Tick (R. Appendiculatus) Salivary glands bioactive molecules with a biochemical study of the anticoagulant in relation to vector control (Doctoral dissertation).
[38] Ware, F. L., & Luck, M. R. (2017). Evolution of salivary secretions in haematophagous animals. Bioscience Horizons: The International Journal of Student Research, 10, hzw015.
[39] Edman, J. D. (1964). Sources, rate of digestion and utilization of vertebrate blood in mosquitoes (Diptera: Culicidae). Kansas State University.
[40] Nijhout, H. F., & Carrow, G. M. (1978). Diuresis after a bloodmeal in female Anopheles freeborni. Journal of Insect Physiology, 24(4), 293-298.

[41] Naik, B. R., Dinesh, D. S., Siddaiah, M., Daravath, S., & Tyagi, B. K. (2025). Biology of Mosquitoes. In Mosquitoes of India (pp. 219-236). CRC Press.
[42] Li, J. (1994). Egg chorion tanning in Aedes aegypti mosquito. Comparative Biochemistry and Physiology Part A: Physiology, 109(4), 835-843.
[43] Edgerly, J. S., McFarland, M., Morgan, P., & Livdahl, T. (1998). A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito. Journal of Animal Ecology, 67(5), 805-818.
[44] Hallock, A., Buckner, E. A., Telg, R. W., & Poulin, A. R. (2020). Container Mosquito Habitat Community Cleanup: A How-To Guide for Event Organization: ENY-2047/IN1286 12/2020. EDIS, 2020(6), 6-6.
[45] Judson, C. L. (1960). The physiology of hatching of aedine mosquito eggs: hatching stimulus. Annals of the Entomological Society of America, 53(5), 688-691.
[46] Huber, J. T. (1998). The importance of voucher specimens, with practical guidelines for preserving specimens of the major invertebrate phyla for identification. Journal of Natural History, 32(3), 367-385.
[47] Parubrub, A., Reyes, R., Smallridge, C. J., Woods, B., & Haymer, D. (2015). Mitochondrial single nucleotide polymorphisms in Ceratitis capitata (Diptera: Tephritidae) can distinguish sterile, released flies from wild flies in various regions of the world. Journal of economic entomology, 108(1), 301-306.
[48] Das, S., Mukhiya, I. A., Hazra, T., Roy, S., & Das, A. (2024). Mosquito Morphology: Anatomy to Adaptation in the Shadow of Evolution. In Mosquitoes: Biology, Pathogenicity and Management (pp. 37-104). Singapore: Springer Nature Singapore
[49] Keilin, D. (1944). Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. Parasitology, 36(1-2), 1-66.
[50] Baker, J. R., Apperson, C. S., & Arends, J. J. (1986). Insect and other pests of man and animals--some important, common, and potential pests in the Southeastern United States. AG (North Carolina Agricultural Extension Service).
[51] Awasthi, V. B. (2017). Agricultural Insect Pests and Their Control. Scientific Publishers.
[52] Stone, C. M., & Foster, W. A. (2013). Plant-sugar feeding and vectorial capacity. In Ecology of parasite-vector interactions (pp. 35-79). Wageningen Academic.
[53] Gautreau, C. E. (2021). Assessing coastal risk and improving resilience: Cape Jourimain National Wildlife Area (Doctoral dissertation).
[54] Saliternik, Z. (1955). The specific biological characteristics of Anopheles (Myzomyia) sergentii (Theo) and their correlation with malaria control in Israel. Bulletin of Entomological Research, 46(2), 445-462.
[55] Service, M. W. (1997). Mosquito (Diptera: Culicidae) dispersal—the long and short of it. Journal of medical entomology, 34(6), 579-588.
[56] Sarwar, M., & Salman, M. (2015). Insecticides resistance in insect pests or vectors and development of novel strategies to combat its evolution. International Journal of Bioinformatics and Biomedical Engineering, 1(3), 344-351.
[57] Thapa, S., Lv, M., & Xu, H. (2017). Acetylcholinesterase: a primary target for drugs and insecticides. Mini reviews in medicinal chemistry, 17(17), 1665-1676.
[58] Soderlund, D. M. (2020). Neurotoxicology of pyrethroid insecticides. In Advances in Neurotoxicology (Vol. 4, pp. 113-165). Academic Press.
[59] Hadiatullah, H., Zhang, Y., Samurkas, A., Xie, Y., Sundarraj, R., Zuilhof, H., .. & Yuchi, Z. (2022). Recent progress in the structural study of ion channels as insecticide targets. Insect Science, 29(6), 1522-1551.
[60] Suh, P. F., Elanga-Ndille, E., Tchouakui, M., Sandeu, M. M., Tagne, D., Wondji, C., & Ndo, C. (2023). Impact of insecticide resistance on malaria vector competence: a literature review. Malaria Journal, 22(1), 19.
[61] Russell, R. J., Scott, C., Jackson, C. J., Pandey, R., Pandey, G., Taylor, M. C., .. & Oakeshott, J. G. (2011). The evolution of new enzyme function: lessons from xenobiotic metabolizing bacteria versus insecticide-resistant insects. Evolutionary Applications, 4(2), 225-248
[62] Ranganathan, M., Narayanan, M., & Kumarasamy, S. (2022). Importance of metabolic enzymes and their role in insecticide resistance. In New and future development in biopesticide research: Biotechnological exploration (pp. 243-260). Singapore: Springer Nature Singapore
[63] Balabanidou, V., Grigoraki, L., & Vontas, J. (2018). Insect cuticle: a critical determinant of insecticide resistance. Current opinion in insect science, 27, 68-74
[64] Sial, M. U., Zhao, Z., Zhang, L., Zhang, Y., Mao, L., & Jiang, H. (2018). Evaluation of insecticides induced hormesis on the demographic parameters of Myzus persicae and expression changes of metabolic resistance detoxification genes. Scientific Reports, 8(1), 16601.
[65] Montella, I. R., Schama, R., & Valle, D. (2012). The classification of esterases: an important gene family involved in insecticide resistance-A review. Memorias do Instituto Oswaldo Cruz, 107, 437-449.
[66] Dasari, S., Ganjayi, M. S., Oruganti, L., Balaji, H., & Meriga, B. (2017). Glutathione S-transferases detoxify endogenous and exogenous toxic agents-minireview. J Dairy Vet Anim Res, 5(5), 00154.
[67] Esteves, F., Rueff, J., & Kranendonk, M. (2021). The central role of cytochrome P450 in xenobiotic metabolism—a brief review on a fascinating enzyme family. Journal of xenobiotics, 11(3), 94-114.
[68] De Souza, R. F., Amaro, T. R., Palacio-Cortés, A. M., da Silva, M. A. N., Dionisio, J. F., Pezenti, L. F., .. & da Rosa, R. (2024). Comparative transcriptional analysis between susceptible and resistant populations of Aedes (Stegomyia) aegypti (Linnaeus, 1762) after malathion exposure. Molecular Genetics and Genomics, 299(1), 92.
[69] Liu, K., Wang, L., Wen, S., Qiu, X., Cao, P., Liang, P., & Wu, S. (2025). Role of mutation G255A in modulating pyrethroid sensitivity in insect sodium channels. International Journal of Biological Macromolecules, 139455.
[70] Casida, J. E., & Durkin, K. A. (2013). Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annual review of entomology, 58(1), 99-117.
[71] Brengues, C., Hawkes, N. J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., .. & Hemingway, J. (2003). Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and veterinary entomology, 17(1), 87-94.
[72] Belinato, T. A., & Martins, A. J. (2016). Insecticide resistance and fitness cost. Insecticides resistance, 243-261.
[73] Dahmana, H., & Mediannikov, O. (2020). Mosquito-borne diseases emergence/resurgence and how to effectively control it biologically. Pathogens, 9(4), 310.

[74] Fotakis, E. A., Chaskopoulou, A., Grigoraki, L., Tsiamantas, A., Kounadi, S., Georgiou, L., & Vontas, J. (2017). Analysis of population structure and insecticide resistance in mosquitoes of the genus Culex, Anopheles and Aedes from different environments of Greece with a history of mosquito borne disease transmission. Acta Tropica, 174, 29-37.
[75] Bellini, R., Zeller, H., & Van Bortel, W. (2014). A review of the vector management methods to prevent and control outbreaks of West Nile virus infection and the challenge for Europe. Parasites & vectors, 7, 1-11.
[76] Cuamba, N., Morgan, J. C., Irving, H., Steven, A., & Wondji, C. S. (2010). High level of pyrethroid resistance in an Anopheles funestus population of the Chokwe District in Mozambique. PloS one, 5(6), e11010.
[77] Nolden, M. (2023). *Characterization of cytochrome P450-mediated pyrethroid resistance in Anopheles funestus with special reference to transfluthrin* (Doctoral dissertation, Liverpool School of Tropical Medicine).
[78] Oxborough, R. M. (2015). Laboratory and experimental hut evaluation of mosquito net and indoor residual spray (IRS) insecticides for improved malaria control (Doctoral dissertation, London School of Hygiene & Tropical Medicine).
[79] Trape, J. F., Tall, A., Sokhna, C., Ly, A. B., Diagne, N., Ndiath, O., .. & Rogier, C. (2014). The rise and fall of malaria in a west African rural community, Dielmo, Senegal, from 1990 to 2012: a 22-year longitudinal study. The Lancet infectious diseases, 14(6), 476-488
[80] Corbel, V., Akogbeto, M., Damien, G. B., Djenontin, A., Chandre, F., Rogier, C., .. & Henry, M. C. (2012). Combination of malaria vector control interventions in pyrethroid resistance area in Benin: a cluster randomised controlled trial. The Lancet infectious diseases, 12(8), 617-626.
[81] Aslam, R. W., Naz, I., Quddoos, A., & Quddusi, M. R. (2024). Assessing climatic impacts on land use and land cover dynamics in Peshawar, Khyber Pakhtunkhwa, Pakistan: a remote sensing and GIS approach. GeoJournal, 89(5), 202.
[82] Shah, S. A. A., Nisa, S., Khan, A., & Rahman, Z. U. (2012). Trends and variability in climate parameters of Peshawar district. Science Technology and Development, 31.
[83] Okumu, F. O. (2012). Combining insecticide treated bed nets and indoor residual spraying for malaria vector control in Africa (Doctoral dissertation, London School of Hygiene & Tropical Medicine).
[84] Fatima, T., Habib, A., Khan, A., Riaz, R., ul Haq, M. Z., & Raufi, N. (2023). Mosquito-borne diseases in Pakistan: challenges, strategies, and future prospects. IJS Global Health, 6(6), e0385.
[85] Nájera, J. A., González-Silva, M., & Alonso, P. L. (2011). Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). PLoS medicine, 8(1), e1000412.
[86] Andreazza, F., Oliveira, E. E., & Martins, G. F. (2021). Implications of sublethal insecticide exposure and the development of resistance on mosquito physiology, behavior, and pathogen transmission. Insects, 12(10), 917.
[87] Lee, S. E., Kim, J. E., & Lee, H. S. (2001). Insecticide resistance in increasing interest. Journal of Applied Biological Chemistry, 44(3), 105-112.
[88] Vontas, J., Kioulos, E., Pavlidi, N., Morou, E., Della Torre, A., & Ranson, H. (2012). Insecticide resistance in the major dengue vectors Aedes albopictus and Aedes aegypti. *Pesticide Biochemistry and Physiology*, *104*(2), 126-131.
[89] Oliver, S. V., & Brooke, B. D. (2016). The role of oxidative stress in the longevity and insecticide resistance phenotype of the major malaria vectors Anopheles arabiensis and Anopheles funestus. PloS one, 11(3), e0151049.
[90] Matthews, G. A. (2018). A history of pesticides. Cabi.
[91] Bass, C., Denholm, I., Williamson, M. S., & Nauen, R. (2015). The global status of insect resistance to neonicotinoid insecticides. Pesticide biochemistry and physiology, 121, 78-87.
[92] Enayati, A., Hanafi-Bojd, A. A., Sedaghat, M. M., Zaim, M., & Hemingway, J. (2020). Evolution of insecticide resistance and its mechanisms in Anopheles stephensi in the WHO Eastern Mediterranean Region. Malaria Journal, 19, 1-12.
[93] Yavaşoglu, S. İ., Yaylagül, E. Ö., Akıner, M. M., Ülger, C., Çağlar, S. S., & Şimşek, F. M. (2019). Current insecticide resistance status in Anopheles sacharovi and Anopheles superpictus populations in former malaria endemic areas of Turkey. Acta tropica, 193, 148-157.
[94] Badzohre, A., Oshaghi, M. A., Enayati, A. A., Moosa-Kazemi, S. H., Nikookar, S. H., Talebzadeh, F., .. & Vatandoost, H. (2023). Ace-1 Target Site Status and Metabolic Detoxification Associated with Bendiocarb Resistance in the Field Populations of Main Malaria Vector, Anopheles stephensi in Iran. Journal of Arthropod-Borne Diseases, 17(3), 272.
[95] Susanna, D., & Pratiwi, D. (2022). Current status of insecticide resistance in malaria vectors in the Asian countries: a systematic review. F1000Research, 10, 200.
[96] Ladonni, H. (1988). Genetics and biochemistry of insecticide resistance in Anopheles stephensi (Doctoral dissertation, University of Liverpool).
[97] Hemingway, J., Small, G. J., Monro, A., Sawyer, B. V., & Asap, H. K. (1992). Insecticide resistance gene frequencies in *Anopheles sacharovi* populations of the Cukurova plain, Adana Province, Turkey. Medical and veterinary entomology, 6(4), 342-348
[98] Hien, A. S., Soma, D. D., Hema, O., Bayili, B., Namountougou, M., Gnankiné, O., .. & Dabiré, K. R. (2017). Evidence that agricultural use of pesticides selects pyrethroid resistance within Anopheles gambiae sl populations from cotton growing areas in Burkina Faso, West Africa. PLoS One, 12(3), e0173098.
[99] Priester, T. M., Georghiou, G. P., Hawley, M. K., & Pasternak, M. E. (1981). Toxicity of pyrethroids to organophosphate-carbamate-and DDT-resistant mosquitoes.
[100] Aïzoun, N., Gnanguenon, V., Azondekon, R., Anagonou, R., Aïkpon, R., & Akogbéto, M. (2014). Status of organophosphate and carbamate resistance in Anopheles gambiae sensu lato from the Sudano Guinean area in the central part of Benin, West Africa. J. Cell. Anim. Biol, 8(4), 61-68.
[101] Rathor, H. R., Toqir, G., & Reisen, W. K. (1980). Status of insecticide resistance in anopheline mosquitoes of Punjab Province, Pakistan. The Southeast Asian Journal of Tropical Medicine and Public Health, 11(3), 332-340.
[102] Mohsin, M., Shafi, J., Naz, S. I., Aslam, S., & Hassan, S. (2021). Variability in susceptibility status of malaria vectors and other Anopheles species against different insecticides in district Faisalabad. Central Punjab, Pakistan.
[103] Casimiro, S. L. R. (2003). Susceptibility and resistance to insecticides among malaria vector mosquitoes in Mozambique (Doctoral dissertation).
[104] Mutunga, J. M., Anderson, T. D., Craft, D. T., Gross, A. D., Swale, D. R., Tong, F., .. & Bloomquist, J. R. (2015). Carbamate and pyrethroid resistance in the akron strain of Anopheles gambiae. Pesticide biochemistry and physiology, 121, 116-121.
[105] NYACHWAYA, L. M. (2019). I56/21120/2012 (Doctoral dissertation, Kenyatta University).

[106] Hakizimana, E., Karema, C., Munyakanage, D., Iranzi, G., Githure, J., Tongren, J. E., .. & Koenraadt, C. J. (2016). Susceptibility of Anopheles gambiae to insecticides used for malaria vector control in Rwanda. Malaria journal, 15, 1-11.
[107] Aïzoun, N., Aïkpon, R., Gnanguenon, V., Oussou, O., Agossa, F., Padonou, G. G., & Akogbéto, M. (2013). Status of organophosphate and carbamate resistance in Anopheles gambiae sensu lato from the south and north Benin, West Africa. Parasites & vectors, 6, 1-6.
[108] Dossou, C., Tchigossou, G., Koto, M., Atoyebi, S. M., Tossou, E., Adanzounon, D., .. & Djouaka, R. (2024). Organophosphate and carbamate susceptibility profiling of Anopheles gambiae sl. across different ecosystems in southern Benin. Wellcome Open Research, 9, 424.
[109] Lagos, S. W. N. (2012). Evidence of carbamate resistance in urban populations of Anopheles gambiae ss mosquitoes resistant to DDT and deltamethrin insecticides in.
[110] Wahedi, J. A., Ande, A. T., Oduola, A. O., & Obembe, A. (2021). Bendiocarb resistance and kdr associated deltamethrin and DDT resistance in Anopheles gambiae sl populations from North Eastern Adamawa State, Nigeria. Ceylon Journal of Science, 50(1).
[111] Iga, J., Ochaya, S., Echodu, R., Opiyo, E. A., Musiime, A. K., Nakamaanya, A., & Malinga, G. M. (2023). Research Article Sibling Species Composition and Susceptibility Status of Anopheles gambiae sl to Insecticides Used for Indoor Residual Spraying in Eastern Uganda.
[112] Oyewole, I. O., Mustapha, M., Kolawole, A. R., Adedeji, O. C., Adeogun, D., & Awolola, S. (2018). Susceptibility pattern of anopheles mosquito to different classes of insecticides in selected communities in Ila-Orangun, southwest Nigeria.
[113] Tchounwou, P. B., Patlolla, A. K., Yedjou, C. G., & Moore, P. D. (2015). Environmental exposure and health effects associated with malathion toxicity. Toxicity and hazard of agrochemicals, 51, 2145-2149.
[114] Winteringham, F. P. W. (1962). Action and inaction of insecticides. Journal of the Royal Society of Arts, 110(5074), 719-740.
[115] Rathor, H. R., Nadeem, G., & Khan, I. A. (2013). Pesticide susceptibility status of Anopheles mosquitoes in four flood-affected districts of South Punjab, Pakistan. Vector-Borne and Zoonotic Diseases, 13(1), 60-66.
[116] Vatandoost, H., Hanafi-Bojd, A. A., Nikpoor, F., Raeisi, A., Abai, M. R., & Zaim, M. (2022). Situation of insecticide resistance in malaria vectors in the World Health Organization of Eastern Mediterranean region 1990–2020. Toxicology Research, 11(1), 1-21.
[117] Abbasi, E., Vahedi, M., Bagheri, M., Gholizadeh, S., Alipour, H., & Moemenbellah-Fard, M. D. (2022). Monitoring of synthetic insecticides resistance and mechanisms among malaria vector mosquitoes in Iran: A systematic review. Heliyon, 8(1).
[118] Chouaibou, M. S., Chabi, J., Bingham, G. V., Knox, T. B., N'Dri, L., Kesse, N. B., .. & Jamet, H. V. P. (2012). Increase in susceptibility to insecticides with aging of wild Anopheles gambiae mosquitoes from Côte d'Ivoire. BMC infectious diseases, 12, 1-7.
[119] Hemingway, J. (1983). Biochemical studies on malathion resistance in Anopheles arabiensis from Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene, 77(4), 477-480.
[120] Sadia Abbas, S. A., Shabab Nasir, S. N., Muhammad Fakhar-e-Alam, M. F. E. A., & Malik Saadullah, M. S. (2019). Toxicity of different groups of insecticides and determination of resistance in Aedes aegypti from different habitats.
[121] Zahirnia, A. H., Vatandoost, H., Nateghpour, M., & Djavadian, E. (2002). Insecticide resistance/susceptibility monitoring in Anopheles pulcherrimus (Diptera: Culicidae) in Ghasreghand district, Sistan and Baluchistan province, Iran. Iranian Journal of Public Health, 31(1-2), 11-14.
[122] Howell, P. I. (2018). Genetic components of insecticide resistance in Anopheles arabiensis. The University of Liverpool (United Kingdom). Howell, P. I. (2018). Genetic components of insecticide resistance in Anopheles arabiensis. The University of Liverpool (United Kingdom).
[123] Dhiman, S., Yadav, K., Rabha, B., Goswami, D., Hazarika, S., & Tyagi, V. (2016). Evaluation of insecticides susceptibility and malaria vector potential of Anopheles annularis sl and Anopheles vagus in Assam, India. PLoS one, 11(3), e0151786.
[124] Akiner, M. M., Caglar, S. S., & Simsek, F. M. (2013). Yearly changes of insecticide susceptibility and possible insecticide resistance mechanisms of Anopheles maculipennis Meigen (Diptera: Culicidae) in Turkey. Acta tropica, 126(3), 280-285.
[125] Fagbohun, I. K., Idowu, E. T., Otubanjo, O. A., & Awolola, T. S. (2020). Susceptibility status of mosquitoes (Diptera: Culicidae) to malathion in Lagos, Nigeria. Animal Research International, 17(1), 3541-3549.