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Susceptibility of *Anopheles maculipennis* to different classes of insecticides in West Azarbaijan Province, Northwestern Iran

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PEER REVIEW

Peer reviewer

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Comments

This valuable research work carries lot of significance in controlling *An. maculipennis*, which is carrier of many deadly diseases causing organisms. In the 916 specimens of *An. maculipennis* examined against the insecticides, *An. maculipennis* appeared tolerant to permethrin, deltamethrin and dielderin, but displayed resistance against propoxur, bendiocarb and malathion.

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ABSTRACT

Objective: To determine the susceptibility status of *Anopheles maculipennis (An. maculipennis)* against the major insecticides used in the health sectors in West Azarbaijan Province, Northwestern Iran.

Methods: Unfed 3-5 days old adult females of *An. maculipennis* were collected across the West Azarbaijan Province and were subjected to evaluation of their susceptibility following World Health Organization recommended protocol against six insecticides (permethrin, deltamethrin, propoxur, bendiocarb, malathion and dieldrin) belonging to four different classes.

Results: In this study, 916 specimens of *An. maculipennis* were examined against the insecticides which indicated that *An. maculipennis* was tolerant to permethrin, deltamethrin and dielderin, but displayed resistance against propoxur, bendiocarb and malathion.

Conclusions: The pattern of resistance in *An. maculipennis* could be attributed to the agricultural landscapes, agricultural pesticides used and the exposure of the mosquitoes to insecticides. Logical cooperation is needed between the agriculture and health sectors to ensure the judicious use of pesticides in each sector and the management of probable resistance.

KEYWORDS

Anopheles maculipennis, Insecticide, Resistance, Iran

1. Introduction

A wide range of vector-borne diseases are caused by mosquitoes. Different species of mosquitoes have the potential of transmitting a diverse number of diseases. *Anopheles maculipennis (An. maculipennis)* is one such species which has been reported as the vector of some important mosquito-borne diseases such as malaria[1], filarial nematodes[2], West Nile virus[3], Sindbis virus[4], among others. In addition to the role of this species in the transmission of different diseases, the wide geographical distribution of *An. maculipennis* from Northern Europe[5], through Northern Africa and the Middle East[6-8], makes it imperative to study this species.

The complexity of the classification of this species has led it to be recognized as a species complex comprising of twelve species of which six [*Anopheles atroparvus, Anopheles labranchiae,*

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An. maculipennis, Anopheles messeae, Anopheles persiensis and (*Anopheles sacharovi*) *An. sacharovi*] have been reported from Iran[9]. A new species (*Anopheles persiensis*) has since been described for the first time from Iran[10]. Proper morphologic-based identification of different sibling species of *An. maculipennis* complex is nearly impossible judgments about different biological and behavioral aspects of sibling species which is reasonably difficult.

Recent years have seen several control measures employed against mosquitoes of which the most widely used method is the use of chemical insecticides[11]. However, with emerging resistance in mosquitoes, environmental issues and their residues have floundered the advantages of this method[12,13]. Monitoring of insecticide susceptibility status of medically important species against the routinely used compounds is one of the most recommended processes for the rational use of insecticides and decreasing the risk of emerging resistant species[14].

The susceptibility status of *An. maculipennis* against different insecticide chemicals have been studied. In one of this studies, seasonal variation in susceptibility of this species to dichlorodiphenyltrichloroethane (DDT) was observed[15]. Another study carried out in Northern Iran attributed the resistance to DDT, dieldrin, susceptibility of malathion, lambda-cyhalothrin and deltamethrin[16]. Recently, the resistance of this species against DDT and the resistance surveillance category for malathion, permethrin and deltamethrin in Turkey was reported[17].

Considering the geographical location of West Azarbaijan Province, North-Western Iran, which has common border lines with several countries like Turkey, Azarbaijan and Iraq and also the shared social and cultural relations among the countries, the proper study of vectors and their control in this region is necessary. These political and social factors are emergencies, such as possible political and humanitarian crises of which Soviet crisis could lead to the outbreak of malaria or other mosquito-borne in the region[18]. On the other hand, according to the agricultural prosperity of the region and the widespread use of pesticides, the knowledge on the status of the mosquitoes against insecticides would be of great importance to the selection and use of pesticides in case of potential emergency.

The aim of this study was to determine the susceptibility of *An. maculipennis* against major insecticides used in West Azarbaijan Province, Northwestern Iran.

2. Materials and methods

2.1. Study areas

All samples were collected from north, central and south parts of West Azarbaijan Province (Figure 1). West Azerbaijan Province is located in the northwest of Iran, bordering the countries: Turkey, Iraq, Armenia, Azerbaijan, and the provinces of East Azerbaijan, Zanjan and Kurdistan.

2.2. Sample collection and species identification

Larvae collection was carried out from different habitats using the standard (350 mL dipper) dipping method in 7 localities of three counties across West Azerbaijan Province (Figure 1)[19].

Figure 1. The map of Iran and location of West Azarbaijan Province.

Anophelinae larvae collections were conducted during June–October of 2013 and were allowed to mature into adults. The unfed 3-5 days old adult females were used for tests. Also for collecting the blood fed, semi-gravid and gravid stages of adult *An. maculipennis*, samples were collected from barns[19]. Collected samples were transferred to the laboratory and the collected blood fed, semi-gravid and gravid samples were kept individually in paper cups to lay eggs. Their eggs were kept in the optimum conditions to become larvae, pupae and adults, respectively. The emerged 3-5 days old female adults were selected for susceptibility tests.

2.3. Adult susceptibility test

The susceptibility tests were carried out using the recommended method by World Health Organization[20]. The sugar-fed 3-5 days old adult female *An. maculipennis* were selected from reared larvae and/or from laid eggs. The female *An. maculipennis* were transferred to hold tubes and after completing an hour of rest and removal of dead and damaged samples, the specimens were exposed for 60 min to toxicant tube containing insecticide impregnated papers which were supplied by World Health Organization with specified discriminating concentration. After 1 h exposure, the specimens were transferred to clean holding tubes and were kept in insectariums with optimum conditions and sugar solution was supplied. With the exception of toxicant exposure, there was a control group in all experiments. The number of dead and alive samples was counted and recorded for all holding tubes.

2.4. Statistical analysis

Bioassay data were considered for each insecticide. The mortality rate was calculated as the percentage of individuals that died within 24 h after one hour of exposure. Bioassay outcomes were assessed according to World Health Organization[20]. Those with an overall mortality $\geqslant$ 98% were considered susceptible, those with mortality < 98% but > 90% were considered potentially resistant, and those with mortality < 90% were strongly suspected to be resistant.

3. Results

In the 916 unfed 3-5 days old female *An. maculipennis,* 300 were

Table 1

The results of susceptibility test of adult *An. maculipennis* against different insecticides in West Azarbaijan Province, Iran.

Group	Insecticide (discriminating concentration, 1 h exposure period)	Replicates	No. of mosquito tested	No. of mosquito dead after 24 h exposure to insecticide	Mortality rate (%±SD)
Pyrethroid	Permethrin (0.75%)	5	115	109	95.0 ± 1.0
	Deltamethrin (0.05%)	4	87	82	96.4 ± 1.5
Carbamate	Propoxur (0.10%)	4	98	5	5.0 ± 0.4
	Bendiocarb (0.10%)	4	96	8	8.3 ± 0.4
Organophosphate	Malathion (5.00%)	5	120	60	50.0 ± 0.6
Chlorinated hydrocarbon	Dieldrin (4.00%)	4	100	91	91.0 ± 2.0
Control	-	15	300	6	2.0 ± 1.0

used in control groups and 616 were exposed to different studied insecticides (Table 1). Each of the insecticides was evaluated at least thrice.

In this study, the susceptibility status of *An. maculipennis* were examined against six insecticides belonging to four main classes of insecticides (permethrin, deltamethrin form the class of pyrethroids, propoxur, bendiocarb form the class of carbamate, malathion form the class of organophosphate and dieldrin form the class of organochlorine).

The results of experiments are shown in Table 1 which revealed the higher susceptibility to deltamethrin, permethrin, and dieldrin at 96.4%, 95% and 91% mortality, respectively. The lowest susceptibility was seen against propoxur and bendiocarb at 5% and 8.3% mortality (Figure 2).

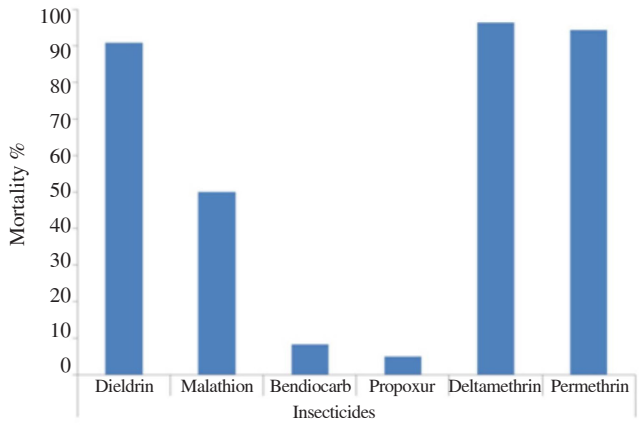

Figure 2. Mortality of *An. maculipennis* adult females to different insecticides in West Azarbaijan Province, Northwestern Iran.

Considering the World Health Organization criteria[20], the studied species, *An. maculipennis*, was tolerant to permethrin, deltamethrin and dielderin, while it is resistant to propoxur, bendiocarb and malathion.

4. Discussion

Considering the presence of *An. maculipennis* in northwestern part of Iran[7], the present study is the first evaluation of susceptibility status of *An. maculipennis* against six insecticides belonging to four main classes of pesticides in West Azarbaijan Province, northwestern of Iran.

An. maculipennis was subjected to the evaluation for probable resistance against five insecticides of three major classes of pesticides in Astara Region, Guilan Province, north of Iran during 1998-1999[16]. This species was shown to be resistant to DDT and dieldrin which is roughly consistent with the result of present study in the case of dieldrin. The results of our study show that *An. maculipennis* is at the borderline of resistance against dieldrin with a mortality rate of 1% and its resistance needs confirmation by other methods. Both studies show comparable results that indicated the species' susceptibility to deltamethrin.

In the case of malathion, the results of the present and previous study are different[16]. Thus, the present study suggests resistance to malathion among *An. maculipennis* of northwestern of Iran while the previous study on the population of the north of Iran indicated susceptibility to malathion.

Differences in the pattern of resistance of *An. maculipennis* against the studied insecticides in these two different areas (Northwestern Iran in this study, and north of Iran) could be due to differences in geographical conditions, the patterns of agriculture, agricultural pesticide use and exposure of mosquitoes to different insecticides[16].

Another study conducted in this region reported the resistance profile of another species (*An. sacharovi*) which is a member of *An. maculipennis* species complex against different insecticides[18]. Comparing the results of present study with the aforementioned one shows the similar tolerance levels to dieldrin in both the species. Also similar results were observed for resistance to permethrin and deltamethrin. In contrast, the susceptibility status of these species incuding *An. maculipennis* in present study and *An. sacharovi* to propoxur, bendiocarb, and malathion were different[18].

The result of another study conducted in another part of Iran (Ardebil Province, north of Iran) suggested that *An. sacharovi* was resistant to DDT and dieldrin and susceptible to malathion and propoxur. The results of the present study indicated the need of further investigations for confirmation the resistance of *An. maculipennis* against permethrin, deltamethrin, and dieldrin with mortality rates between 90%-98%[20]. Similar susceptibility status against the insecticides belonging to the main classes with similar mode of action (pyrethroid-hydrochloride and carbamate-organophosphate) could be due to the exposure to pesticides and development of cross resistance against a wide range of insecticides that possibly act by similar mechanism of actions.

It is necessary to note that the pattern of use of pesticides in agriculture influences the development of resistance in pests such as mosquitoes[21]. Due to the geographical location of the region, its significant water resources and agricultural development, a logical cooperation is needed between agriculture and health sections in order to improve the pattern of use of pesticides in each section and management of probable resistance.

Finally, considering the medical importance of this species, its wide range of distribution and the diseases which could be transmitted by this species or the probability of establishment of these diseases in northwestern of Iran, further studies are needed to understand the mechanism of resistance of this species against insecticides using molecular and biochemical methods.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

An. maculipennis is one of the important mosquito species of Iran, Northern Europe, Northern Africa and the Middle East. The species is carrier of many disease causing organisms and thus is of medical importance. This species is becoming tolerant and resistant to insecticides and thus the present investigation carries lot of significance.

Research frontiers

The present research work depicts that resistance in *An. maculipennis* could be due to the exposure of the mosquitoes to insecticides in the agriculture field.

Related reports

Earlier study on five insecticides of three major classes of pesticides in Astara Region, North of Iran indicates that the species is resistant to DDT and dieldrin which is roughly consistent with the result of present study in the case of dieldrin.

Innovations and breakthroughs

An. maculipennis showed higher susceptibility to deltamethrin, permethrin, and dieldrin at 96.4%, 95% and 91% mortality, respectively. The lowest susceptibility was seen against propoxur and bendiocarb at 5% and 8.3% mortality.

Applications

The literature survey and the present investigation indicate that the species which is carrier of malaria and many other nematode and viral parasites is becoming resistant to many pesticides being used in the study area.

Peer review

This valuable research work carries lot of significance in controlling *An. maculipennis*, which is carrier of many deadly disease causing organisms. In the 916 specimens of *An. maculipennis* examined against the insecticides, *An. maculipennis* appeared tolerant to permethrin, deltamethrin and dielderin, but displayed resistance against propoxur, bendiocarb and malathion.

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