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Evaluation of biological control agents for mosquitoes control in artificial breeding places

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ABSTRACT

Objective: To evaluate the entomological impact of chlorpyrifos-methyl, *Bacillus thuringiensis*, and *Gambusia affinis* on mosquitoes control in artificial breeding places. **Methods:** A Latin square design with 4 replicates was performed in order to evaluate the efficacy of chlorpyrifos-methyl, *Bacillus thuringiensis*, and *Gambusia affinis* on larva. The larvicide was applied at the dosage of 100 mg a.h/ha, *Bacillus thuringiensis* at the recommended dosage and 10 fishes per m^2 were applied at 1×1 m^2 artificial breeding sites. The larval densities for both anopheline and culicine were counted according to larvae /10 dippers prior and 24 h after application. **Results:** All three control agents are effective for mosquito density reduction, and the difference between the three agents and the control is significant ($P<0.05$). There is also significant difference among chlorpyrifos-methyl, *Bacillus thuringiensis* and *Gambusia affinis*. *Bacillus thuringiensis* exhibited more reduction on mosquito larval density than fish and larvicide ($P<0.05$). **Conclusions:** *Bacillus thuringiensis* in comparison with two other agents is the appropriate method for larviciding in the breeding places. Although long term assessing for biological activities as well as monitoring and mapping of resistance is required.

1. Introduction

Malaria is still considered as a major public health problem in Iran. There are different measures for malaria vector control in Iran based on Integrated Vector Management including indoor residual spraying, using impregnated bed net, larviciding by larvicides, biological agents and larvivourous fishes.

Mosquitoes belonging to the family of culicidae and are responsible for the transmission of the pathogens of some most life-threatening and debilitating diseases of human societies, like malaria, yellow fever, dengue hemorrhagic fever, filariasis which are rising and resurgent in many tropical and subtropical areas[1]. Malaria is a major health problem in southeast of Iran. It is unstable with two seasonal peaks mainly in spring and autumn[2]. Therefore malaria control is increasingly recognized as a key role in poverty reduction in high burdened countries[3]. In south of Iran, five anopheline mosquitoes including *Anopheles stephensi*, *Anopheles dthali*, *Anopheles fluviatilis*, *Anopheles superpictus* and *Anopheles culicifascies* are known to be main malaria vectors[4] .

One of the methods for malaria control is the control of adult mosquito, but in recently years, it has becoming increasingly difficult[5] because of resistance to some insecticides in vectors. Resistance of *Anopheles stephensi* to DDT was first recognized in 1957[6] then to dieldrin in 1960[7] and malathion in 1976[8]. Recent efforts were focused on using biological control agents[9].

The present study was carried out in a semi-filed condition to evaluate the entomological impact of chlorpyrifos-methyl, *Bacillus thuringiensis*, and *Gambusia affinis* on mosquitoes control in artificial breeding places.

2. Materials and methods

2.1. Study area

The investigation was carried out in Kazeroon district, located in west of Fars province, southwest of Iran.

This district has subtropical weather and population is prone to mosquito borne disease due to the existence of several species of malaria vectors including *Anopheles stephensi*, *Anopheles dthali* and *Anopheles fluviatilts* as well as some culicine mosquitoes.

2.2. Larvicides

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For malaria control in Iran, the main methods are the use of chlorpyrifos-methyl (Reldan), *Bacillus thuringiensis* as biolarvicides in breeding places. *Gambusia affinis* as larvivorous fish have also been introduced into the breeding sites [3]. WHO recommended test procedure for larviciding [3,10] was used too.

2.3. Test design

A Latin square designe with 4 replicates including *Bacillus thuringiensis* (A), chlorpyrifos-methyl (B), control (C), *Gambusia affinis* (D) was performed in the breeding places of mosquitoes. The larvicide was applied at the dosage of 100 mg a.h/ha, *Bacillus thuringiensis* at the recommended dosage which is being used in the country and 10 fishes per m^2 were applied at 1×1 m^2 artificial breeding sites. For the control, nothing was applied. The larval densities for both anopheline and culicine were counted according to larvae/10 dippers prior and 24 h after application.

2.4. Data analysis

For comparing the efficacy of different larval control agents Stata software was employed. ANOVA test was performed to determine whether there is a statistically significant difference among control agents. $P<0.05$ was considered to be statistically significant.

3. Result

A Latin square design with 4 replicates including Bacillus thuringiensis (A), chlorpyrifos-methyl (B), control (C), Gambusia affinis (D) were performed in the breeding places of mosquitoes (Figure 1).

A	D	C	B
C	B	A	D
B	A	D	C
D	C	B	A

Figure 1. Latin square design for evaluation of different mosquito control agents.

The larval densities were significant different between pre-treatment and post-treatment ($P<0.05$) indicating that all three control agents are effective for mosquito density reduction (Table 1) and the difference is significant in comparison with the control ($P<0.05$). There is also significant difference among the reduction efficacy of chlorpyrifos-methyl, *Bacillus thuringiensis* and *Gambusia affinis*, *Bacillus thuringiensis* exhibited more reduction on mosquito larval density than fish and larvicide ($P<0.05$).

Table 1
Mosquito larval densities in the pre-treatment and post-treatment.

Control agent	Pre-treatment			Post-treatment		
	Culicine	Anopheline	Total	Culicine	Anopheline	Total
Bacillus thuringiensis	300	121	421	0	0	0
Gambusia affinis	284	221	505	33	46	79
Chlorpyrifos-methyl	112	309	421	0	14	14
Control	92	249	386	109	233	342

4. Discussion

Results showed that *Bacillus thuringiensis* in comparison with two other agents is the appropriate method for larviciding in the breeding places. Although chlorpyrifos-methyl exhibited mortality against mosquito larvae, it leads to environmental pollution and side effects on non-target organisms too[11]. The use of larvivorous fish such as *Gambusia affinis* for biological control of mosquitoes in the breeding places specially ricelands has several advantages, although they eat eggs and young of other aquatic fishes, other amphibians is also important for consideration of such mosquito control activities[12].

Conflict of interest statements

We declare that we have no conflict of interest.

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