EFFECT OF THE BIOLOGICAL INSECTICIDE, BACILLUS THURINGIENSIS (SEROTYPE H-14) ON MOSQUITO TRANSMISSION OF DISEASES

A THESIS

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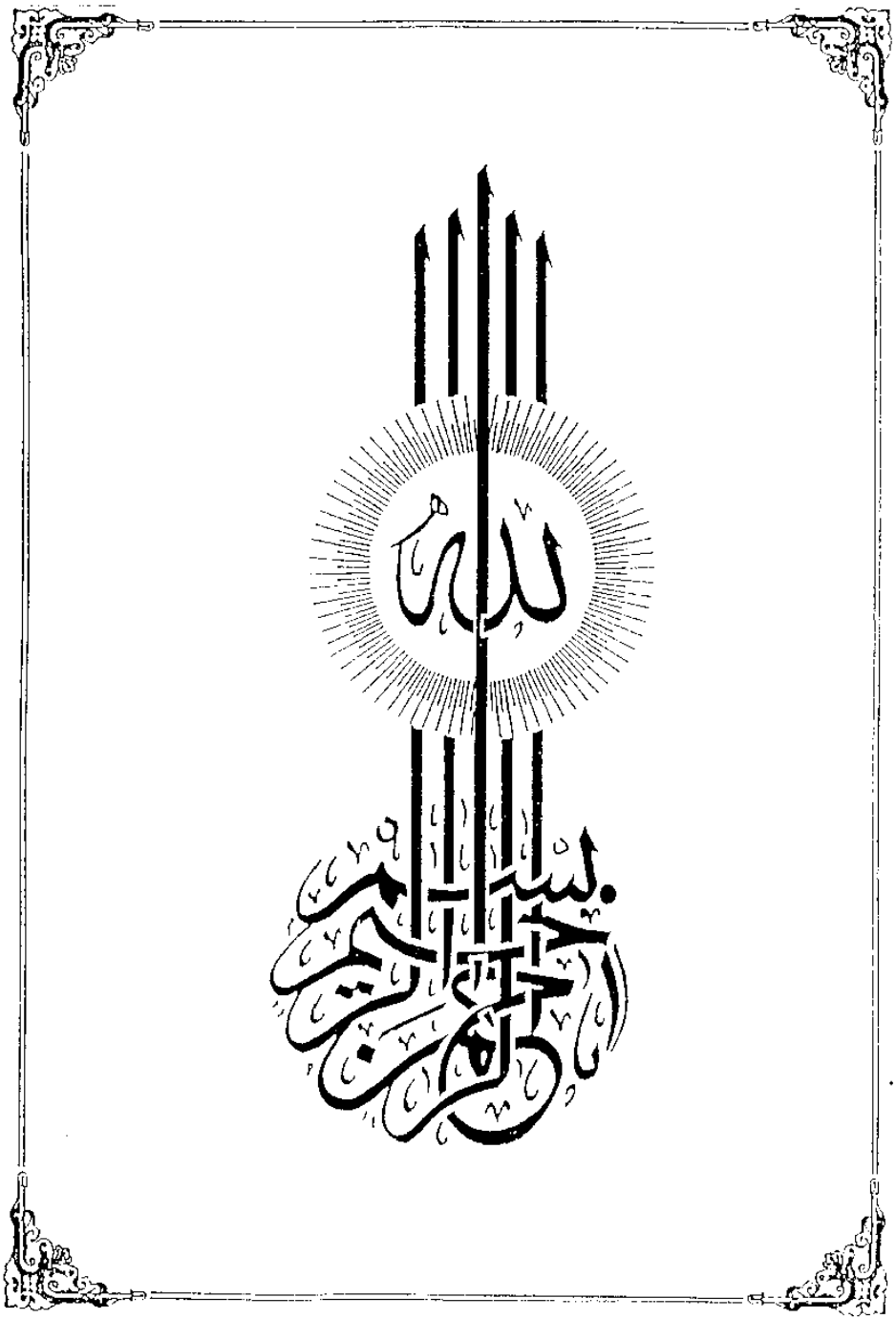
TO MY HUSBAND



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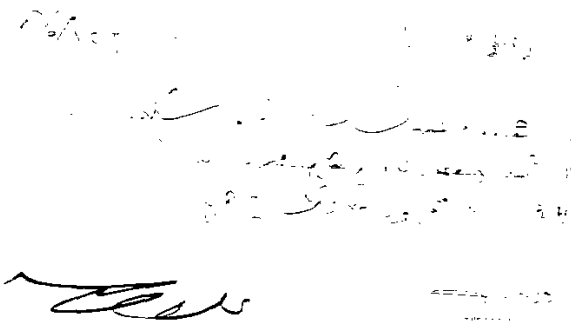

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1- INTRODUCTION

Mosquito-borne diseases, particularly malaria constitute important public health problems and obstacles to social and economic progress in many developing countries. Inspite of the direct impact of chemical insecticide application in malaria control programmes, yet, the global malaria situation has deteriorated in many countries, mainly due to insecticide resistance. The development of biological control of vectors has extended during the past decade. The mosquito pathogenic bacteria, Bacillus thuringiensis H-14 and Bacillus sphaericus proved to be very promising agents. However, these microbial agents are used as larvicides.

The objective of anti-larval measures is to keep mosquito densities at an epidemiologically negligible level which might mean zero in some areas because transmission can continue at very low densities of the vector. The success of utilizing microbial control agents against agricultural pests was mainly due to that the larval stages cause the direct damage while in case of vectors, adults only are responsible for disease transmission. The present investigation tried to evaluate the impact of the bacterial mosquito larvicides on the biology, fecundity and transmission capacity of anopheline vectors of malaria as the decrease in disease transmission is the ultimate goal of vector control planning.

Aim of the present study:

Part I:

1. Selection of a highly virulent bacterial larvicide formulation to the Egyptian mosquito species.
2. Determination of LT 50 and LC 50 for the fourth larval instar in order to select the larvicidal concentration and exposure time needed to yield sufficient numbers of adults from the treated larvae.
3. Studying the effect of the sublethal infection of the used bacteria on the biology and fecundity of female mosquitoes.

Part II:

Experimental transmission studies on treated and non-treated mosquitoes with the bacterial larvicide during larval stages, by feeding on infected laboratory animals.

II- LITERATURE REVIEW

1) Colonization of anopheline mosquitoes

Gerberg (1970), succeeded to maintain a colony of Anopheles gambiae Giles using the following procedure: Eggs were collected on wet filter paper placed on wet cotton wool in a petri dish. The filter paper was removed daily and placed on damp cotton wool in a closed glass container for 36-48 hrs. The eggs were washed off on water and hatching begins within 30 minutes. Powdered meat was provided as food instead of yeast since it had been published that adult emergence and survival rates significantly increased by using meat meal. A fine mesh tea strainer was used for applying the food. Larvae were reared in an enamel tray 35 x 30 x 5 cm at 26.5 C^{o} and require 7-13 days to pupate. Two hundred newly hatched larvae were placed in 2 liters of distilled water. They were fed on alternate days. The author collected the pupae daily and 300 placed in a 7.5 cm diameter aluminum pot with a least amount of water. Distilled water was added to keep the water level constant. Pupae were maintained at 26.5 $\pm$ 0.5 C^{o}. Adult emergence was completed in about 2 days. Eight hundred pupae were placed in a cage. He kept adults for the first 7 days in cages 30 x 30 x 30 cm and then transferred to larger stock cages 75 x 38 x 40 cm. The adults were maintained in an insulated, temperature controlled (26.5 C^{o}) (70-90% RH) darkened room. Adults were maintained on sugar solution or corn syrup on cotton wool and were offered a blood meal on the day after emergence, and then on alternate days. Rabbits were used as a source of blood meals. The back of the rabbit was shaved and the unanaesthetized rabbit was placed in a box in the cage. Mating occurs at dusk or in a darkened room or cage. Maximum mating occurs 3-5 days after emergence. Fertilized females

took blood meals more readily than unfertilized females. About 15.6% survived 20 days. The author also maintained a colony of Anopheles stephensi using the following procedure: He collected the eggs in plastic containers 12.7 x 17.8 x 6.4 cm containing a layer of cotton balls covered with paper toweling. About 300 ml of water were added to the container to the level required to wet, but not to cover the surface of the paper toweling. Oviposition containers were placed in the adult cages 48 hours after the females have been provided with a blood meal. The containers were removed after 24 hours exposure to gravid females. The eggs were washed gently into plastic hatching containers 31.7 x 22.8 x 10.2 cm, half filled with water at 27 C^{o}. About 72 hours later hatching was completed. The hatch rate varied from 79.1 to 90.9%. Larvae were counted by the aliquot method. For mass rearing, the author placed 15,000 larvae in trays 137.6 x 76.2 x 5.1 cm (0.69 larvae per sq cm surface area or 1 larva per 1.45 sq cm). The trays were filled with well water to a depth of 1.9 cm, 24 hours prior to the introduction of larvae. The larval food consisted of a 50 : 50 mixture of dog chow and porcine liver. The author used the following schedule:

Day	0	-	(Day of introduction) .13 mg/larva
Day	1	-	.13 mg/larva
Day	2	-	.13 mg/larva
Day	3	-	.20 mg + .045 mg Fleischmann's dried brewers' yeast/larva
Day	4	-	.26 mg/larva
Day	5	-	.40 mg/larva
Day	6 - 9	-	.53 mg/larva

The author mentioned that larval development was completed in six to nine days, using the previously mentioned feeding schedule, and with the rearing medium at 27 C^{o}. Pupation occurs over a 4 - day period with about 5 percent pupation on day six, 35 percent on day seven, 50 percent on day eight and 10 percent on day nine. The pupae were drained into metal boxes with the overflow screened to prevent loss of specimens. The pupae were separated by means of a pipette or small piece of wire screen. The author mentioned that the ice water technique, where pupae used to gather above the surface of the iced water for easily separation. They can also be separated by mechanical means based on size. About 1000 pupae were placed in a plastic tray, 12.70 x 17.78 x 6.35 cm and introduced into a cage for subsequent adult emergence. Adult mosquitoes were held and maintained in cages of 30 x 30 x 30 cm size, constructed of aluminum framing and 20 mesh wire screening. The front of the cage was fitted with a stockinette sleeve and the top with a nylon hammock. The adults emerged within 36- 48 hours at a temperature of 27 C^{o}, after which time the pupal containers were covered and removed from the cages. The newly emerged adults were furnished sucrose by placing cotton balls, soaked in 10 percent sucrose, in the nylon hammock. Four well-soaked cotton balls were provided per cage, and replaced daily. Sucrose was provided to the adults continuously except during the short intervals required to furnish the blood meals. Blood meals were provided by placing a restrained or anaesthetized rabbit, back down, on the nylon hammock for 30 to 60 minutes. The rabbit's back was clipped or shaved. Best egg production was obtained by providing females with two blood meals. These blood meals were supplied at 72 and 84 hours after adult emergence. Mating occurred readily in 1 ft^{3} cages maintained at 27 C^{o}, 80% RH and a light schedule of 14 hours artificial light and 10 hours of darkness. No crepuscular