Prospects of Microbial Larvicides for Mosquito Control Under Field Conditions

A THESIS

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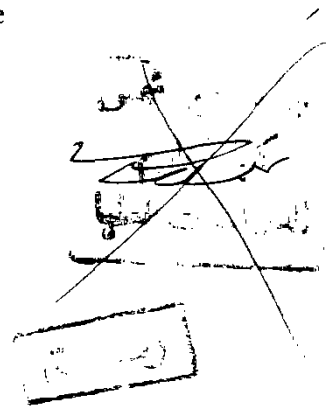

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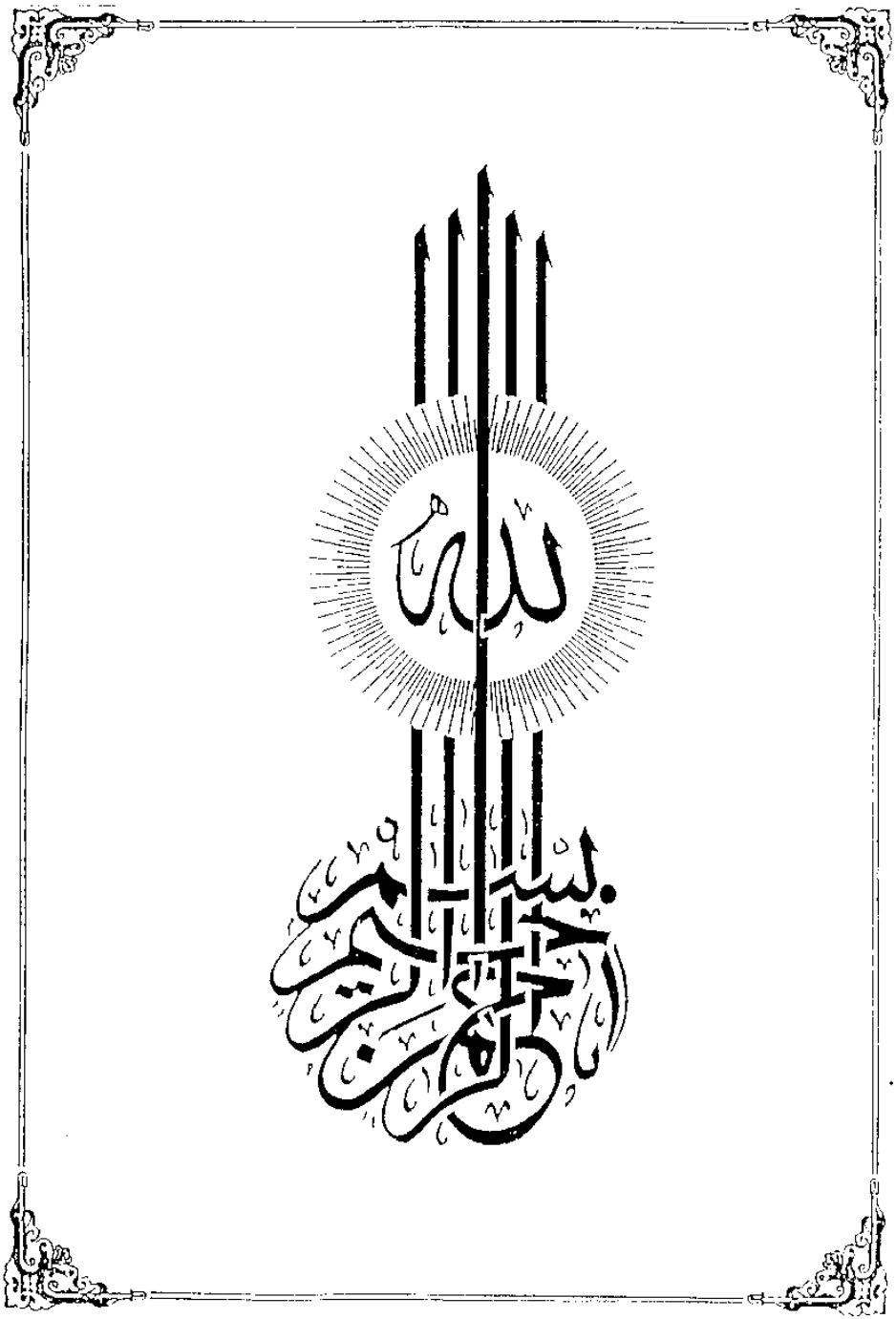
بسم الله الرحمن الرحيم

«وقل اعملوا فسيرى الله عملكم ورسوله و المؤمنون»

صدق الله العظيم



BIOGRAPHY

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CONTENTS

INTRODUCTION

The failure of chemical pesticides to continue their control of insect disease vectors has caused scientists to turn their attention to search for alternative methods.

Due to the continued price increase of insecticides, vector resistance and the public concern about environmental contamination, anti-larval measures using biological agents are now being encouraged, either alone or in integrated control programmes against mosquito vectors.

Among the most promising biological agents for mosquito control are Bacillus thuringiensis H-14 and Bacillus sphaericus.

The present investigation aimed at the evaluation of these biological agents under natural field conditions.

Preliminary experiments were carried out under laboratory conditions to study the effect of some factors which might affect the efficacy of these biological agents before applying them in the natural breeding places.

The second aim of this study was to test these biological agents on small-scale field trials to evaluate their activity and persistence under natural conditions.

The last step was a large-scale mass application to study the effect of these larvicides on the reduction of newly emerged adults that survived larval treatment which are the stage responsible for disease transmission.

LITERATURE REVIEW

A. Bacillus sphaericus Neide and its pathogenicity to mosquito larvae :-

Kellen et al. (1965) isolated a strain of Bacillus sphaericus Neide from moribound fourth-instar larvae of Culiseta incidens (Thomson) collected near Fresno, California.

The bacterium was a gram-variable, aerobic, spore-former and was facultatively parasitic to mosquitoes in laboratory tests. Tests were performed with larvae of 10 species of mosquitoes, all of which were susceptible to the bacterium.

Davidson et al. (1975) determined numbers of viable bacteria in second-instar Culex pipiens quinquefasciatus Say larvae following ingestion of pathogenic strain SSII-1 and non-pathogenic Bacillus sphaericus. The non-pathogenic B. sphaericus recovered from larvae declined rapidly after cessation of feeding as did numbers of pathogenic SSII-1 cells fed at LD_{20} dosage. Pathogenic cells when fed at LD_{70} dosage, the number of B. sphaericus in larvae was increased following initial decline. When chloroform treated SSII-1 cultures, in which all bacteria except spores were dead, were fed at LD_{70} and LD_{98} dosages, no viable B.sphaericus was recovered from larvae. The authors added that, in all SSII-1 treatments, other bacterial flora multiplied rapidly in larvae following onset of mortality.

Ramoska et al. (1977) elucidated that Bacillus sphaericus strain 1593, 1404 and SSII-1 were assayed for infectivity against field collected larvae of Psorophora columbiae, [Dayer &Knab] Culex nigripalpus [Theobald] and Aedes taeniorhynchus [Lynch Arribalzaga] in southwest Florida.

Their results indicated that, all the three strains were highly active against the Psorophora and Culex species. Aedes taeniorhynchus was also found susceptible but required higher doses to achieve lethal responses. Tests were also conducted on the rate of infection and the differences in susceptibility of different instars to B. sphaericus. The authors stated that nearly 75% of the mortality occurred within 48 hrs. post-incubation with the bacterium.

Mulligan et al. (1978) found that Bacillus sphaericus Neide strain 1593-4 was virulent against the southern house mosquito, Culex quinquefasciatus Say, and possessed longer residual activity than strain SSII.1 in pond test at Fresno, California.

Further testing of strain 1593-4 provided excellent control of a natural population of Culex tarsalis Coquillett at 10^4 cells/ml

Infectivity of the bacterium to a mixed population of Cx. tarsalis and southern house mosquito was decreased in a treatment of a dairy drain. The authors also noticed effective control of a mixed population of Aedes melanimon Dyar and Ae. nigromaculis Ludlow was not obtained at 10^4 Cell/ml. in 0.02 ha pasture plots, although laboratory tests showed

that Ae. nigromaculis was high susceptibility to the bacterium compared to other Aedes species. They added that the field tests of strain 1593-4 showed no deleterious effect on non-target organisms at 10^5 Cells/ml.

Myers and Yousten (1978) related the pathogenicity of Bacillus sphaericus SSII.1 to be due to toxin. mediated rather than an infectious process. Chloroform or ultraviolet-light treatments that decreased the viable count of SSII.1 cells by 4 or 5 logs did not significantly alter the ability of the bacterial cells to kill larvae. They presented also that three lines of evidence indicated that toxic activity was not related to sporulation: **1)** Cells grown in either a complex or a defined medium were toxic to all ages; **2)** When supplemental Mn^{2+} was excluded from complex medium, the culture yeilded few spores but was of equal toxicity to a culture containing many spores; and **3)** Several early blocked oligo sporogenous mutants were isolated that had toxic activities comparable to that of the parent. The authors added that the toxin was shown to be relatively unstable because activity was destroyed by heat and decreased by refrigeration.

Myers et al. (1979) assayed two strains of Bacillus sphaeircus SSII.1 and 1593 for toxic activity against second instar larvae of Culex pipiens quinquefasciatus. They found that strain 1593 developed a level of toxicity 3000 times that of strain SSII.1. The authors stated that although the toxic activity of B. sphaericus SSII.1 was relatively unchanged

throughout growth, an increase in activity of strain 1593 occurred as the bacteria began to sporulate; the toxin of strain 1593 was shown to be more stable than that of strain SSII-1.

Unlike the spores of strain SSII.1, the spores of B. sphaericus 1593 were found to be highly toxic.

Ramoska and Pacey (1979) found that the effectiveness of Bacillus sphaericus (Strain 1593) for the control of mosquito larvae in the laboratory was inversely related to the amount of food available to the larvae. The rate of bacterial uptake differed between larvae of Culex quinquefasciatus and Anopheles albimanus.

Larvae of the former species consumed lethal quantities rapidly (5min. On wards) after bacterial application, whereas, those of the latter species did so less rapidly, resulting in low mortality even after incubation for 90 min. with a high concentration (7 X 107 Cells/ml.)

Balaraman (1980) compared two strains of Bacillus sphaericus isolated indigenously from sick moribund mosquito larvae in Pondichary to a strain of B. sphaericus which was commercially produced in U.S.A and supplied by WHO, regarding their efficacy in killing larvae of three species of mosquito vectors of diseases (Culex fatigans, Anopheles stephensi and Aedes aegypti).

The author found that the indigenous isolated strain was as efficient for use as a larvicide as the strain supplied by the WHO.