BACTERIOPHAGES AS FACTORS AFFECTING THE EFFICIENCY OF THE BIOLOGICAL MOSQUITO LARVICIDES BACILLUS THURINGIENSIS H-14 AND BACILLUS SPHAERICUS

A Thesis

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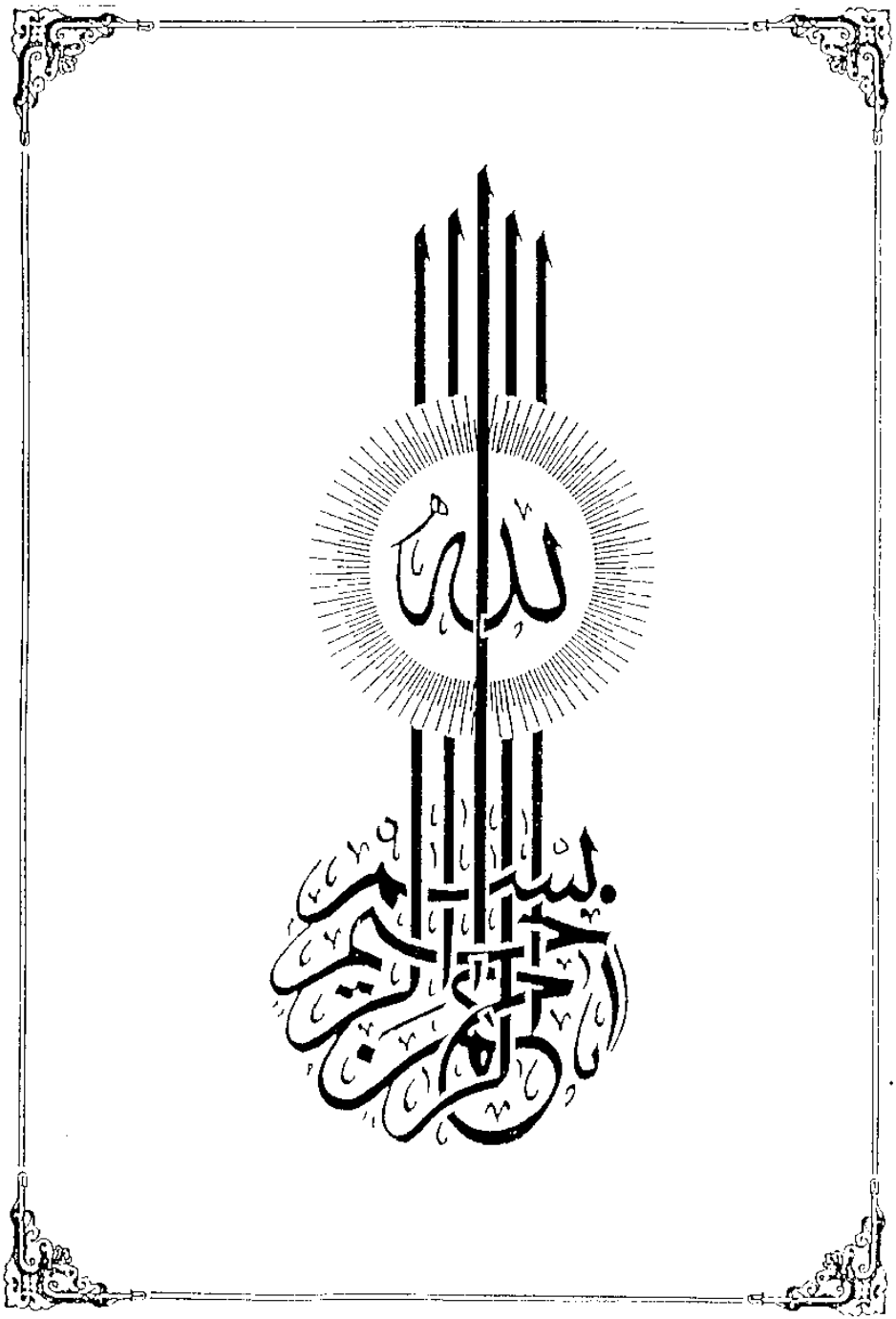

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The failure of chemical pesticides to continue their control of insect disease vectors has caused scientists to turn their attention to the *biogenesis of entomocidal toxins* (that is the production of insect toxins by microorganisms). During the past decade, bacterial larvicides were formulated commercially for the control of mosquito larvae, particularly, *Bacillus thuringiensis* H-14 and *Bacillus sphaericus*. The insecticidal activity is generally associated with a parasporal crystal produced during sporulation.

The effectiveness of these bacterial larvicides under field conditions is highly dependent on the length of time the active material remains in the appropriate area. The persistence of these larvicides under field conditions has lately been described by several workers, Davidson et al. 1984; WHO (report), 1985; El Sayed, 1988, and many others. It has been demonstrated that toxicity to mosquito larvae disappeared after different post-spraying periods depending on the types of aquatic larval habitats. However, similar types of larval breeding places, sometimes, gave different persistence potentials of the applied bacterial larvicides. Accordingly, a biotic factor was suspected to play a role in the stability or inhibition of the larvicidal activity which is the presence of bacteriophages that could lysate the entomopathogenic sprayed bacteria.

The present study aimed at investigating the role naturally existing bacteriophages, may play in the inhibition of the larvicidal activity of the mosquito-bacterial larvicides under natural breeding places.

II. LITERATURE REVIEW

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Raun et al. (1966) isolated an agent causing lysis of the vegetative cells of *B. thuringiensis* from a single corn-borer larva. A similar agent was also isolated from a commercially produced spore powder of *B. thuringiensis*. Since the agent was filtrable and transmissible to *B. thuringiensis* and caused lysis of the cells, it met all requirements for a bacterial virus or a bacteriophage. Because the bacteriophage had been isolated from *B. thuringiensis*, it seemed likely that the phage might act as a bacteriostatic mechanism that reduced the pathogenicity of the bacterium in corn-borer larvae. It had been suggested that the bacteriophage might be valuable as an immunogenic factor in the resistance of silk-worms to causal agents of bacterial diseases. The authors performed injection and feeding tests to determine the role of the phage in the resistance of treated corn-borers to the bacterium. They stated that the bacterial-virus did not greatly alter the final mortality figures, but it did slow down the effect of the bacterium. This delay in mortality of the phage-treated larvae was probably due to the time required for a phage-resistant strain of *B. thuringiensis* to reproduce to lethal numbers. Standard tests of bacterial growth confirmed that resistant colonies had developed in 24 to 48 hours.

Colasito and Rogoff (1969a) stated that by using a strain of *B. thuringiensis* var. *galleriae*, serotype V, esterase type 5, as a host, six phages, grouped in 3 types, were studied. The

characteristics of the three groups of phages were easily differentiated. However, sufficient differences were observed between individual phages within a group. The bacteriophages were isolated from various sources. The authors stated that examination of the micrographs indicated 3 morphological types among the 6 phages examined. The host range differs from *B. cereus*, to *B. thuringiensis* var *sotto*, *B. thuringiensis* var *alesti* and *B. thuringiensis* var *galleriae*. It was found that serological relations within the morphological groups were fairly consistent. They recommended that further exploration must be done. The data obtained suggest either a difference in the number of adsorption sites on the host cell surface for phage GV-6 as opposed to the other phages or a difference in GV-6 affinity for an adsorption site common for all the phages tested. They determined latent periods and average burst sizes for each phage. Burst sizes ranged from 50 infected cells in GV_1 to 200 infected cells in GV-6. They showed that maximal burst sizes were obtained with Ca^{++} supplementation, minimal latent period and maximal efficiency were obtained with Ca^{++} supplementation. They stated that there were differences in the inactivation of phages when held at 60°C or held in ice cold broth. All phages were quite stable under a variety of conditions except GV-6. They observed that stability was maximal between pH 6 and pH 8, and all the phages were inactivated at pH 4 and pH 10. They concluded that according to size, morphology, serology, host range and thermal inactivation, these six phages can be separated into

3 groups: Group I GV_1, GV_2 and GV_4; Group II GV_3 and GV_5; Group III GV_6. In the 1st two groups, individual phages can be distinguished regarding the plaque type, host range, adsorption rate, latent period and Ca^{++} requirement.

Colasito and Rogoff (1969b) isolated eight phages lysogenic for *B. thuringiensis* by induction of the host strains, and they divided them into 3 groups: Group I GT-1 to GT-5; Group II GT-6 and GT-7; and Group III GT-8. They were examined as for their morphology, host range, serum neutralization, adsorption rates, one step growth characteristics and thermal inactivation rates. The 3 groups are distinguishable as morphology and host range. Group II and III showed a high degree of homology with regard to their host range, serum neutralization, one step growth and thermal inactivation characteristics. The bacterial cultures used included a type species of *B. thuringiensis* var *galleriae*, esterase type 5, serotype V. They used different methods of induction (ultraviolet induction, H_2O_2 induction and Mitomycin C induction). These methods lead finally to complete lysis. The lysates were then manipulated and used in different examinations for serum neutralization studies, antisera were also prepared, using guinea pigs. The authors obtained the following results:

1. Group I included polyhedral-head and contractile tail variety. Group II including phages which are polyhedral-head, non-rigid tail and a conspicuous end-apparatus on