FACTORS INFLUENCING THE EFFICIENCY OF SOME MICROBIAL ENTOMOPATHOGENS ON SOME LEPIDOPTEROUS PLANT PESTS

A THESIS

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1. INTRODUCTION

The past two decades have witnessed a marked revival of interest in the microbial method of insect pest control. This resulted from a growing realization of the fact that supplements and alternatives to other control methods, particularly the use of chemical insecticides, are needed to overcome some of the faults and weaknesses now recognized as inherent in the latter methods.

The scope of microbial control investigations is now considerably extended. Such extension is expressed in many ways, mostly noticed in the increasing use of entomopathogens against various insect pests. It is also obvious in the increase in basic research dealing with the pathogens themselves, their virulence and factors influencing their efficiency ... etc.

The study of environmental factors significant in the field of insect pathology is an important aspect. Such factors play a considerable rule in the development of pathogens, their subsequant infection of the host, their survival and virulence within the host and to alimited extent in the host susceptibility. The factors to be studied should include the radiation, temperature, humidity, chemical insecticides, biological factors and mechanical injuries.

بسم الله الرحمن الرحيم

5- Testing the influence of other microorganisms (polyhedrosis virus) on the viability and virulence of *Bacillus thuringiensis* using the eri-silk worm, *Philosamia ricini* Boisd. and the cotton leaf worm, *Spodoptera littoralis* Boisd.

6- Testing the influence of mechanical injuries on the incidence of natural diseases and mortality among the eri - silk worm and the cotton leaf worm.

II. LITERATURE REVIEW

The improvement and success of the utilization of the entomopathogenic microorganisms in the microbial insect control attempts depend mainly on the surrounding environmental conditions. So the application of this method in insect control depends on the understanding of interrelationships between host insect, pathogen and environment.

1- Test insects :

1) The eri-silk worm, *Philosamia ricini* Boisd.

Chowdhury (1960), cleared out that *Attacus ricini* Boisd. is a synonym of *Philosamia ricini*.

Lefroy and Ghosh (1907), stated that eri-silk worm, *Philosamia ricini* Boisd. suffers from the diseases of mullberry silk worm, pebrine, flacherie, grasserie, muscardine and added that in one brood, worms died with symptoms pointing the pebrine and in one lot the worms died from diseases showing the symptoms of muscardine. Chowdhury (1960), reported that *Attacus ricini* Boisd. suffers heavily from pebrine and flacherie, but it is very resistant to grasserie. Mogahed (1967), isolated fungus identified as *Asperigillus lutescens* from diseased caterpillars.

2) The cotton leaf worm, Spodoptera littoralis Boisd.

The insect was found to be slightly susceptible to Bacillus thuringiensis strains (Hafez, Afify and Merdan,1969) and (Afify and Merdan,1969). Also the insect was found to be highly susceptible to polyhedrosis virus (Abul-Nasr 1956).

3) The imported cabbage worm, Pieris rapae L.

The insect was found to be highly susceptible to Bacillus thuringiensis varieties and strains under both laboratory and field experiments, (Heimpel and Angus 1959); (Heimpel and Lloyed,1959); (McEwen, Glass, Davis, and Splittstoesser,1960); (Jaques and Fox,1960); (Abdel-Rahman 1966); (Atwa,1969) and others. Also the insect was found to by susceptible to polyhecrosis virus (Tanada,1953).

2- Entomopathogens :

1) Bacteria

Berliner (1911), was the first to isolate Bacillus thuringiensis var. thuringiensis strains from diseased larvae of the Mediterranean flour moth, Anagasta (Ephestia) Kühniella Zell.

Ellinger and chorine (1930), indicated that Bacillus thuringiensis is synonymous with Bacillus cereus.

These Berliner strains have been applied to a large group of insects as effective entomopathogens during the last four decades.

Husz (1928, 1929, and 1930) suggested the use of Bacillus thuringiensis for controlling the European corn borer, Pyrausta nubilalis (Hbn.).

Mattes (1927), could infect Ephestia larvae with Bacillus thuringiensis through the mouth.

Metalnikov and Chorine (1929), found Bacillus thuringiensis to be pathogenic to a large group of insect species and was mainly virulent to lepidopterous larvae.

Steinhaus (1949), tested the pathogenicity of Bacillus thuringiensis against several insect species and found that it could be used as a virulent and effective bacterial insecticide.

From these and other studies, Bacillus thuringiensis var. thuringiensis strains could be considered as effective against a large group of lepidopterous larvae; e.g. the alfalfa caterpillar; colias philodice eurytheme Boisd. (Steinhaus, 1951); the European cabbage worm Pieris brassicae L. (Kreig, 1957 and Lemoigne et al;1956) the European corn borer

Pyrausta nubilalis Hbn. (Hautouret, [illegible]); the [illegible] caterpillar *Malacosoma neustria* (Van damme and van Der Laan, [illegible]); Other lepidopterous pests in crucifers (Canada , [illegible], [illegible]); the silk worm, *Bombyx mori* L, the brown tail moth, *Euproctis phocarrhoea* and the gypsy moth, *Lymantria dispar* (Vankova, 1957).

It was also found to have an effect on the imported cabbage worm *Pieris rapae* L., the cabbage looper *Trichoplusia ni* Hbn., and the red banded leaf roller, *Argyrotaenia velotinana* walker (Mc Ewen, Glass, Davis and splittstoesser 1960); the ailanthus web worm *Atteva aurea* and the fall web worm *Hyphantria cunea* (Hall and Onnoha, 1962); the Indian meal moth *Plodia interpunctella* Hb. and the imported cabbage worm *Pieris rapae* L. (Abdel-Rahman, 1966); the cotton leaf worm, *Spodoptera littoralis*, the lesser cotton leafworm, *Laphygma exigua*, the pink ball worm *Pectinophora gossypiella* saund, the cut worm *Agrotis ypsilon* Rott. (Afify, Hafez and Merdan, 1969); the imported cabbage worm, *Pieris rapae* L. (Afify, Abdel-Rahman and Atwa, 1970).

2) Virus

Polyhedrosis virus affecting nearly 200 species of Lepidoptera, Hymenoptera and Diptera have been reported (Hughes, 1957, Martignoni and Langston, 1960). This type of virus disease occurs mainly in Lepidoptera. The general aspects of the infection caused by nuclear polyhedrosis have been discussed by a number of writers (Paillot 1930; Lotje, 1939; Bergold, 1943; 1953a; 1958a; Steinhaus, 1946, 1949a; Jahn, 1958; Smith, 1958, 1959; Krieg, 1961).

The occurrence of virus infection in insects under natural conditions may vary according to several environmental factors , the imported cabbage worm Pieris rapae L., (Tanada, 1953); the gypsy moth, Prothetria dispar L. (Wallis, 1957); the gamma noctuid, Plusia gamma (Vago and Cayrol, 1955); the california oak worm, Phryganidia california (Packard); the cotton leafworm, Spodoptera littoralis (Boisd.) (Abul-Nasr, 1956); the silk worm Bombyx mori L.; (Yokokawa and Yamaguch, 1960); the greater wax moth, Galleria mellonella (David and Martin 1962); the bollworms Heliothis zea (Ignoffa, 1966); and (Robert and Canerday, 1968); the Eastern tent caterpillar, Malacosoma americanum (Smirnoff, 1967a); greater wax moth Galleria mellonella L. (David and Martin, 1962); the bollworm , Helioths zea and the tobacco bud worms Heliothis virescencs. (Bullock et al, 1970); the bollworm Heliothis zea (Boddie) and the fall army worm, spodoptera frugiperda (Hamm and Young, 1971).

3- Physical environmental factors :

1) Irradiation

Latarjet & Miletic (1953), reported that radiation of longer wavelength (blue light) can inactivate bacteriophages that the effect in this case is probably due to the production of toxic substances in the irradiated medium.

Aruga (1956), and Aruga and Yoshitake (1961), have provided evidence of inhibited development of viruses in irradiated insect larvae, but these investigators did not provide histological evidence.

Jafri (1963 and 1964), showed that the adults of certain rustured flour beetle, Tribolium castaneum and the confused flour beetle, Tribolium confusum Diw, exhibit an increased susceptibility to infection by Bacillus thuringiensis Berliner following exposure to sublethal and lethal doses of ionizing radiation.

Jafri (1965), indicated that when the confused flour beetle, Tribolium confusum Diw, receive Bacillus thuringiensis Berl. following exposure to sublethal and lethal doses of X-rays their life span is shortened to a week or at the most to a fortnight only. The insects did not give any significant increased or decreased in susceptibility when they receive Bacillus thuringiensis Berliner immediately after an interval of 24 and 144 hours following exposure to sublethal and lethal doses of X-rays.

In (1966), Jafri found a decreased susceptibility in the silk worm *Bombyx mori* L. larvae infected with the polyhedrosis virus *Borrelinavirus bombycis*, following exposure to sublethal and lethal doses of X-rays.

The same auther, (1968), observed that the sublethal and lethal doses of ionizing radiation appeared to inhibit the development of virus of VND ("virose à noyaux denses") in the greater wax moth, *Galleria mellonella* L. larvae. In the same year, he exposed larvae of the same insect (two weeks old) to X-ray doses of 1,000, 10,000, 30,000 and 60,000 r. After twenty four hours, some of the irradiated larvae were infected with the virus VND (virose à noyaux denses). The larvae receiving 1,000 and 10,000 r showed a somewhat increased susceptibility to infection. The time of the death of 50% of the larvae (LT_{50}) receiving VND virus after exposure to 30,000 and 60,000 r was found to be several days later than non-irradiated infected larvae. It appears that sublethal and lethal doses of ionising radiation (30,000 and 60,000 r) inhibit the development of VND.

[illegible]) Ultraviolet irradiation :

Burgerjon and Yamviras (1959), used ultraviolet rays in destroying about 95% of spores of Bacillus thuringiensis Berl. in their tests on the Mediterranean flour moth, Anagasta (Ephestia) Kühniella Zell. and the European cabbage worm, Pieris brassicae (L).

David and Martin (1962), stated that the exposure for 65 minutes to ultraviolet light did not affect polyhedrosis, but the thirteen individual of greater wax moth Galleria mellonella Lin., 5 minutes exposure to ultraviolet light increased mortality by polyhedrosis.

Yamviras (1962), reported a definite reduction in the pathogenicity of Bacillus thuringiensis Berliner to larvae of the Mediterranean flour moth, Anagasta kühniella Zell, when the spores were subjected to ultraviolet radiation.

According to Cantwell & Franklin (1966), spores of Bacillus thuringiensis var. thuringiensis Berliner had deleterious effects when exposed on filters or glass slides to ultraviolet light, he stated that " a lamp of short-wave ultraviolet is very useful in killing the spores that remain when a clean preparation of Bacillus thuringiensis crystals is being prepared".

Raun et al.(19[illegible]), found that ultraviolet irradiation of a spore powder of Bacillus thuringiensis var. thuringiensis Berliner for 72 hours eliminated its pathogenicity for both insect, the fall army worm, Spodoptera frugiperda and the European corn borer Ostrinia nubilalis Hübner.

David and Magnus (1967), pointed out that ultraviolet irradiated virus which has been immediately exposed to intense visible light after ultraviolet radiation and before feeding to the larvae causes very significantly more deaths than ultraviolet irradiated virus which has not been subsequently exposed to visible light.

Gudauska et al (1968), found that infectivity of Trichoplusia nuclear polyhedrosis viruses was greatly reduced by exposures of 4 - 10 minutes to ultraviolet at 2 inches. Total inactivation did not occur after 60 minutes, but did after 120 minutes. Effect of ultraviolet on both viruses (Heliothis and Trichoplusia nuclear-polyhedrosis viruses) was progressively lessened as distance from light source to virus increased. No reduction in infectivity of either virus occurred at an exposure distance of 32 inches.

David (1969), indicated that when the known granuloses virus of the European cabbage worm, Pieris brassicae L. was exposed to ultraviolet radiation from a germicidal lamp (predominantly 253.7 nm) it was then rapidly inactivated in pure films than in films of crude virus, where coloured and solid impurities were present. In both cases inactivation occurred more rapidly in wet films than in dry films. When the inactivating effect of ultraviolet of different wave lengths was compared using a highly purified virus preparation, it was found that this decreased progressively as the wavelength was increased but that inactivation was still brought about by, at any rate, the shorter was ultraviolet radiation (291.5 - 320 nm) present in sunlight as it reaches the earth.

Bullock et al.,(1970), irradiated a suspension of polyhedra of Heliothis polyhedrosis virus at 16 microwatts/ cm^2 with narrow-band ultraviolet energy at wavelengths of 257 nanometer (nm), 307.5 nm and 364 nm and with broad-band energy in the visible and near infra-red range. They assayed for virulence with 20 mg larval boll worms, Heliothis Zea and tobacco bud worms Heliothis virescencs. They found that wavelengths of 257 nm and 307.5 nm significantly inactivated virus, and 364 nm and the visible range may have inactivated it: Mortality was reduced 45 and 86.9 % in boll worm Heliothis Zea and tobacco bud worm Heliothis virescencs, respectively, when they were assayed with a polyhedral suspension (1250 viral inclusions/mm^2 of diet surface) treated with monochromatic energy at 307.5 nm.

ii) <u>Infra-red radiation</u> :

As far as the author is a ware no reliable information was found concerning the effect of infra-red radiations on the entomophagous microorganisms.

iii) <u>Gamma radiation</u> :

Duxbury and Sadun (1963), found an increased susceptibility of the irradiated malaria carrier mosquitoes, *Anopheles quadrimaculatus* with gamma radiation to infection with *Dirofilaria uniformis* Cantwell & Franklin (1966), found that radiation by gamma source (400 R/min) was very time consuming since 400 minutes were needed to inactivate 97% of the spores.

Smirnoff (1967a), suggested that irradiation with doses of 100,000 rad or more are lethal to third and fourth instar larvae of the Eastern tent caterpillar, *Malacosoma americanum*. Exposure to radiations of 2,000,000 r. completely inactivates the polyhedra of the Eastern tent caterpillar, *Malacosoma americanum*. Development of the virus seems to be accelerated in larvae exposed to low doses of radiation.

The same author (1967c), recorded that growth of six entomophagous bacterial varieties were inhibited with gamma irradiation in the range 228, 280 to 456, 560 rads and suppressed at 500,000 rads and over *Bacillus thuringiensis* var. *entomocidus* was the most sensitive.