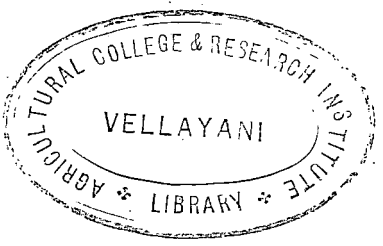
STUDIES ON THE SUSCEPTIBILITY OF THE COMMON CATERPILLAR PESTS TO INFECTION BY *Bacillus thuringiensis* BERLINER



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THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri M.J. Thomas under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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C O N T E N T S

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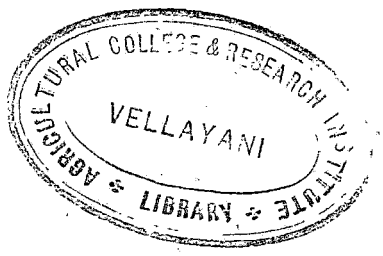
INTRODUCTION



INTRODUCTION

Insects are subject to attack by a large number of parasitic and predacious species and disease causing microorganisms. Utilization of these natural enemies to control insect pests is 'applied biological control'. Biological control using microorganisms is termed 'microbial control'. Although the term is of recent origin the idea it designates dates back to over a hundred years ago. The illustrious zoologist Elie Metchnikoff (1879) is credited with having first suggested the use of microorganisms to control harmful insects.

The microorganisms associated with insects are bacteria, fungi, virus, protozoa and nematodes. Some of these have proved promising in the control of various plant and animal pests. Some of the microorganisms are amenable to be cultured in enormous numbers to meet urgent demands. By virtue of certain rare attributes like non-interference in the natural balance, lack of hazards to other animals and absence of risk of causing development of resistance in insects, microbial preparations are becoming increasingly popular both for research as well as for practical application in insect control.

Early attempts to use microorganisms were made with fungi and not until the 20th century were the bacteria utilized in controlling insect pests. Landmarks in the use of bacteria begin with the period 1909-1915 when d' Herelle (1911, 1912, 1914, 1915) created considerable interest and excitement by claiming successful control of the migratory grasshoppers with the use of Coccobacillus acridiorum. But the failure to duplicate this success by subsequent workers led to the abandoning of the effort. In the late 1920s and 1930s several workers in Hungary, Yugoslovia and elsewhere obtained encouraging results with the use of bacteria, especially spore forming organisms like Bacillus thuringiensis Berl. However, for some unknown reasons these successes were not followed up. The third stage is marked by the success achieved against the japanese beetle Popillia japonica by the use of the 'milky disease' organisms Bacillus popillae and B. lentimorbus. Later in 1949, Steinhaus renewed the investigations with B. thuringiensis and related species. This was followed by exhaustive studies by workers like Faldini and Pastrana (1952), Angus (1956 a, b), Grison and Beguin (1954), Tanada (1956,1959) Okka (1957), Rabb et al (1957), Heimpel and Angus (1958) and many others.

As a result of these studies it is now known that

B. thuringiensis Berl. is a large and motile bacterium which is gram positive, rodshaped, crystalliferous, spore forming and entomogenous and can be used as an effective 'living insecticide'. The outstanding advantages of the bacterium have been found to be its long history of absence of toxicity to warm blooded animals, wide spectrum of activity, compatibility with most pesticides, stability of virulence enabling its long storage, comparative rapidity of action, and amenability to commercial production. These virtues have acquired for the organism the highest place among other microbial insecticides.

Recent investigations by the workers, Bennefoi and Beguin (1959), Fisher and Rosner (1959), Jaques and Fox (1960) Mc Ewen et al. (1960), Patel and Cutcomp (1960), Steinhaus (1951, 1960) and Smirnoff (1963) have brought to light many informations regarding its toxicity, mode of action, virulence, compatibility, residual action and other aspects. Effect of environmental factors on the progression of infection, the histopathological changes in the host, methods of increasing susceptibility of host insects etc., are fields which have not been probed to in detail. Quantitative data on the susceptibility of different insect species is meagre and far between.

In India researches in this field have just been

initiated only. The works so far done are of De and Konar (1956), Majumder *et al.*(1956), Rao *et al.*(1962), Venkatraman *et al.* (1962), Sekhar and Gopinath (1962) and Ramamurthy (1963 a,b) and these are mainly of a screening nature. In Kerala, Ayyar (1961) reported the susceptibility of five caterpillar pests.

In view of the immense possibilities of the use of *B. thuringiensis* in controlling crop pests and the lack of information on its effect on the different caterpillar pests in this country, the present studies were taken up.

In these studies the susceptibility of twentysix species of caterpillar pests affecting various crops has been ascertained by screening tests. Dosage mortality relation between 16 of these caterpillars and spores of *B. thuringiensis* has been worked out in full. The different species of caterpillars have shown different susceptibilities to infection by *B. thuringiensis* and the bacterial preparation has proved promising to control some of these pests.

A brief review of work done so far with *Bacillus thuringiensis* is also presented.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

A bacterial disease of the larvae of Mediterranean flour moth, Ephestia kuhniella Zeller was recorded in Germany by Berliner in 1911. Later in1915 he named the causative organism as Bacillus thuringiensis. Mattes (1927) also isolated the bacterium from the diseased larvae of the same insect.

There is some disagreement on the taxonomic status of B. thuringiensis and its related species. Ellinger and Chronine (1930), Smith et al .(1946), Toumanoff (1953,1955) and Toumanoff and Le Corroller (1959) consider B. thuringiensis as a variety of B. cereus F. & F. But Steinhaus (1951 a, 1951 b), Steinhaus and Jerrel (1954) and Heimpel and Angus (1958, 1960 a) treat it as a distinct species.

According to Bergy's Manual of Determinative Bacteriology (1957), B. thuringiensis has been given the following systematic position.

Class	: Schizomycetes
Order	: Eubacteriales
Family	: Bacillaceae
Genus	: Bacillus
Species	: thuringiensis

The bacterium has been described in detail in the Bergy's Manual. The vegetative cells of the bacterium are

rod shaped with square ends, measuring 1.0 to 1.2 by 3.0 to 5.0 microns, usually occurring in short to long chains and not capsulated. They are motile and gram positive. The sporangia are not definitely swollen, spores lying obliquely in them. The sporangium contains crystalline inclusion bodies. Spores are ellipsoidal and measure 1.0 to 1.5 microns.

I. Toxic Mechanism.

Different views have been expressed by various workers regarding the toxic mechanism of the bacterium. Some believe that the toxicity is attributable to the presenceof a crystal formed during sporulation, while others think that the spore alone is enough. A third concept in this regard is that both the spore and the crystal are essential for the toxic effect.

The presence of a crystal in the sporangium together with the spore was first observed by Berliner (195) and later by Mattes (1927). The relationship of the crystal with the toxicity was first indicated to by Angus (1953). He found that injection of the toxin into the alimentary canal of silkworms caused cessation of feeding, paralysis of the midgut and eventual death. Hannay (1953) observed that during spore formation, the spores invariably are accompanied by diamond shaped crystals and concluded that they may be associated

with pathogenicity. Further works by Angus (1954 a,1956 b) also demonstrated the highly toxic character of the crystals to certain insects. He found that the midgut pH of the susceptible insects was alkaline (9.0 to 10.5), a condition necessary to dissolve the crystals.

Heimpel (1955a), from his studies with many bacteria pathogenic to the larch sawfly, Pristiphora erichsonii, fuled out the possibility that the pathogenecity is dependent on the presence of the crystals. But investigations by Vankova (1957), Bennefoi and Beguin (1959) and Patel and Cutcomp (1961) yielded results revealing the toxic nature of the crystal. For example Bennefoi and Beguin (1959) demonstrated that the pure extracts of crystals are 2.5 times as effective as a standard spore preparation. They also observed the gut pH of susceptible insects is above 9.

Heimpel and Angus (1959) recorded that in the larvae of Anagastra kuhniella, the spores germinate in the presence of crystalline toxin and grow in the midgut before causing death, indicating that both the spore and the crystal are necessary to cause death.

McConnel and Richards (1959) and Tamashiro (1960) reported another toxin which is heat-stable and toxic when injected into insects but not when ingested.

Lipa (1962) observed in Pieris brassicae that the infected larvae died out of septicemia, the dead

larvae showing a large number of bacterial cells. Similar results were also obtained by Tanada and Reiner (1962) on Heliothis zea. Smirnoff (1963) in his elaborate experiments with Choristoneura fumiferana recorded typical septicemia and concluded that the crystals are not essential to pathogenicity.

Cameron (1963), reviewing the whole position, summarised his views in the following words. "The present day knowledge of the relative function and importance of the spores and crystals in causing death of different insect species is far from complete; sometimes one or the other is almost entirely responsible, but in other cases there appears to be an interaction between the two."

Properties of the crystal : The crystal is typically diamond-shaped as observed by Hannay (1953) and it may take a rhombohedral to cuboidal shape in some cases as noted by Stieinhaus and Jerrel (1954). According to Hannay (1954), it is soluble in dilute alkalies and insoluble in water and organic solvents. Investigations by Hannay and Fitz James (1955) proved the presence of at least 17 amino acids in it.

II. Pathogenesis.

Pathogenesis or the course of infection as it takes place in the insect hosts has been studied in detail

by Mattes (1927) in E. kuhniella. He observed the following successive stages: (1) ingestion of the spores and their germination in the midgut; (2) formation of a large number of vegetative cells in themidgut; (3) damaging the gut epithelium by the enzymatic activity of the bacillus as it flourishes in the gut contents; (4) migration of the bacteria between the host cells into the body cavity; (5) abundant growth of the bacillus in the haemolymph; (6) disintegration of internal tissues as the result of bacterial enzymes;(7) appearance of external symptoms of the disease; (8) penetration of the bacteria into the tissues of the nervous system resulting in death; (9) further disintegration of all tissues and drying or 'mummification' of the larval remains: (10) formationcof spores of the bacteria.

III. Virulence.

The term virulence refers to the ability of a micro-organism to invade and injure the tissues of the body of its host. Variations in the virulence are rather frequent among entomogenous bacteria and have caused inconsistency in the results.

Mattes (1927) recorded that the spores of Bacillus thuringiensis remain viable for at least six years. Steinhaus (1949) suggested that the virulence can be enhanced by (1) passing them through susceptible hosts,

(2) causing them to dissociate into more virulent strains, (3) introducing together with microorganisms, substances that may increase their invasive power and (4) mixing them with other micro organisms that may render them more capable of invading tissues. Again he (1951) showed that the culture of the organism on artificial media and their long storage upto fifteen years caused no loss of virulence. Lemoigne et al.(1956)also noted no loss of virulence in laboratory cultures for three years.

However, Majumder, et al. (1956) reported that the virulence was lost on artificial culturing, but regained when passed through susceptible hosts.

Steinhaus (1959 a) observed that,when a maxture of Serratia marcescens and B. thuringiensis was fed to Galleria mellonella, the former inhibited the development of the latter.

IV. Factors Affecting Infection and Virulence.

a. Environmental facoors:

It is widely recognized that temperature and humidity are the principal factors that affect the activity of entomogenous microorganisms.

Husz (1929) reported that the spores remain alive even when the atmospheric temperature was below $0^{\circ}C$ for

long periods; however 30°C, was found to be more favourable to rapid development and quick pathogenic effect. Angus (1953) and Steinhaus (1954) pointed out that the bacterium possesses its maximum virulence after sporulation which in turn was observed to be maximum at a range of 35 to 37°C. McConnel and Cutcomp (1954) working on european corn borer larvae obtained a positive correlation between temperature within a range of 20-35°C. and the rapidity of infection. Steinhaus (1959) observed that very high or low temperatures and relative humidities had little effect on virulence. Ignoffo (1962 b) in his extensive studies with Pectinophora gossypiella obtained a direct relationship of the progression of mortality in larvae with the temperature level at which they were maintained. The relative humidity was not found to be an influencing factor. The optimum temperature on the basis of LT 50, was 40.1° C. Smirnoff (1963) studied the effect of temperature in detail. He found that the incubation period and the amount of mortality vary with temperature.

Kushner and Harvey, quoted by Cameron (1963), found that at certain times of the year the foliage of certain trees contain a component that inhibits the germination of B. thuringiensis in the insect gut, thus affording protection against infection.

b. Culture medium:

The medium in which the organism is grown may influence the virulence of the pathogen. Husz (1929) observed appreciable difference in the virulence of the bacterium grown in cultures of different pH concentrations, those having pH 7 or 7.2 imparting more virulence. Metalnikov and Chorine (1929 b) reported that older cultures were more virulent than young ones. Steinhaus (1951) observed that spore production was more in solid medium than that in liquid medium. He also noted that addition of 1% dextrose to the medium increased the rate of spore formation while the addition of 2% proteosepeptone or 5% sodium chloride retarded it.

V. Feeding Habits of the Host in Relation to Mortality.

The bacterium acts as a stomach poison and its effects are more marked on chewing insects. As early as in 1927, Mattes observed that larvae of Ephestia kuhniella, if protected by web, are not affected by the spores. De and Konar (1956) attributed the low mortality in Trogoderma granarium to the internal feeding habit of the larvae. Steinhaus (1957 a), Burgerjon and Klingler (1959) and Jaques and Fox (1960) in their experiments on Pyrausta nubilalis, Torix viridiana and Heliothis zea respectively, suggested that the ineffectiveness of the bacterium in the field is due

to the boring habits of the larvae. Pointing (1962) established a relation between the low effect of the bacterium on Rhyacionia buoliana and itspeculiar habit in rejecting plant tissues while boring into the bud.

Cameron (1963) concluded that the bacterium is not effective against borers and internal feeders.

VI. Persistence and Residual action in the Field.

The residual effect of a pathogenic material is an important factor especially when it is applied against an insect having overlapping generations. No conclusive data is available to show the persistence and residual toxicity of B. thuringiensis.

Chorine (1930) concluded that the bacterium cannot be used as a prophylactic measure as its effectiveness is lost too soon. Weisman (1941) found that its effectiveness was not affected upto eight days, but was lost in sixteen days due to rains. However, Lemoigne et al. (1956) observed that the spores have persisted even after the rains have fallen and in one case they have prevented cabbage worm infestations for longer periods than that with two applications of DDT. Hall and Andres (1959) have reported that the bacterium without sticking agents displayed a residual effect only for 5 to 10 days against cabbage insects, whereas Vankova (1962) found

that the effectiveness of sprays with commercial preparations lasted for two weeks.

Hall (1961) contented that the residual effect is dependent not only upon the host-parasite relationship, but also on the plant growth and environmental factors. Smirnoff (1963) found that the effectiveness of spray diminished with time whether protected or unprotected from rain. He has put forward two possible explanations for this; (1) the spores germinate on the foliage and subsequently die, as suggested by Stephens (1957) in the case of B. cereus and (ii) some volatile substances released by the foliage may inactivate the spores.

VII. Compatibility with Adjuvants, Insecticides and Fungicides.

Although knowledge of the effect of additives in spray or dust formulations of insect pathogens is limited, various standard wetting and sticking agents have been used successfully in combination with microbial materials.

Adjuvants: Weisman (1941) found that effectiveness of the spray of spore materials can be increased by adding a wetting agent, though lime sulphur added as an adhesive has a retarding effect. Tanada (1956) supported this view by showing that addition of Triton B-1956, as a wetting agent to spray, at a concentration of 1:800, had no adverse effect on the virulence.

Angus (1954b) and Kreig (1957) have reported successful mixing of methyl cellulose with bacterial pathogen as an adhesive. Further investigations by Angus (1959) showed that foliage treated with B. thuringiensis and a venyl latex sticker was toxic to silkworm larvae even after considerable weathering and washing. Gunther (1960) was successful in adding molasses as an adhesive. Jaques and Fox (1960) observed that skim milk powder and geon latex increased the effectiveness of the pathogen against Pieris rapae on cabbage, but failed to increase the effect on Operophthera brumata and Alsophila pometaria on apple. Geering and Lloyd (1962) in laboratory trials found that Lovo (amine stearate) spray additives produced significant improvement inthe efficacy, leading to improved susceptibility, reduced evaporation and improved adhesion of deposits. Cage tests using Pieris brassicae also showed a four-fold increase in dosage efficiency.

Pesticides: B. thuringiensis has been combined with some of the recent potent insecticides, fungicides and antibiotics.

Martouret (1959) reported that Copper oxychloride and Zinc dithiocarbamate are compatible with B.thuringiensis. Genung (1960) mixed Toxaphene with the pathogen and applied against Trichoplusia ni , but the combination was not significantly better than Bacillus or Toxaphene alone.

McEwen et al. (1960) studied the compatibility of B. thuringiensis with Parathion, Sevin, Demeton, TEPP, Trithion, Meneb, Captan, Cocs and Chloranil. Of these chloranil alone had some adverse effects.

Toumanoff and Lapied (1954) observed that B. thuringiensis showed varied reactions to antibiotics. Tanada (1959) pointed out that Penecillin is innocuous, while streptomycin affects most of the entomogenous bacteria.

VIII. Methods of Infection and Transmission.

In nature the pathogenic bacteria infect their hosts mainly through the oral route and to a lesser extent through congenital transmission. Transmission may also occur through sting of parasites, bites of predators or through cannibalism. There is little evidence for the infection through respiratory route in insects though it is a common method in bacterial pathogens of man.

Natural dispersal occurs through the infected hosts, by the mechanical transportation through bodies of insects and other animals and through wind, rain and streams.

Metalnikov and Metalnikov (1935) in their studies with the wax moth larvae observed that the parasite Diborachys sp. can transmit Bacillus ephestiae (=Bacillus thuringiensis) through its ovipositor. Investigations of

Polivka (1956) have shown that the spread of the disease occurs through insect parasites, predators, other animals and birds.

Majumder *et al.* (1956) obtained some spread of the disease by liberating infected larvae among the population of the lablab pod borer *Adisura atkinsoni*.

Tanada (1961) is of opinion that in oral infection the host acquires the pathogen by cannibalism or by feeding on food contaminated with : (1) the decomposed remains of infected hosts, (2) the faeces from infected host and (3) the infectious microorganisms carried to the food plants by wind, rain, other animals and man.

Although there are increasing reports on the congenital transmission of certain pathogens like viruses and protozoa, only a few bacteria have been reported to be transmitted in this manner. There appears no such incidence in the case of *B. thuringiensis*.

IX. Methods of Application.

The microbial agents are usually applied for insect control in the following four major ways: (1) application alone as a spray, dust or bait, (2) introduction and colonisation into an insect population, (3) application in combination with chemical

insecticides and (4) application co-ordinated with insect parasites, predators and other microorganisms.

Husz (1930) working on Pyrausta nubilalis observed that dusting gave results equal to that of spraying. Field experiments conducted by Hall and Andres (1959) showed that coverage of the material on the plant is extremely important and that this factor may explain the efficacy of dust over spraying.

Dunn (1960) and Briggs (1960) obtained good control of Musca domestica which develop in droppings of animals, by incorporating B. thuringiensis spores into the feed of Steers and Chicken. Similar results were obtained by Burns et al. (1961) by feeding the spores to caged layers in their ration.

The time, number and method of application, as well as the complete coverage of the plant are important factors in increasing the efficiency of microbial control as emphasized by Hall (1961) and Tanada and Reiner(1962). The latter authors observed that a thorough dust application was most effective against Pyrausta nubilalis.

Smirnoff(1963) in his experiments with "Thuricide" found that aqueous suspensions were more effective than oil emulsions against Choristoneura fumiferana.

Regarding the appliances, Tanada (1959) stated that most of the conventional sprayers and dusters, mist blowers and aircraft have been found suitable for field application of the microbial insecticides.

X. Toxicity to Higher animals and Plants.

There has not been any reported case of toxicity of Bacillus thuringiensis to higher animals and the harmlessness has been proved beyond doubt in many instances.

Berliner (1915) found no indication of the bacillus being pathogenic to any vertebrate. Later Steinhaus (1951), by his elaborate toxicological tests, established the harmlessness of the bacterium to warmblooded animals. He pointed out that oral consumption of a culture of the bacillus by a human volunteer produced no untoward result of any kind. The harmlessness of B. thuringiensis to higher animals was further evinced by the works of Lemoigne et al. (1956) , Grison and Milaire (1959) and Fisher and Rosner (1959).

There is an apprehension among some scientists that Bacillus thuringeinsis may mutate to B. anthracis or similar forms which are pathogenic to vertebrates and plants. But Steinhaus (1959 c) considering the

question in detail has expressed the view that the chances of such a change are very little.

The only recorded observation on phytotoxicity seems to be that of Creigton et al. (1961) on Tobacco. In field tests, a wettable powder preparation at 10 lb. per acre, applied by hand to buds, was found to injure tender foliage. At 4 lb. per acre it was less phytotoxic.

XI. Effect on Natural enemies of Insects.

Instances of deleterious effect on insect parasites and predators are rather few. Solaman (1940) reported a case wherein the Hymenopteran parasite Microbracon hebetor on hosts severely attacked by Bacillus thuringiensis became infected and quickly succumbed.

On the contrary the harmlessness of the bacterium on natural enemies of insects has been established in several cases. Bilotti (1956) investigated the effects of the bacterium on the parasites of Pieris brassicae . He observed that the development of Anilastus ebinius and Apanteles glomerata was normal, the only difference being that the larvae of Apanteles left their hosts to pupate somewhat earlier than those in the control. However, he noted considerable mortality among larvae of the Syrphid, Xanthandrus comtus in test plots. Tanada (1956) did not observe any adverse effects on insect parasites and predators of the Lepidopterous pests of cruciferous

plants. According to Rabb (1957) the spores did not affect the adults and colonies of Polistes exclamans, an important natural enemy of hornworms. Helson (1960) reported that the bacterium did not have any harmful effect on the Syrphid and Hymenopterous enemies of cabbage insects. Investigations by Ayyar (1961) revealed the harmlessness of the bacterium to Trichospilus pupivora, Apanteles sp., Microbracon sp., and Scymnus sp. Tamashiro (1960) recorded an interesting observation that bracon parasitised larvae of Corcyra cephalonica were more susceptible to infection by the bacterium.

XII. Honey bees and the Toxicity of the Bacterium.

Reports so far do not show that the bacterium has any adverse effect on honey bees. Studies in this line by Martouret (1959), Ayyar (1961) and Wilson (1962) lead to the conclusion that the bacterium is harmless to the bees.

XIII. Spectrum of Insects Susceptible.

Bacillus thuringiensis by virtue of its great potentialities as a microbial insecticide has been tried against a large number of insects. Large scale applications of the bacterium against the alfalfa caterpillar in California, against Colias lesbia in Argentina and

against cabbage pests in U.S.A. have yielded spectacular results. A list of insects against which the bacterium has been tried is given in Table I.

TABLE I

A list of insect pests whose susceptibility to Bacillus thuringensis has been investigated by various workers.

Insect	Degree of effectiveness.	References
Order Lepidoptera:		
Arctiidae :		
Arctia caja	xxx	Martouret 1959
Estigmene acrea	xx	Hall and Dum 1958
Hypantria cunea	-	Weiser and Veber 1954
	xxx	Vasiljevic 1957,1961
	xxx	Hall and Geoffrey 1962
Pericallia ricini	-	Rao et al. 1962
Utetheisa pulchella	-	Ramamurthy 1963 a
Boarmiidae:		
Betropis crepuscularia	-	Morris 1962.
Crambidae:		
Chilo zonellus	-	Ramamurthy 1963 a
Proceras indicus	xxx	Ramamurthy 1963 a.
Scirpophage nivella	xxx	Ramamurthy 1963 a.
Cryptophasidae:		
Nephantis serinopa	x	Ayyar 1961.

1	2	3
Eupterotidae:		
Eupterote fabia	xx	Sekhar and Gopinath 1962
E. mollifera	-	Rao et al. 1962
Galleridae:		
Galleria mellonella	xxx	Tamashiro 1960
	xxx	Rao et al. 1962
Gelechiidae:		
Gnorimoschema operculella	xx	Toumanoff and Grison 1954
Pectinophora gossypiella	xxx	Metalnikov and Metalnikov 1933, 1935
	xxx	Ignoffo 1962 a
Geometridae:		
Alsophila pometaria	xxx	Jaques and Fox 1960
	xxx	Jaques 1961
	xxx	Quinton & Daone 1962
Melanolophia imitata	xx	Morris 1962
Operophtera brumata	xxx	Weisman 1941
	xxx	Jaques & Fox 1960
	xxx	Jaques 1961
	xxx	Quinton & Doane 1962.
Semiothisa pervolgata	x	Ramamurthy 1963 a
Thamnonoma wauaria	xxx	Isokova 1958
Hyponomeutidae:		
Hyponomeuta sp.	xxx	Wiesman 1941
H. mallinellus	xxx	Isakova 1958, Schwezoa 1959, Gunther 1960, Franz 1961.

1	2	3
Lasiocampidae:		
Dendrolimus sibiricus	xxx	Talalaev 1958, 1959
D. superans	xxx	Talalaev 1962
Malacosoma americana	xxx	Jaques & Fox 1961
M. neustria	xxx	Toumanoff & Grison 1954
	xxx	Van Damme & Vander Lann 1959
	xxx	Vander Laan & Wassink 1962
Lymantridae		
Euproctis lunata	xx	Venkatraman *et al.*1962
Lymantria dispar	xxx	Metalnikov & Chorine 1929 a
	xxx	Cantwell *et al.* 1961
	xxx	Metalnikov 1930
Stilpnotia salcis	xx	Mentalnikov 1930
Lionetiidae:		
Bucculatrix thurberiella	xx	Hall & Dunn 1958
Noctuidae:		
Adisura atkinsoni	xxx	Majumder *et al.* 1956
Alabama argillacea	xxx	Figueiredo *et al.*1960
Heliothis peltigera	xxx	Martouret 1959
H. virescens	-	Rabb *et al.* 1957
	-	Guthrie *et al.*1959
	-	Greighton *et al.*1961
H. zea	xx	Hall & Dunn1958
	-	Jaques & Fox 1960
	xxx	Tanada & Riener 1962

1	2	3
Laphygma exigua	-	Hall & Andres 1959
	x	Hall & Dunn 1958
Plusia chalcites	xx	Helson 1960
P. peponis	xxx	Ayyar 1961
Spodoptera mauritia	xx	Ayyar 1961
Trichoplusia ni	xxx	Tanada 1956
	xx	Hall and Dunn 1958
	xxx	Grigarick & Tanada 1959
	xxx	Hall & Andres 1959
	xxx	Mc Ewen et al.1960
	xxx	Hall et al.1961
	x	Semel 1961
Notodontidae:		
Datana ministra	xxx	Jaques and Fox 1961
Nymphalidae:		
Vanessa urticae	xxx	Metalnikov and Chorine 1929
Olethreutidae:		
Spilonota ocellana	xxx	Jaques & Fox 1961
	xxx	Legner & Oatman 1962
Papilionidae:		
Papilio demoleus	xxx	Ramamurthy 1963 a
Phyllocnistidae:		
Phyllocnistis citrella	xxx	Ramamurthy 1963 a,b.
Pieridae:		
Colias lesbia F.	xxx	Faldini & Pastrana 1952

1	2	3
Colias philodice eurytheme	xxx	Steinhaus 1951
	xxx	Stern et al.1959
	xxx	Hall & Stern 1962.
Pieris brassicae	xxx	Metalnikov & Metalnikov 1935
	xxx	Tamanoff & Grison 1954
	xxx	Bilotti 1956
	xxx	Lemoigne et al.1956
	xxx	Burgerjon 1957
	xxx	Kreig 1957
	xxx	Isakova 1958
	xxx	Martouret 1959
	x	Bohm 1961
	xxx	Lipa 1962
	xxx	Vankova 1962
Pieris rapae	xxx	Tanada 1956
	xxx	Hall and Andres 1959
	xxx	Mc Ewen & Hervey 1959
	xxx	Mc Ewen et al.1960
	xxx	Fox & Jaques 1961
Plutellidae:		
Plutella maculipennis	xxx	Tanada 1956
	xxx	Oka 1957
	xxx	Menn 1960
	xxx	Fox & Jaques 1961

1	2	3
Pterophoridae:		
Platyptilia carduidactyla	xxx	Tanada & Riener 1960
Pyralidae:		
Acleris variana	xxx	Morris 1962
Cnaphalocrocis medinalis	-	Ramamurthy 1963 a
Corcyra cephalonica	xxx	Tanada 1960
	XXX	Venketaraman *et al.*1962
Ephestia cautella	xx	Godavaribai *et al.*1960
E. kuhniella	xxx	Berliner 1915
	xxx	Jacobs 1960
Hellula undalis	xxx	Tanada 1956
Leucinodes orbonalis	-	Ramamurthy 1963 a
Margaronia indica	xxx	Ayyar 1961
Plodia interpunctella	xxx	Kantak 1959
Stomoptervx subsecivella	xxx	Ramamurthy 1963 a,b
Sylepta silicalis	xxx	Figueiredo *et al.*1960
Udea rubigalis	xx	Hall & Dunn 1958
Pyraustidae:		
Pyrausta nubilalis	xxx	Husz 1928,1929,1930
	xxx	Chorine 1929
	xxx	Metalnikov *et al.*1930
	x	Mc Connel & Cutcomp 1954
	xx	Steinhaus 1957
	xxx	Martouret 1959
	xx	Hudon 1962
	-	Eckstein 1934

1	2	3
Saturnidae:		
Actias selene	x	Ramamurthy 1963 a
Sphingidae:		
Protoparce sexta	xxx	Rabb *et al*.1957
	xxx	Guthrie *et al*.1957
	xxx	Creighten 1961
P.quinquemaculata	xxx	Rabb *et all*.1957
Thaumetopoedae:		
Thaumetopoea pityocampa	xxx	Grison and Beguin 1954
	xxx	Martouret 1959
	xxx	Androic 1961
T. Processionea	xxx	Grison & Beguin 1954
T. wilkinsoni	xxx	Moore *et al*.1962
Tortricidae:		
Amorbia assigana	xx	Hall & Dunn 1958
Archips crataegana	xxx	Kadler *et al*.1959
Argyrotaenia mariana	xxx	Jaques & Fox 1961
A. Velutinana	xxx	Mc Ewen *et al*.1960
Carpocapsa pomonella	-	Weisman 1941
	x	Tadic & Vasiljevic 1957
	x	Madsen & Hoyt 1958
	xx	Mc Ewen *et al*.1960
	xx	Jaques 1961
Choristoneura murinana	xxx	Franz 1961
Totrix sp.	xx	Nelson 1960
Zygaenidae:		
Harrisinia brillians	x	Hall 1955

1	2	3
Order Orthoptera:		
Acrididae:		
Melanoplus bivittatus	x	Baird 1958
M.bilituratus	x	Baird 1958
Schistocerca gregaria	x	Venkatraman *et al*.1962
Order Hymenoptera:		
Tenthredinidae:		
Athalia proxima	xxx	Venkataraman *et al*.1962
Order Coleoptera:		
Bostrichidae:		
Rhizopertha dominica	-	Steimbaus & Bell 1953
Curculionidae:		
Calandra granaria	xx	Steinhaus & Bell 1953
C. Oryzae	xx	do ,,
Hypera brunneipennis	xx	Hall & Dunn 1958
	-	Stern 1961
Dermestidae:		
Trogoderma granaria	-	De & Konar 1956
Tenebrionidae:		
Euchocerus cornatus	xx	Shepherd 1924
Tribolium confusum	-	Steinhaus & Bell 1953
Chrysomelidae:		
Altica ambiens	x	Hall & Dunn 1958
Galerucella xanthomelaena	x	Hall & Dunn 1958
Coccinellidae:		
Epilachna ocellata	xxx	Venkataraman *et al*.1962

1	2	3
Scarabaeidae:		
Holotrichia conferta	xxx	Sekhar and Venkataramiah 1963
Oryctes rhinoceros	-	Cumber 1957
Order Diptera:		
Muscidae:		
Musca domestica	xxx	Hall et al.1959
	xxx	Figueiredo et al.1960
	xxx	Briggs 1960
	xxx	Dunn 1960
	xxx	Menn 1960

The effectiveness is rated as

— = none, x = low, xx = moderate, xxx = high.

XIV. Host Susceptibility and Development of Resistance.

The widespread development of resistance in insects to chemical insecticides has caused some concern that similar problems of resistance to entomogenous micro-organisms also may appear in due course. However, there is no positive evidence todate that insects have become absolutely resistant to infection from applied bacterial pathogens or their by-products.

The phenomenon, that young larvae are most susceptible than elder larvae to most diseases, has been found to

hold good in the case of Bacillus thuringiensis also. Investigations of McConnel and Cutcomp (1954) with Pyrausta nubilalis, Vasiljevic (1957) with Hyphantria cunea and Ayyar (1961) with Nephantis serinopa and Spodoptera mauritia have yielded results in this line.

The susceptibility of certain insects to B. thuringiensis and its close relatives is apparently associated with the pH of the midgut. Angus (1954 a,1956b) from his critical studies on the toxicity of the crystal, found that the susceptible insects had a midgut pH of 9.0 to 10.5

While most explanations of host resistance have been based on selection, the possibility of the occurrence of an immunity must not be overlooked. Metalnikov and Chorine (1929 b) showed that Pyrausta nubilalis can be easily immunized against moderately virulent bacteria, but not against more virulent ones as B. thuringiensis.

Briggs (1956) demonstrated that vaccination of some Lepidopterous larvae resulted in the formation of a heat stable antibacterial principle and it conferred an increased tolerance to pathogenic bacteria, but he failed to duplicate the result by feeding the inoculum. Stephens (1962 a,b) persued this type of investigations using Galleria mellonella and was able to isolate the factor.

XV. Dosages to Control Insects.

In applied insect pathology the goal of the entomologist is to determine the minimum dosage at which a pathogen will give effective control of a particular insect pest. Early in 1956 Lemoigne et al. observed that a concentration of 200 million spores per ml. applied at the rate of 126 gallons per acre gave complete kill of Pieris brassicae.

Tanada (1956) reported that concentrations of 0.25 gm., o.5, gm., and 1 to 2 gm. spores per gallon of water were required for effective control of Pieris rapae, Plutella maculipennis and Trichonlusia ni respectively.

Oka (1957) obtained 95% control of Plutella maculipennis with a concentration of 2,500 million spores per cc., while Grison (1957) found that a concentration of 200 million spores per cc. gave effective control of caterpillar pests of cabbage.

Hall and Andres (1959) from their critical studies concluded that a minimum dose of 25 x 10^{12} spores per acre was required to give 80% kill of Trichoplusia ni and Pieris rapae . Mc Ewen and Harvey (1959) used a commercial preparation containing 100,000 million spores per gram at a dosage of 0.3 lb.per acre and found effective against Pieris rapae.

Kantack (1959) observed that 12.5 million spores per gram of grain was effective against Plodia interpunctella.

Stern et al. (1959) got excellent control of Colias eurytheme by using a preparation containing 40,000 million spores per gram applied at 1.8 oz.per acre.

Hall (1961) stated that for use in initial field tests of the sporeforming crystalbearing bacteria a series of graduated dosages up to a level of 4.5×10^{13} spores per acre (that is up to approximately 1 lb. of material of a concentration of 100 billion spores per gram or the equivalent toxicity per acre) applied as a dust or spray might be suitable.

Hudon (1962) observed that 2 to 4 lb. per acre of a material containing 25×10^9 spores per gram was necessary for control of Pyrausta nubilalis. Hull and Geoffrey (1962) found that a suspension of 75 million spores per 100 cc. is an effective dosage against ailanthus webworm.

Quinton and Doane (1962) reported that 4 lb. of a material containing 75×10^9 spores per gram was effective against Alsophila pometaria.

Smirnoff (1963) obtained best results against Choristoneura fumiferana when 50 grams of "Thuricide"

(containing 30 x 10^9 spores per gram) per gallon of water was applied at the rate of 10 gallons per acre. Ramamurthy (1963 b) obtained good control of groundnut leaf folder Stomopteryx subsecivella with a spore suspension containing 400 million spores per millilitre.

Dosage-mortality analyses in the case of crystalliferous bacteria are complicated by the action of the toxic crystal. Several workers have reported variability in effectiveness as the spore concentration varies . Only very few attempts have been made to determine quantatively the host susceptibility. McConnel and Cutcomp (1954) for example, worked out the median lethal dose for Pyrausta nubilalis as 50,000 spores per cc. Baird (1958) worked out the LD_{50} for Melanoplus bilituralis as 0.002 mg. (5 x 10^9 spores per gm) and Menn (1960) calculated LD_{50} for Plutella maculipennis as 1.1 x 10^8 spores per 100 cc. The LD_{50} and LT_{50} of the pink boll worm larvae have been worked out by Ignoffo (1962 a). LD_{50} for long and short cycle larvae were 49.7 and 49.9 spores per larva ie 1.9 spores per mg and 2.1 spores per mg. body weight respectively.

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MATERIALS AND METHODS

MATERIALS AND METHODS

A. MATERIALS.

(a) Bacterial spores used.

A wettable powder preparation of spores of *Bacillus thuringiensis* Berl. known under the commercial name 'Larvatrol W.P. 25 x 10^9' containing 25 x 10^9 viable spores per gram was used in the present investigations. This was obtained from the Agricultural products Company California, U.S.A.

(b) Spraying tower: (Plate 1)

A spray settling tower constructed on the principles of ' Potters tower' was used for the spraying.

(c) Glass wares.

These consisted of specimen tubes (7.5 cm x 2.5 cm), glass vials, conical flasks, pippette 1 cc., 2 cc. and 5 cc. capacity, Petridishes (9.5 cm.diameter), measuring cylinder 100 cc. capacity, glass rod, and beakers 50 cc., 100 cc. and 250 cc. capacity.

(d) Rearing equipments.

Rearing equipments used were Hurricane chimneys 15 cm. x 5 cm., glass jars (18 cm. x 10 cm. x 27 cm.) camel hair brushes etc.

(e) Test insects used.

Caterpillars of twentysix different species of moths were used for the experiments and they are listed below:

List of Test Insects.

Sl.No.	Name	Family	Host plant
1.	Euchromia polymena L.	Amatidae	Sweet potato
2.	Diacrisia obliqua W.	Arctiidae	Cowpea
3.	Pericallia ricini F.	,,	Banana
4.	Nephantis serinopa M.	Cryptopasidae	Coconut
5.	Eupterote mollifera W.	Eupterotidae	Moringa
6.	Argina cribraria C.	Hypsidae	Sunnhemp
7.	Euproctis fraterna M	Lymantridae	Castor
8.	Psalis securis H.	,,	Paddy
9.	Achoea janata L	Noctuidae	Castor
10.	Orthaga exvinacea M.	,,	Mango
11.	Phytometra peponis F.	,,	Snakegourd
12.	Polyorycta dimidialis S	,,	Cowpea
13.	Prodenia litura F.	,,	Bhindi
14.	Spodoptera mauritia B.	,,	Paddy
15.	Melanitis ismene C	Nymphalidae	,,
16.	Papilio demoleus L.	Papilionidae	Citrus
17.	Cnaphalocrocis medinalis G.	Pyralidae	Paddy
18.	Dichocrocis punctiferalis G.	,,	Castor
19.	Glyphodes marginata H.	,,	Tabernae montana
20.	Hymenia fascialis C.	,,	Amaranthus
21.	Margaronia indica S.	,,	Bittergourd
22.	Nacoleia vulgalis G.	,,	Greengram
23.	Psara bipunctalis F.	,,	Amaranthus
24.	Sylepta derogata F.	,,	Bhindi
25.	Nymphula depunctalis G.	Pyraustidae	Paddy
26.	Pyrausta Phoenicealis H.	,,	Coleus sp.

B. METHODS.

(a) Conditioning of the test caterpillars.

Healthy and active caterpillars of uniform size were used for the experiments. These caterpillars were starved for 24 hours before being used. Starving of the caterpillars was considered to ensure uniform feeding by the individual caterpillars when they were subsequently supplied with food materials sprayed with the spore suspension of B. thuringiensis and thus minimise variation due to differences in the amount of spores consumed.

(b) Preparation of spore suspensions.

Five different concentrations were tried in each experiment. In each case a suspension containing the highest concentration was prepared which served as a stock suspension from which the lower concentrations were obtained by dilution with sterile distilled water. Following are the details of an example.

1 gm. 'Larvatrol' __ 100 cc. water = 25×10^9 spores per 100 cc. - E

10 cc. E + 10 cc. water = 12.5×10^9 spores/100 cc. - D

10 cc. E + 30 cc. water = 6.25×10^9 ,, - C

10 cc. E + 70 cc. water = 3.125×10^9 ,, - B

10 cc. E + 150 cc. water = 1.5625×10^9 ,, - A

(c) Applicationof spore suspension to host materials.

Young leaves of the host plant concerned were selected, washed well with tap water and air dried.

Both the sides of the leaves were then sprayed with the measured spore suspension under a spraying tower. For this the leaf or leaves were placed in a petridish on the platform of the spraying tower and sprayed over wit 1 cc. of the suspension. The spray deposit was then allowed to dry after which the opposite side of the leaves also were sprayed with the suspension in the similar way and allowed to dry.

(d) Feeding the caterpillars with the spore deposits.

The sprayed leaves were transferred to hurricane chimneys. Ten caterpillars selected at random from the stock kept ready for the purpose, were introduced into each of the chmineys using a fine camel hair brush. The chimneys were then closed with muslin. The caterpillars were allowed to feed on the sprayed foliage for 24 hours. Subsequently the surviving larvae were transferred daily to fresh untreated folliage.

(e) Assessment of effects of infection:

The lethal effects caused by the bacterial infection was assessed by observing the mortalities of caterpillars at intervals of 24 hours. Any other effect such as cessation of feeding and discolouration also were observed.

(f) Experimental conditions.

All the experiments were conducted under laboratory conditions of temperature and humidity. Appendix I gives the monthly temperature and humidity for the period of the experiments.

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EXPERIMENTS AND OBSERVATIONS

EXPERIMENTS AND OBSERVATIONS

Preliminary Screening Tests.

With a view to ascertain the overall susceptibility of the common caterpillar pests occurring in Kerala as a first step in planning more objective and precise tests, a series of screening experiments were conducted by feeding *Bacillus thuringiensis* Berl. spores to the insects. Only one concentration of the spore suspension containing 12.5×10^9 spores per 100 cc. was utilised in all cases. The dip method used by Jaques (1961) was followed in these tests. A parallel control was run in each case with caterpillars fed on the food materials dipped in sterile distilled water.

The caterpillars used were collected from the field and starved for 24 hours prior to the experiments. Only healthy caterpillars of uniform size were utilised in all cases. Mortality counts were recorded at intervals of 24 hours. These experiments were conducted during the period between 7-8-1963 and 26-11-1963. Details of the experiments and observations are presented in Table II.

It will be observed from the Table that the susceptibility of different caterpillars is varying. The Bacterium causes cent per cent mortality among

TABLE II

Mortality of different species of caterpillars fed on the host material dipped in 12.5×10^9 spores per 100 cc. suspension of *Bacillus thuringiensis*.

Sl. No.	Insect species	No. of caterpillars used		percentage mortality after					
		Treated	Control	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs
(1)	(2)	(3)				(4)			
1	*Dichocrocis punctiferalis*	25	20	48.0	64.0	72.0	80.0	80.0	88.0
2	*Sylepta derogata*	30	15	53.3	63.3	73.3	86.7	93.3	100.0
3	*Psara bipunctalis*	20	20	15.0	35.0	80.0	85.0	95.0	95.0
4	*Glyphodes marginata*	20	10	70.0	85.0	85.0	100.0	--	--
5	*Psalis securis*	40	10	5.0	50.0	70.0	75.0	80.0	80.0
6	*Melanitis ismene*	10	10	10.0	50.0	80.0	80.0	80.0	--
7	*Orthaga exvinacea*	40	10	10.0	20.0	35.0	55.0	70.0	70.0
8	*Cnaphalocrocis medinalis*	20	20	10.0	20.0	45.0	80.0	80.0	--
9	*Prodenia litura*	30	30	0	0	6.7	6.7	6.7	--
10	*Polyorycta dimidialis*	15	15	0	26.7	53.3	60.0	66.7	--
11	*Pyrausta phoenicealis*	20	15	15.0	80.0	100.0	--	--	--
12	*Margaronia indica*	25	10	60.0	80.0	80.0	88.0	100.0	--

Continued...

Continued.

(1)	(2)	(3)		(4)					
13	Phytometra nononis.	20	10	60.0	70.0	80.0	85.0	85.0	--
14	Bupteroto mollifera	40	10	0	0	0	0	0	0
15	Nephantis serinopa	30	10	0	6.7	6.7	33.3	33.3	33.3
16	Diacrisia obliqua	30	10	6.7	16.7	16.7	20.0	20.0	23.3
17	Nacoleia vulgalis	15	10	6.7	66.7	100.0	--	--	--
18	Papilio demoleus.	15	10	13.3	80.0	93.3	100.0	--	--
19	Hymenia fascialis.	40	10	75.0	75.0	80.0	82.5	100.0	--
20	Argina cribraria	30	10	0	26.7	33.3	40.0	60.0	60.0
21	Achoea janata	20	10	0	60.0	95.0	100.0	--	--
22	Euchromia polymena.	50	20	0	6.0	6.0	42.0	56.0	56.0
23	Spodoptera mauritia.	30	10	6.7	10.0	20.0	33.3	53.3	63.3
24	Nymphula depunctalis	20	10	15.0	20.0	20.0	60.0	80.0	85.0
25	Pericallia ricini	20	10	5.0	5.0	20.0	45.0	45.0	45.0
26	Euproctis fraterna.	30	10	0	0	0	3.3	3.3	3.3

caterpillars of Sylepta derogata, Glyphodes marginata, Pyrausta phoenicealis, Margaronia indica, Nacoleia vulgalis, Papilio demoleus, Hymenia fascialis and Achoea janata within 24 to 144 hours. Caterpillars of Neohantis serinopa, Diacrisia obliqua and Pericallia ricini record only less than 50% mortality. The organism causes practically negligible amount of mortality among caterpillars of Prodenia litura and Euproctis fraterna and no mortality in Eupterote mollifera.

Typical symptoms of bacterial infection were noticed in all cases of death except in Prodenia litura and Euproctis fraterna.

Main Experiments.

A series of sixteen experiments were conducted to assess the effect of different graded concentrations of spores of Bacillus thuringiensis Berl. on different species of caterpillar pests commonly occurring in Kerala. The effect was assessed in terms of mortality caused to the caterpillars as a result of the bacterial infections. The details and results of experiments are given in the following pages.

Experiment. I.

Dosage mortality relationship between Bacillus thuringiensis spores and caterpillars of Glyphodes marginata

Experimental Details:

Treatments:

A. 1.5625×10^9 Viable spores per 100 cc.
B. 3.125×10^9 ,,
C. 6.25×10^9 ,,
D. 12.5×10^9 ,,
E. 25.0×10^9 ,,
Control: Distilled water only

Replications: 3 each

Number of caterpillars in each replication: 10

Test insect: Caterpillars of the fourth instar were used for the experiment. These were reared from the egg masses collected from Tabernae montana plants and conditioned as detailed under materials and methods.

Period during which the experiment was conducted : 5-3-1964 to 11-3-1964.

Temperature during the period: Mnm. 78° - 82° F.
Mxm. 82° - 86° F.

Relative humidity during the period: 63% - 90%

Procedure: The caterpillars were fed on the spores sprayed on the leaves as described earlier under materials and methods.

T A B L E III

Percentage mortality of G.marginata caterpillars fed on Taberme montana leaves sprayed with different concentrations of spore suspensions of B. thuringiensis.

Concentrations of spore suspension (Spores per 100 cc)	Log.Concentration.	* Per cent mortality after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
A. 1.5625×10^9	9.194	13.3	20.00	20.00	26.7	26.7
B. 3.125×10^9	9.495	40.0	50.00	53.00	53.3	56.7
C. 6.25×10^9	9.796	60.0	80.00	83.00	86.7	90.0
D. 12.5×10^9	10.097	90.0	100.00	100.00	100.0	100.0
E. 25.0×10^9	10.398	100.0	100.00	100.00	100.0	100.0
Control		0	0	0	0	0

* Treatment mortalities corrected using Abbott's formula.

Results:

Results of the experiment are presented in Table III which gives the percent mortalities in caterpillars caused by different concentrations of Bacillus thuringiensis spores at varying periods after ingestion. The same results are depicted in Fig. (1). It will be observed that the highest concentration (E) causes cent per cent mortality among the caterpillars within a period of one day after ingestion. The highest two concentrations viz. 12.5×10^9 and 25×10^9 appear to be more or less equitoxic to the caterpillars. In other cases mortality increases with increase in concentration and even after five days none of these concentrations produce complete mortality.

Symptoms of infection: Infected caterpillars developed a brown colour at the anterior 1/3 of the body. They stopped feeding and remained motionless on the leaves. Diarrhoea and vomiting of ingested food were also evident. Soon after death the cadavers turned dark brown and dried up without liquifying, giving an offensive smell.

Experiment. 2

Dosage mortality relationship between B. thuringiensis spores and caterpillars of Argina cribraria.

Experimental details:

Test insect: Fourth instar caterpillars were used for the experiment. They were reared from the

Fig. (1) Graph showing dosage mortality realtionship between B. thuringiensis spores and caterpillars of Glyphodes marginata.

Fig. (2) Graph showing dosage mortality relationship between B. thuringiensis spores and caterpillars of Argina cribraria.

Fig. (3) Graph showing dosage mortality relationship betweenB. thuringiensis spores and caterpillars of Euchromia polymena.

Fig. (4) Graph showing dosage mortality relationship between B. thuringiensis spores and caterpillars of Sylepta derogata.

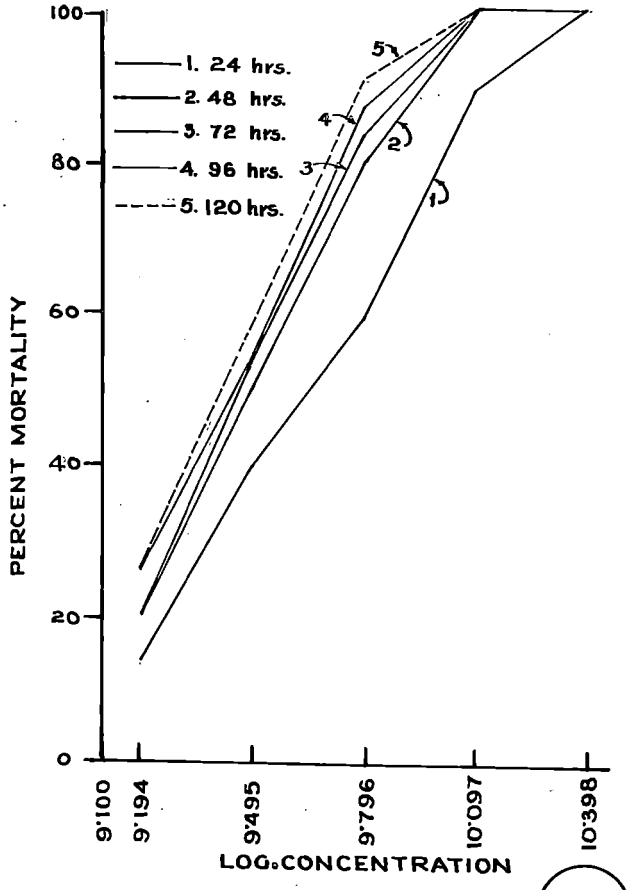

FIG. 1

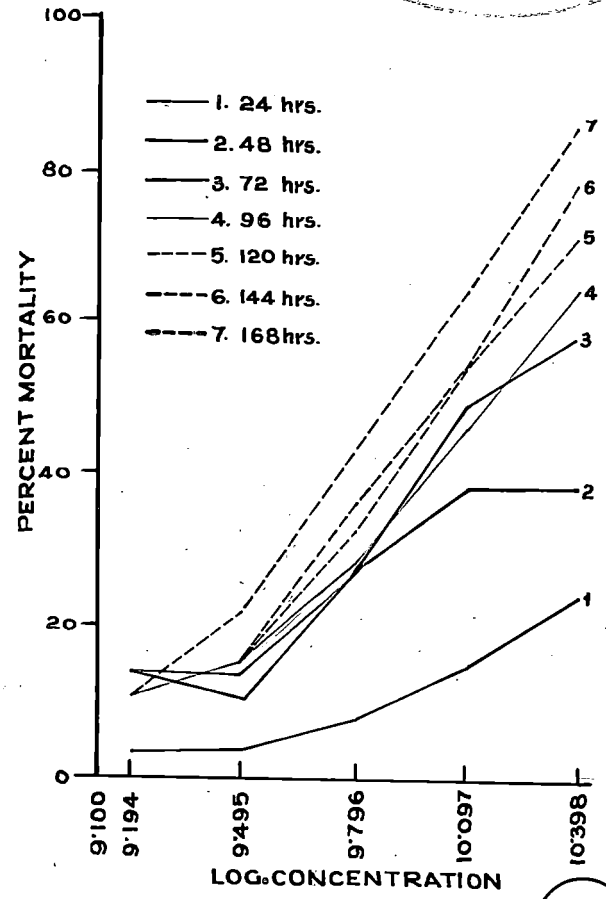

FIG. 2

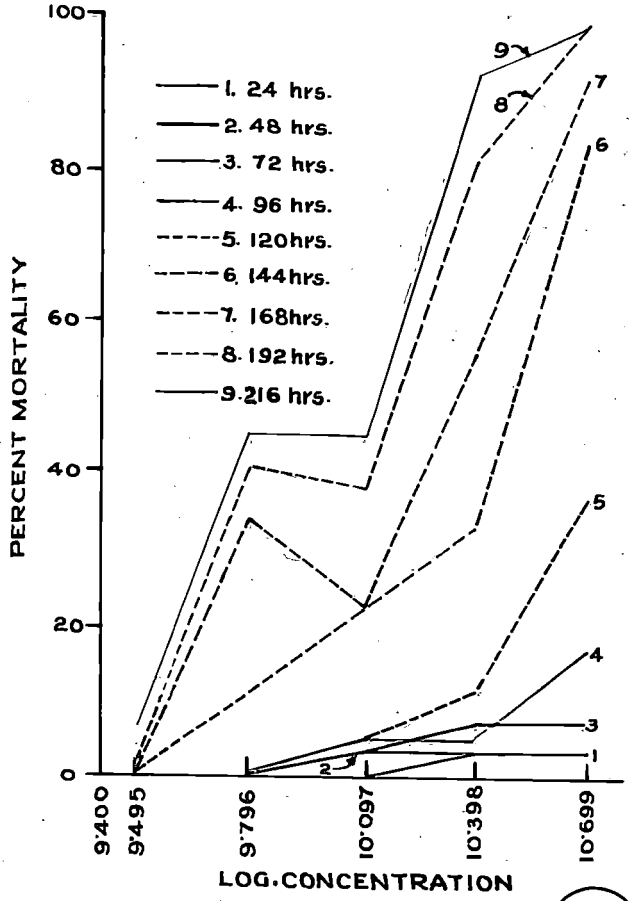

FIG. 3

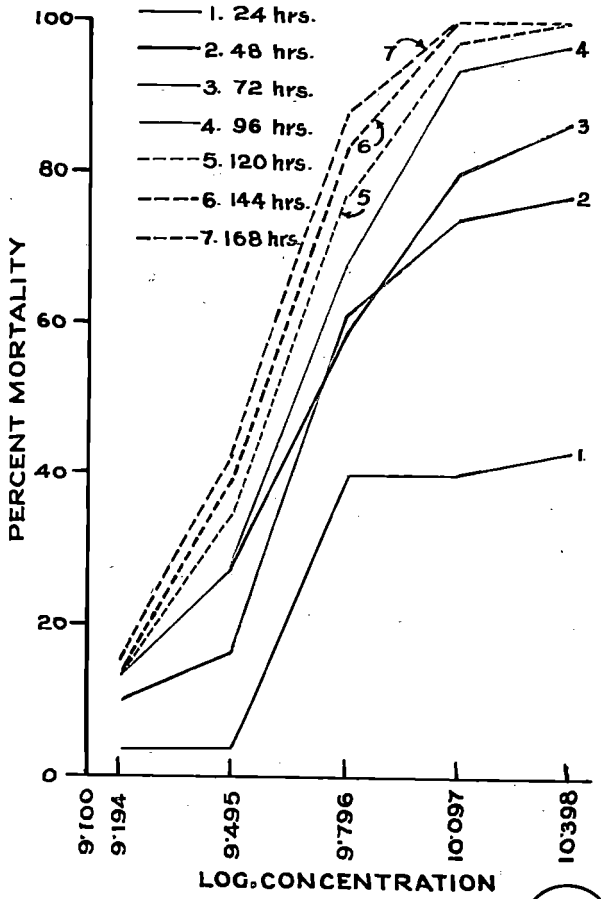

FIG. 4

small caterpillars collected from sunhemp crop in the farm, and conditioned in the laboratory.

Period during which the experiment was conducted:	29-10-1963 to 5-11-1963.
Temperature during the period:	Mnm. 76° - 80° F Mxm. 78° - 84° F
Relative humidity during the period:	62% - 92%

Other details same as in Experiment I.

Results:

Results of the experiment are given in Table IV and represented in Fig. (2). It is observed that even after seven days, none of the concentrations under test causes complete mortality of the caterpillars. Further, only the highest two concentrations cause more than 50% mortality. The increase in mortality with lapse of time is graduâl and it is more or less static in the two lower concentrations (A) and (B).

Symptoms of infection: Cessation of feeding was the most conspicuous symptom in infected caterpillars. Infected caterpillars were found to be lethargic and became moribund six hours before death. The dead caterpillars

T A B L E IV

Percentage mortality of A. cribraria caterpillars fed on sunnhemp foliage sprayed with different concentrations of spore suspension of B.thuringiensis

Concentrations of spore suspension (Spores per 100 cc.)	* per cent mortality after						
	24 hrs.	48 hrs	72 hrs.	96 hrs.	120 hrs	144 hrs	168 hrs.
A. 1.5625×10^9	3.5	13.9	13.9	10.9	10.9	10.9	10.9
B. 3.125×10^9	3.5	10.3	13.9	14.3	14.3	14.3	21.4
C. 6.25×10^9	6.9	27.6	27.6	28.5	32.2	35.7	42.9
D. 12.5×10^9	13.9	38.0	48.3	46.4	53.6	53.6	64.3
E. 25.0×10^9	24.2	38.0	58.6	64.3	71.4	78.6	85.7
Control	3.3	3.3	3.3	6.7	6.7	6.7	6.7

* Corrected as in Experiment 1.

became dark brown in colour and dried up within 24 hours. A foul smell was also evident.

Experiment. 3

Dosage mortality relationship between Bacillus thuringiensis spores and caterpillars of Euchromia polymena.

Experimental details:

Treatments:

A. 3.125×10^9 Viable spores per 100 cc.
B. 6.25×10^9 "
C. 12.5×10^9 "
D. 25.0×10^9 "
E. 50.0×10^9 "
Control: Distilled water only.

Test insect: Caterpillars of the third instar were used for the experiment. They were reared in the laboratory from egg masses collected from sweet potato plants in the farm.

Period during which the experiment was conducted:	7-11-1953 to 16-11-1963
Temperature during the period:	Mnm. 78^0 - 82^0 F Mxm. 82^0 - 86^0 F
Relative humidity during the period:	57% - 88%

Rest of the details are as in Experiment I.

Results:

Results of the experiment are presented in Table V and depicted in Fig.(3). It is evident that the bacterium has very little and that too slow effect on the caterpillars. Only the highest concentration could bring about 100% mortality and that too after 8 days. Mortality in the initial periods is very low. However, a sharp increase in mortality is evident after 6 days of feeding. Percentage kill in the three lower concentrations A,B & C does not reach even 50%. Increase in mortality is co-extensive with increase in spore concentration.

Symptoms of infection: Infected caterpillars developed a uniform black colour and showed a reduction in feeding which was very marked after 5 days. In the initial stages they showed restlessness and later became lethargic, fell down to the bottom and died; symptoms of diarrhoea also were evident. Increased mortality on the sixth day coincided with the occurrence of moulting. The cadavers turned dark brown, flascid, lost their normal shape, and disintegrated under light pressure, giving an offensive smell.

Experiment. 4.

Dosage mortality relationship between B. thuringiensis spores and caterpillars of Sylepta derogata.

Test insect: Caterpillars of the third instar were used for the experiment. These were reared from small caterpillars collected from bhindi crop

T A B L E V

Percentage mortality of E. polymena caterpillars fed on sweet potato leaves sprayed with different grades of spore concentrations of B. thuringiensis

Concentrations of spore suspension (spores per 100 cc.)	log. concentration.	* Per cent mortality after								
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.	192 hrs	216 hrs.
A. 3.125×10^{0}	9.495	0	0	0	0	0	0	0	0	3.7
B. 6.25×10^{9}	9.796	0	0	0	0	0	11.1	33.3	40.8	44.4
C. 12.5×10^{9}	10.097	0	3.3	3.3	3.5	3.7	22.2	22.2	37.0	44.4
D. 25.0×10^{9}	10.398	3.3	3.3	6.7	3.5	11.1	33.3	55.6	81.4	92.6
E. 50.0×10^{9}	10.699	3.3	3.3	6.7	17.8	37.0	85.2	92.6	100.0	100.0
Control	0	0	0	0	6.7	10.0	10.0	10.0	10.0	10.0

* Corrected as in Experiment 1.

in the farm and conditioned in the laboratoty.

Period during which the experiment was conducted:	12-11-1963 to 19-11-1963.
Temperature during the period	Mnm. 78° - 82° F Mxm. 80° - 86° F
Relative humidity during the period:	62% - 88%

Rest of the details as in Experiment 1.

Results:

Results of the experiment are presented in Table VI which gives the per cent mortality caused by ingestion of different concentrations of Bacillus spores at varying periods after feeding. The same results are depicted in Fig. (4). It can be observed that the highest two concentrations of spores cause cent percent mortality 5 days after feeding. In general the mortality is more or less proportionate to the spore concentration. Increase in mortality with time appears to be gradual.

Symptoms of infection: A sudden stoppage of feeding was noticed in infected caterpillars. They became lethargic and did not web or roll the leaves. They developed dark brown spots on the ventral side of the first three abdominal

s

T A B L E VI

Percentage mortality of S. derogata caterpillars fed on bhindi leaves sprayed with different grades of spore concentrations of B. thuringiensis.

Concentrations of spore suspension (spores per 100 cc.)	* Percentage mortality after						
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.
A. 1.5625×10^9	3.3	10.0	13.9	13.9	13.9	13.9	14.3
B. 3.125×10^9	3.3	16.7	27.6	27.6	31.0	34.5	39.2
C. 6.25×10^9	40,0	60.0	58.6	65.6	75.9	82.7	85.7
D. 12.5×10^9	40.0	73.3	72.3	93.1	96.6	100.0	100.0
E. 25.0×10^9	45.3	76.7	86.2	96.6	100.0	100.0	100.0
Control	0	0	3.3	3.3	3.3	3.3	6.7

* Corrected as in Experiment 1.

segments. Within a few hours after death the whole body turned dark brown or almost black. The cadavers lost their normal shape, became soft and disintegrated under light pressure, giving an offensive smell.

Experiment.5

Dosage mortality relationship between B. thuringiensis spores and caterpillars of Orthaga exvinacea.

Experimental details.

Test insect: Caterpillars of fourth instar were used for the experiment. These were reared from small caterpillars collected from the mango orchard and conditioned in the laboratory.

Period during which the experiment was conducted:	14-11-1963 to 24-11-1963
Temperature during the period:	Mnm. 78° - 82° F Mxm. 80° - 86° F
Relative humidity during the period:	64% - 89%

Rest of the details are as in Experiment 1

Results:

Results of the experiment are presented in Table VII, which gives the per cent mortalities caused by ingestion of Bacillus spores after varying periods of

T A B L E VII

Percentage mortality of *O. exvinacea* caterpillars fed on mango leaves sprayed with different grades of spore concentrations of *B.thuringiensis*

Concentrations of spore suspension (spores per 100 cc.)	* per cent mortality after									
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.	192	216	240
A. 1.5625×10^9	0	3.3	10.0	10.0	16.7	20.0	20.0	26.7	26.7	26.7
B. 3.125×10^9	3.3	10.0	10.0	13.3	16.7	20.0	30.0	30.0	30.0	30.0
C. 6.25×10^9	10.0	13.3	23.3	30.0	33.3.	36.7	40.0	50.0	56.7	56.7
D. 12.5×10^9	16.7	23.3	26.7	36.7	43.3	43.3	56.7	66.7	73.3	76.7
E. 25.0×10^9	16.7	26.7	33.3	36.7	43.3	53.3	63.3	80.0	90.0	93.3
Control	0	0	0	0	0	0	0	0	0	0

* Corrected as in Experiment 1.

feedings. The same results are represented in Fig.(5) . It will be observed that even after 10 days, none of the spore concentrations cause cent per cent mortality. It is also evident that increase in mortality is not co-extensive with increase in spore concentration. When the concentration of spore suspension increases 16 fold, the mortality increases hardly 4 fold. The lowest two spore concentrations ((A) and (B)) do not cause 50% mortality even after 10 days, there being no further mortality with these concentrations after 6 days. A sharp increase in mortality is evidenced in the higher concentrations after seven days.

Symptoms of infection: A gradual reduction in the rate of feeding was observed from second day onwards. Infected caterpillars became lethargic and sluggish in movement. A reduction in size also was noticed. They usually fell down to the bottom and died. The cadavers turned dark brown and shortened in length.

Experiment 6.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of *Nymphula depunctalis*

Experimental details:

Test insect: Caterpillars of second instar were used for the experiment. These were reared from very small caterpillars collected from paddy field.

Fig. (5) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Orthaga exvinacea.

Fig. (6) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Nymphula depunctalis.

Fig. (7) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Cnaphalocrocis medinalis.

Fig. (8) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Nephantis serinopa.

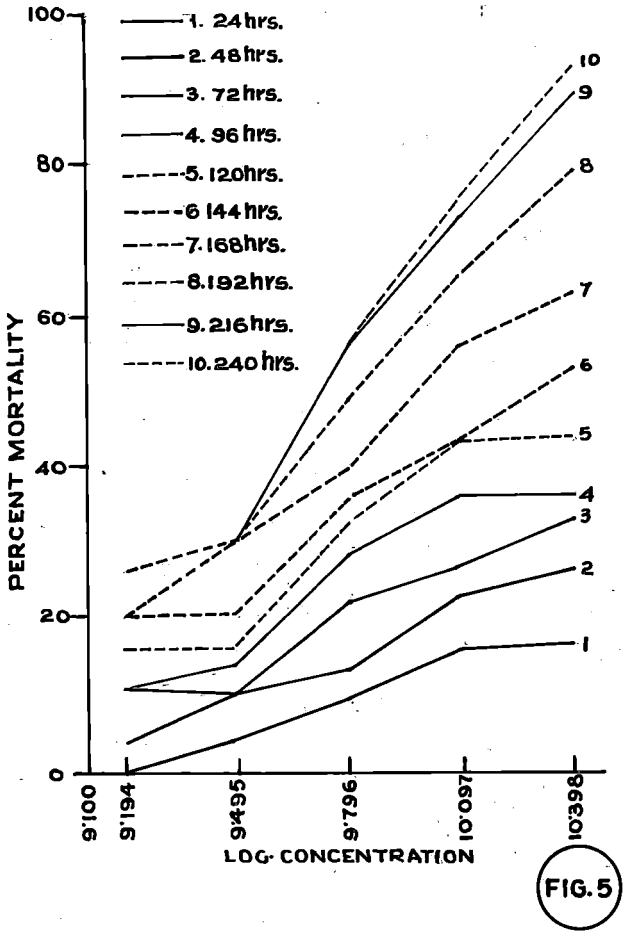

FIG. 5

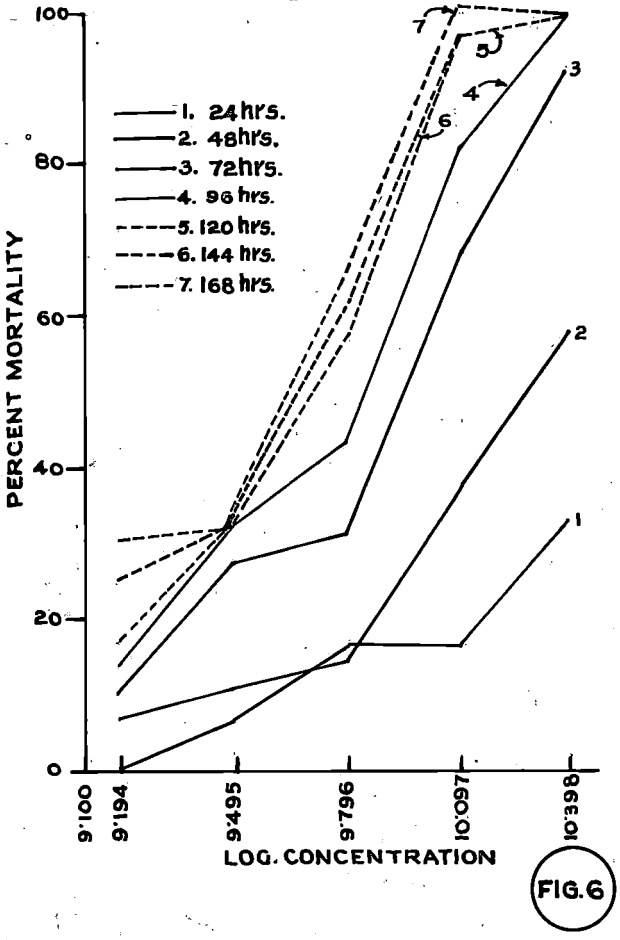

FIG. 6

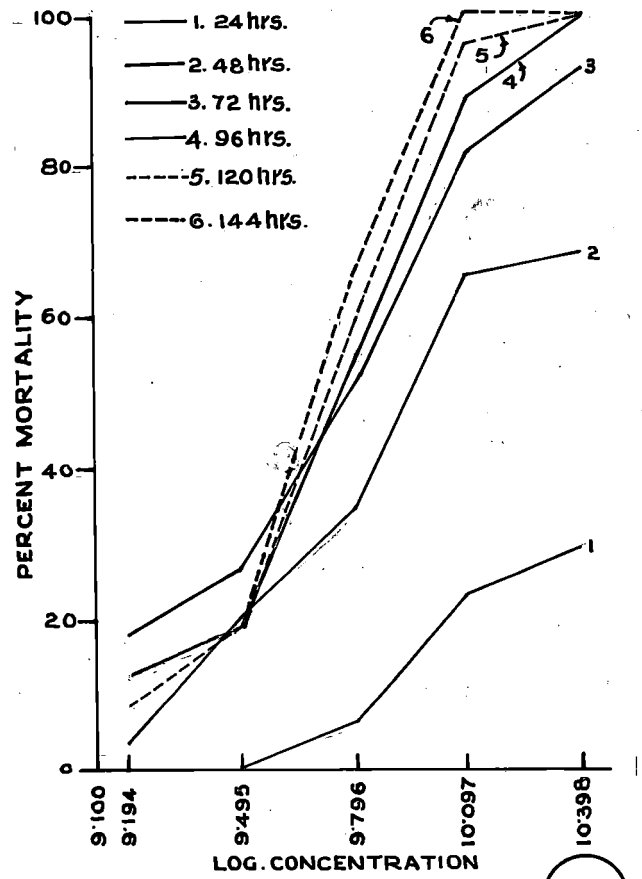

FIG. 7

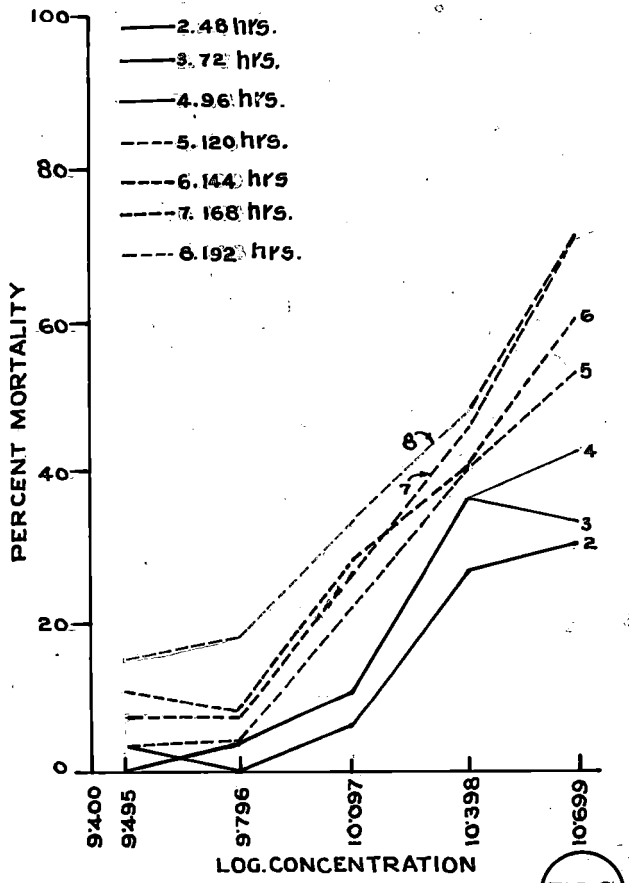

FIG. 8

Th 38

Period during which the experiment was conducted:	18-11-1963 to 25-11-1963.
Temperature during the period:	Mnm. 78° - 80° F Mxm. 80° - 84° F
Relative humidity during the period	64% - 86%

Procedure: Two weeks old paddy seedlings were transplanted in flower pots - three in each pot - and the spore suspension sprayed uniformly on the plants using a hand atmoizer. The control was sprayed with sterile distilled water alone. The caterpillars were then put on to the plants and the plants enclosed in hurricane chimneys. Care was taken to see that there was standing water in the pots. Each pot served as one replication in one treatment. After 24 hours the surviving caterpillars were removed to fresh plants in pots.

Rest of the details as in Experiment I.

Results:

Results of the experiment are presented in Table VIII. The same results are represented in Fig.(6). It is obvious from the Table that only the highest two concentrations(E)and(D)cause cent percent mortality. The lowest two concentrations (A) and (B) do not cause even 50% mortality even after 7 days. On the whole it appears that increase in mortality is directly proportional to the

T A B L E VIII

Percentage mortality of N.depunctalis caterpillars fed on paddy leaves sprayed with different grades of spore concentrations of B.thuringiensis.

Concentrations of spore suspension (spores per 100 cc)	* per cent mortality after						
	24 hrs.	48 hrs	72 hrs.	96 hrs.	120 hrs	144 hrs	168 hrs.
A. 1.5625×10^9	0	6.9	10.3	14.3	17.8	25.0	25.0
B. 3.125×10^9	6.7	10.3	27.6	32.2	32.2	32.2	32.2
C. 6.25×10^9	16.7	13.9	31.0	42.9	57.1	60.7	64.3
D. 12.5×10^9	16.7	37.5	69.0	82.1	96.5	96.5	100.0
E. 25.0×10^9	33.3	58.6	93.1	100.0	100.0	100.0	100.0
Control	0	3.3	3.3	6.7	6.7	6.7	6.7

* Corrected as in Experiment 1.

to the increase in concentration. A sudden increase in mortality is evident two days after ingestion.

Symptoms of infection: Initial symptoms were the appearance of black spots on the thoratic segments and loss of appetite. Infected caterpillars fed very little after 48 hours. They turned dark brown, became moribund, fell down and died within the leaf cases. The dead bodies became soft and disintegrated under light pressure.

Experiment 7.

Dosage mortality relationship between B.thuringiensis spores and caterpillars of Euproctis fraterna.

Expermental details:

Rest insect: Caterpillars of third instar were used for the experiment. These were reared from egg masses collected from castor plants.

Period during which the experiment was conducted:		29-11-1963 to 9-12-1963.
Temperature during the period:	Mnm.	76° - 84° F
	Mxm.	81° - 86° F
Relative humidity during the period:		64% to 93%

Rest of the details are as in Experiment 1.

Results:

No mortality was observed in any of the treatments. The caterpillars fed normally and pupated. Adult emergence was also normal.

Experiment 8.

Dosage mortality relationship between B. thuringiensis spores and caterpillars of Cnaphalocrocis medinalis.

Experimental details:

Test insect: Third instar caterpillars were used for the experiment. They were reared on paddy leaves from small caterpillars collected from paddy fields.

Period during which the experiment was conducted.		3/12-1963 to 9-12-1963.
Temperature during the period.	Mnm.	78° - 84° F
	Mxm.	81° - 86° F
Relative humidity during the period		64% - 89%

Procedure: Same as in experiment 7

Rest of the details as in Experiment. 1.

Results:

Results of the experiment are presented in Table IX. The same results are illustrated in Fig.(7). It will be observed that cent per cent mortality is obtained with the highest concentration (E) on the fourth

T A B L E IX

Percentage mortality of C. medinalis caterpillars fed on paddy leaves sprayed with different grades of spore concentrations of B. thuringiensis.

Concentrations of spore suspension (spores per 100 cc.)	* per cent mortality after					
	24 hrs.	48 hrs	72 hrs.	96 hrs.	120 hrs	144 hrs.
A. 1.5625×10^9	0	3.5	17.3	11.5	7.9	7.9
B. 3.125×10^9	0	20.7	27.6	19.3	19.9	19.9
C. 6.25×10^9	6.7	34.5	51.7	53.9	60.0	65.6
D. 12.5×10^9	23.3	65.6	81.7	88.5	96.0	100.0
E. 25.0×10^9	30.0	68.9	93.1	100.0	100.0	100.0
Control	0	3.3	3.3	13.3	16.7	16.7

* Corrected as in Experiment 1.

day and with the next higher concentration on the sixth day. The lowest two concentrations (A) and (B) do not cause 50% mortality after six days. The mortality rates in these two concentrations remain more or less static. The percentage mortality caused by the lowest concentration (A) is not different from that in the control. A sharp increase in mortality is evidenced after 24 hours.

Symptoms of infection: Infected caterpillars developed a dark brown colour on the ventral aspect of the first three abdominal segments. The colour extended anteriorly and posteriorly causing sluggishness. They showed loss of appetite and failed to construct webs. Four hours after death the cadavers turned completely black in colour, shrunk in size and fell down.

Experiment 9.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of *Nephantis serinopa*.

Experimental details:

Test insect: Caterpillars of the third instar were used for the experiments. They were reared from early instar caterpillars collected from coconut plantations in the farm.

Period during which the experiment was conducted:	4-12-1963 to 12-12-1963
Temperature during the period.	Mnm. 78^{0} - 84^{0} F Mxm. 81^{0} - 84^{0} F
Relative humidity during the period	64% - 88%

Treatments: As in Experiment 3

Rest of the details as in Experiment. 1.

Results.

Results of the experiment are presented in Table X. The same resuts are graphically presented in Fig. (8). It is evident from the table that none of the concentrations causes mortality in 24 hours and does not cause cent percent mortaity even after eight days. The lower three concentrations (A, B and C) do not cause even 50% mortality. The increase in mortality due to increase in concentration is fluctuating. However, increase in mortality in proportion to the concentration is evident. Increase in mortality with time is gradual.

Symptoms of infection: Infected caterpillars showed a marked reduction in feeding and construction of galleries. A dark brown colour developed in the middle 1/3 portion of the body which gradually extended to both ends. They became moribund and died. The cadavers turned darker

T A B L E X

Percentage mortality of *N. serinope* caterpillars fed on coconut leaves sprayed with different grades of spore concentrations of *B. thuringiensis*

Concentrations of spore suspension (spores per 100 cc.)	* per cent mortality after							
	24 hrs.	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	192 hrs.
A. 3.125×10^9	0	3.3	0	3.5	3.5	10.9	7.4	14.8
B. 6.25×10^9	0	0	3.5	3.5	3.5	7.1	7.4	18.6
C. 12.5×10^9	0	6.7	10.9	10.9	21.4	28.5	25.9	33.3
D. 25.0×10^9	0	26.7	35.8	35.8	39.2	39.2	44.4	51.9
E. 50.0×10^9	0	30.0	32.2	42.9	56.7	60.7	70.3	70.3
Control	0	0	6.7	6.7	6.7	6.7	10.0	10.0

* Corrected as in Experiment 1.

and soon hardened, causing a shrinking in size.

Experiment 10:

Dosage mortality relationship between spores B. thuringiensis and caterpillars of Prodenia litura.

Experimental details:

Test insect: Caterpillars of the fourth instar were used for the experiment. They were reared from eggs laid by adults on bhindi plants in the laboratory.

Period during which the experiment was conducted:	10-12-1963 to 15-12-1963.
Temperature during the period:	Mnm. 78° - 80° F Mxm. 81° - 84° F
Relative humidity during the period:	63% - 80%

Rest of the details same as in Experiment 1.

Results.

Results of the experiment are presented in Table XI. The data presented in the Table are not corrected with reference to the mortality in the control as in most cases the mortality variation is irregular and does not follow any relationship with the change in concentration. Further, typical symptoms of infection were not evident. Hence it appears that the bacterium is not infective to the insect.

T A B L E XI

Percentage mortality of P. litura caterpillars fed on bhindi leaves sprayed with different grades of spore concentrations of B.thuringiensis

Concentrations of spore suspension (spores per 100 cc)	Per cent mortality after				
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
A. 1.5625×10^9	0	0	6.7	10.0	10.0
B. 3.125×10^9	0	0	0	0	3.3
C. 6.25×10^9	0	0	6.7	6.7	13.3
D. 12.5×10^9	0	0	3.3	6.7	10.0
E. 25.0×10^9	0	0	6.7	6.7	10.0
Control	0	0	0	10.0	13.3

Experiment 11.

Dosage mortality relationship between B. thuringiensis spores and caterpillars of Hymenia fascialis

Experimental details:

Test insect: Second instar caterpillars were used for the experiment. They were reared from very small caterpillars collected from amaranthus crop in the farm.

Period during which the experiment was conducted:	2-1-1964 to 7-1-1964.
Temperature during the period:	Mnm. 78° - 80°F Mxm. 82° - 86°F
Relative humidity during the period:	64% - 89%

Rest of the details same as in Experiment 1.

Results:

Results of the experiment arepresented in Table XII and in Fig.(9) . It is seen that the highest spore concentration under test causes cent percent mortality among the caterpillars in 3 days and next higher in 5 days. All the concentrations except the lowest one (A) causes more than 50% mortality in 48 hours. It is also clear that increase in mortality is not commensurate

T A B L E XII

Percentage mortality of H. fascialis caterpillars fed on Amaranthus leaves sprayed with different grades of spore concentrations of B.thuringiensis

Concentrations of spore suspension (spores per 100 cc.)	* per cent mortality after				
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
A. 1.5625×10^9	20.0	23.3	27.6	32.2	35.6
B. 3.125×10^9	36.7	53.3	55.2	53.6	64.3
C. 6.25×10^9	53.3	86.7	86.2	92.8	92.8
D. 12.5×10^9	63.3	86.7	89.7	92.8	100.0
E. 25.0×10^9	63.3	93.3	100.0	100.0	100.0
Control	0	0	3.3	6.7	6.7

* Corrected as in Experiment 1.

Fig. (9) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Hymenia fascialis.

Fig. (10) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Margaronia indica.

Fig. (11) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Diacrisia obliqua.

Fig. (12) Graph showing dosage mortality relationship between B.thuringiensis spores and caterpillars of Pericallia ricini.

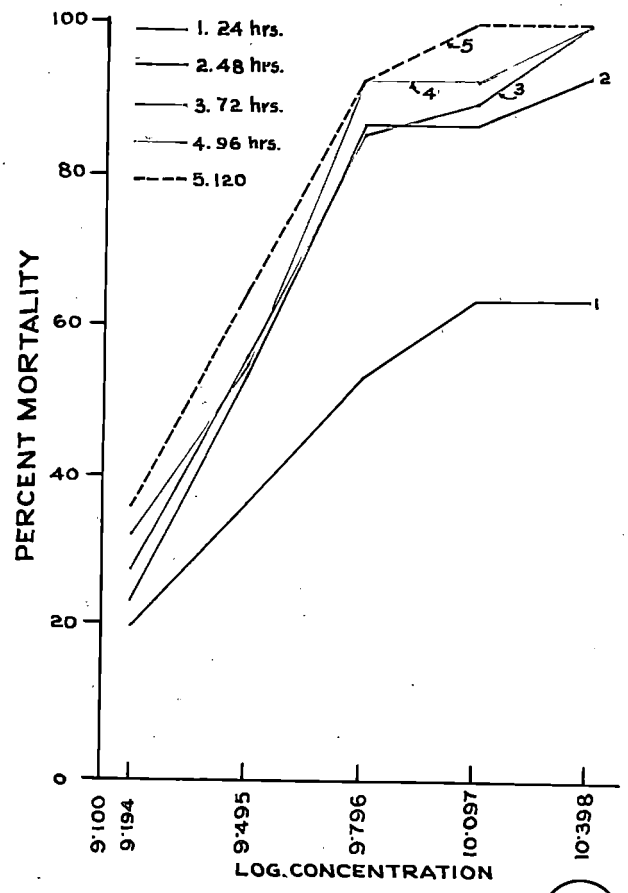

FIG. 9

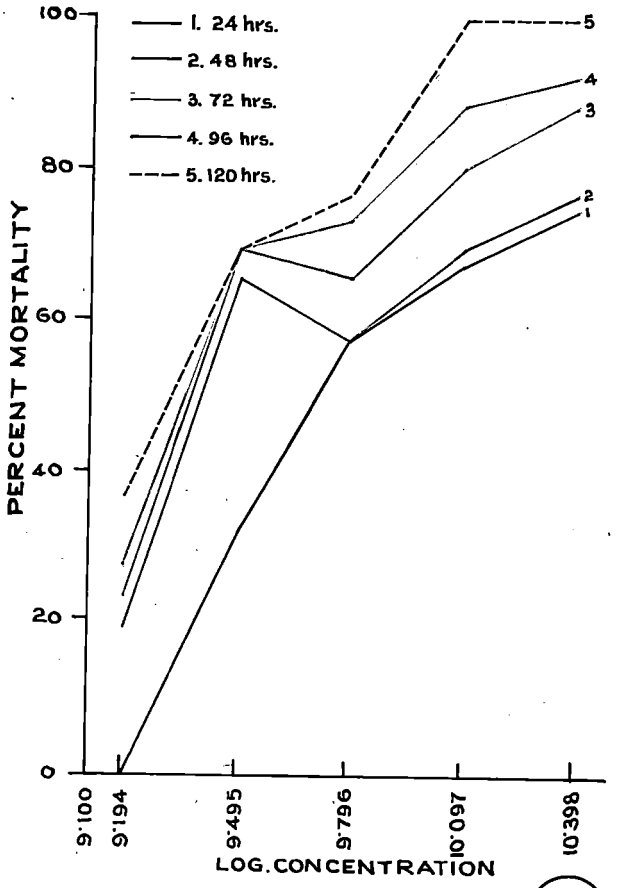

FIG. 10

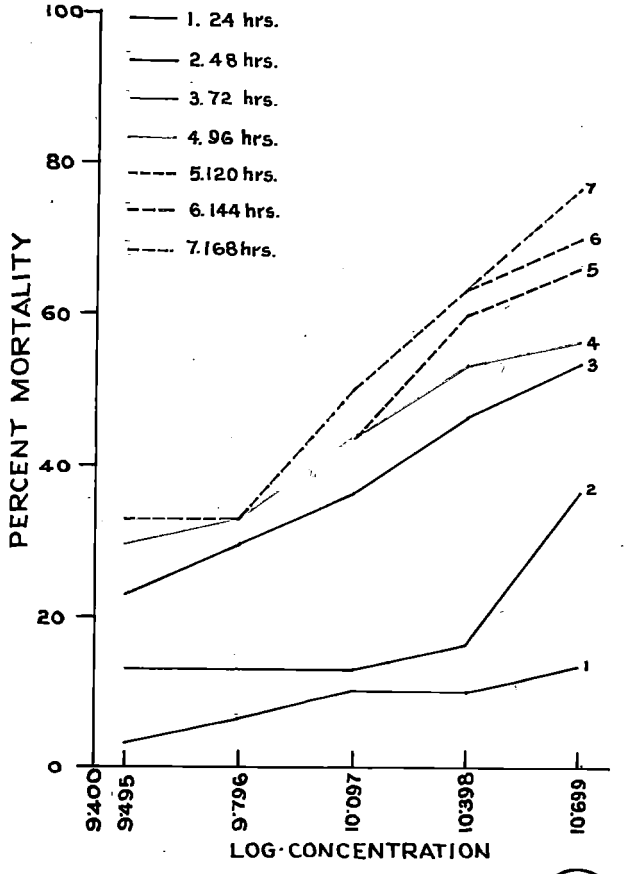

FIG. 11

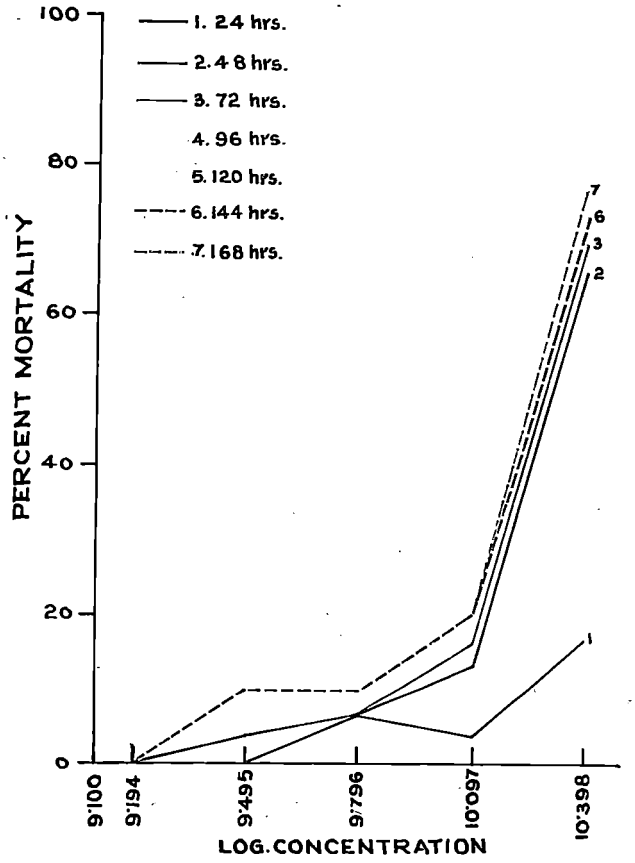

FIG. 12

with increase in spore concentration. A sixteen fold increase in spore concentration causes hardly three fold increase in mortality. Further , mortality increases only slowly with lapse of time.

Symptoms of infection: Infected caterpillars showed a marked reduction in feeding. They did not fold the leaves. They developed a light brown colour, became lethargic and died. The dead caterpillars turned dark brown with deeper shades at the anterior and posterior extremities. They lost their shape and adhered to the leaves.

Experiment 12.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of Margaronia indica.

Experimental details:

Test insect: Third instar caterpillars were used for the experiment. These were reared from small caterpillars collected from bitter gourd plants in the farm.

Period during which the experiment was conducted:	4-1-1964 to 9-1-1964.
Temperature during the period	Mnm. 78° - 80° F Mxm. 84° - 86° F
Relative humidity during the period	47% - 78%

Rest of the details are same as in Experiment. 1.

Results:

Results of the experiment are presented in Table XIII, and in Fig. (10). It will be observed that all the concentrations except the lowest two cause more than 50% mortality in 24 hrs. In the case of the two highest concentrations 100% mortality is achieved in 5 days.

Symptoms of infection: Infected caterpillars stopped feeding, and developed a dark brown colour in the thoracic region which extended anteriorly and posteriorly. Diarrhoea and sluggishness were also noticed. The acadavers turned dark brown and remained flaccid on the leaves. They disintegrated under light pressure giving a bad smell.

Experiment 13.

Dossage mortality relationship between B. thuringiensis spores and caterpillars of Diacrisia obliqua.

Experimental details:

Test insect: Caterpillars of third instar were used for the experiment. These were reared from the small caterpillars collected from cowpea crop in the farm.

Period during which the experiment was conducted:		4-1-1964 to 10-1-1964.
Temperature during the period:	Mnm.	78° - 80° F
	Mxm.	84° - 86° F

T A B L E XIII

Percentage mortality of M. indica caterpillars fed on bitter gourd leaves sprayed with different grades of spore concentrations of B. thuringiensis

Concentrations of spore suspension (spores per 100 cc)	* Per cent mortality after				
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
A. 1.5625×10^9	0	19.3	23.1	27.0	27.0
B. 3.125×10^9	32.2	65.4	69.2	69.2	69.2
C. 6.25×10^9	57.1	57.7	65.4	73.1	76.9
D. 12.5×10^9	67.8	69.2	80.7	88.5	100.0
E. 25.0×10^9	75.0	76.9	88.5	92.3	100.0
Control	6.7	13.3	13.3	13.3	13.3

* Corrected as in Experiment 1.

Relative humidity during the period: 49% - 78%

Rest of the details as in Experiment 3.

Results:

Results of experiment are presented in Table XIV and depicted in Fig.(11). It is observed that no concentration of spores effect cent per cent mortality even after six days. It is also evident that increase in mortality is not commensurate with increase in spore concentration. Thus it is seen that a sixteen fold increase in spore concentration gives hardly threefold increase in mortality percentage. A sharp increase in mortality is evident after the second day. Thereafter the mortality increases only gradually and is more or less constant in the lower two concentrations (A) and (B)

Symptoms of infection: Infected caterpillars stopped feeding 12 hours before death, developed a dark brown or black colour and became moribund, and fell down. The cadavers shrunk in size and appeared stiff.

Experiment 14.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of *pericallia ricini*

Experimental details:

Test insect: Caterpillars of the third instar were used for the experiment. They were reared from eggmasses collected from

T A B L E XIV

Percentage mortality of *E. obliqua* caterpillars fed on Cowpea leaves sprayed with different grades of spore concentrations of *B. thuringiensis*.

Concentrations of spore suspension (spores per 100 cc.)	* per cent mortality after						
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs	144 hrs	168 hrs.
A. 3.125 x 10^9	3.3	13.3	23.3	30.0	33.3	33.3	33.3
B. 6.25 x 10^9	6.7	13.3	30.0	33.3	33.3	33.3	33.3
C. 12.5 x 10^9	10.0	13.3	36.7	43.3	43.3	50.0	50.0
D. 25.0 x 10^9	10.0	16.7	46.7	53.3	60.0	63.3	63.3
E. 50.0 x 10^9	13.3	36.7	53.3	56.7	66.7	70.0	76.77
Control	0	0	0	0	0	0	0

* Corrected as in Experiment 1

Period during which the experiment was conducted.:	7-1-1964 to 14-1-1964.
Temperature during the period:	Mnm. 76° - 80° F Mxm. 82° - 86° F
Relative humidity during the period:	48% - 79%

Rest of the details are as in Experiment.1.

Results:

Results of the experiment are presented in Table XV and graphically represented in Fig.(12). It will be seen that in general the bacterium has little effect on the caterpillars. Even the highest spore concentration does not cause cent per cent mortality in 7 days. Further, only the highest concentration (E) causes more than 20% mortality. It is obvious that increase in spore concentration is commensurate with increase in mortality. Mortality increases suddenly after the first day and then it remains more or less static and does not keep pace with lapse of time. The lowest concentration (A) does not cause any mortality.

Symptoms of infection: The most conspicuous symptom of infection was reduction in feeding followed by stunted growth. The stunting effect was perceptible on the third day after ingestion and was pronounced on the sixth day. The intensity of stunting was generally

T A B L E XV

Percentage mortality of P. ricini caterpillars fed on banana leaves sprayed with different grades of spore concentrations of B. thuringiensis.

Concentrations of spore suspension (spores per 100 cc)	* per cent mortality after						
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.
A. 1.5625×10^9	0	0	0	0	0	0	0
B. 3.125×10^9	0	3.3	3.3	3.3	3.3	10.0	10.0
C. 6.25×10^9	6.7	6.7	6.7	6.7	6.7	10.0	10.0
D. 12.5×10^9	3.3	13.3	16.7	16.7	16.7	20.0	20.0
E. 25.0×10^9	16.7	66.7	70.0	70.0	73.3	73.3	76.7
Control	0	0	0	0	0	0	0

* Corrected as in Experiment 1.

proportional to the dosage . Measurements of length and thoracic width (mean of five values) of infected caterpillars in each treatment were as follows:

	Treatments					
	A	B	C	D	E	Control
Length in mm.	39.00	27.88	17.33	16.00	12.5	41.00
Thoracic width in mm.	3.77	3.03	2.00	2.00	2.00	4.43

Plate (2) also shows clearly this reduction in size of caterpillars due to infection. The dead caterpillars were dark black in colour and dried up without liquifying. They remained on the leaves attached by the anal end. After the seventh day a reversal of effect was noticed as the stunted surviving caterpillars began to grow in size. They pupated and the adults emerged normally.

Experiment. 15.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of *Phytometra peponis*

Experimental details:

Test insect: Third instar caterpillars were used for the experiment. These were reared from small caterpillars collected from snake gourd crop in the farm.

Period during which the experiment was conducted:	11-2-1964 to 16-2-64
Temperature during the period:	Mnm. 80° - 86° F Mxm. 86° - 88° F
Relative humidity during the period:	42% - 58%

Rest of the details as in Experiment 1.

Results.

Results of the experiment are presented in Table XVI and graphically represented in Fig (13). It will be observed that cent per cent mortality is obtained after three days in the highest concentration, whereas it does not reach cent per cent in any other concentration even after five days. Increase in mortality is roughly proportional to increase in spore concentration. A sudden increase in mortality is evident after 2 days.

Symptoms of infection: A significant reduction in feeding was evident in infected caterpillars. They turned completely dark brown. An yellowish green discharge and loose faecal pellets were observed at this stage. In all cases the cadavers were seen hanging down with their anal ends attached to the leaves or sides of the chimneys. They lost their shape and dried up in 48 hours assuming a dark brown to black colour.

T A B L E XVI

Percentage mortality of P.peponis caterpillars fed on snakegourd leaves sprayed with different grades of spore concentrations of B. thuringiensis

Concentrations of spore suspension (spores per 100 cc)	* per cent mortality after				
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
A. 1.5625 x 10^9	7.1	11.5	23.1	19.9	24.0
B. 3.125 x 10^9	14.3	19.3	38.5	43.9	48.0
C. 6.25 x 10^9	28.5	46.1	57.7	55.9	55.9
D. 12.5 x 10^9	53.6	53.9	80.7	84.0	91.9
E. 25.0 x 10^9	82.1	96.2	100.0	100.0	100.0
Control	6.7	13.3	13.3	16.7	16.7

* Corrected as in Experiment 1.

Fig. (13) Graph showing dosage mortality relationship between B. thuringiensis spores and caterpillars of Phytometra peponis.

Fig. (14) Graph showing dosage mortality relationship between B. thuringiensis spores and caterpillars of Spodoptera mauritia.

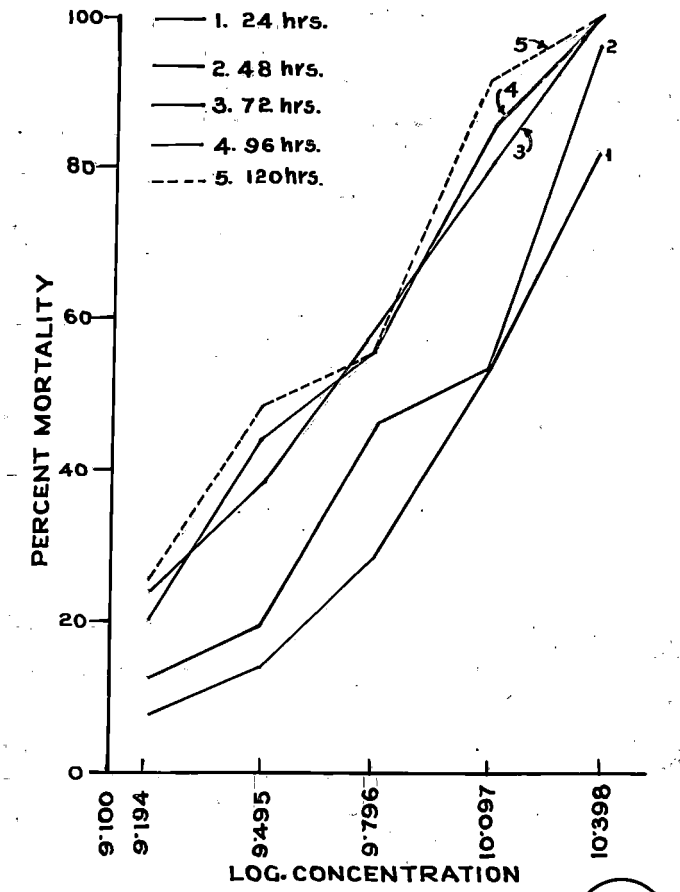

FIG. 13

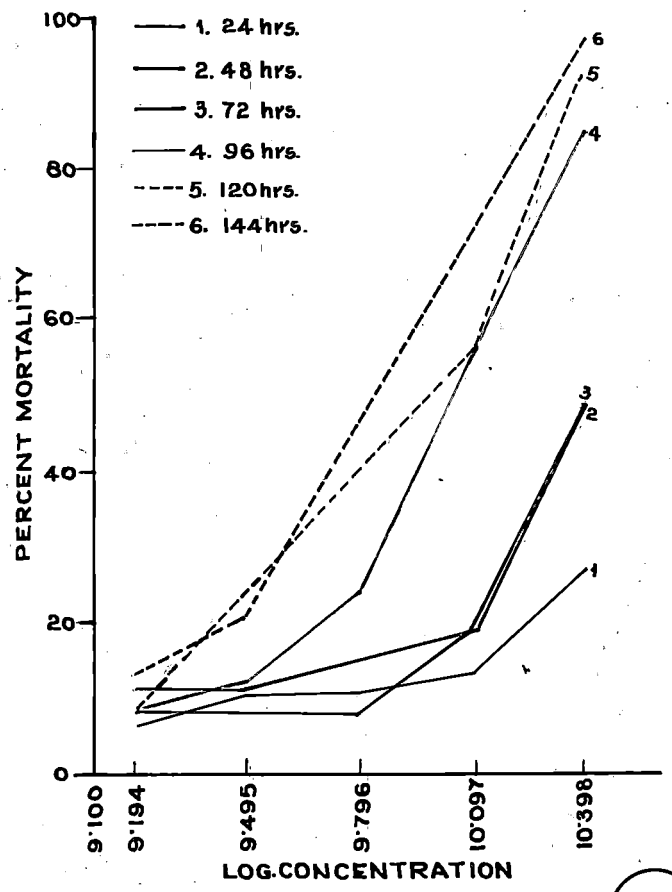

FIG. 14

Pupae of infected caterpillars died in cocoons.

Experiment 16.

Dosage mortality relationship between *B. thuringiensis* spores and caterpillars of *Spodoptera mauritia*.

Experimental details:

Test insect: Caterpillars of third instar were used for the experiment. These were reared from egg masses collected from paddy fields.

Period during which the experiment was conducted:	5-3-1964 to 11-3-1964.
Temperature during the period:	Mnm. 78° - 82° F Mxm 87° - 88° F
Relative humidity during the period	40% - 80%

Rest of the details are as in Experiment 1.

Results:

Results of the experiment are presented in Table XVII and depicted in Fig.(14). It will be observed that even after six days of feeding even the highest concentration of spores does not cause cent per cent mortality among the caterpillars. It is also evident that increase in mortality is not commensurate with

TABLE XVII

Percentage mortality of *S. mauritia* caterpillars fed on paddy leaves sprayed with different grades of spore concentrations of *B. thuringiensis*

Concentrations of spore suspension (spores per 100 cc)	* Per cent mortality after						
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs	168 hrs.
A. 1.5625×10^9	6.9	11.1	7.8	7.9	7.9	12.5	12.5
B. 3.125×10^9	10.3	11.1	7.9	12.0	24.0	20.9	20.9
C. 6.25×10^9	10.3	14.8	7.9	24.0	40.0	45.9	45.9
D. 12.5×10^9	13.9	18.6	19.9	55.9	55.9	70.9	70.9
E. 25.0×10^9	27.6	48.1	48.0	84.0	92.0	95.9	95.9
Control	3.3	10.0	16.7	16.7	16.7	20.0	20.0

* Corrected as in Experiment 1.

increase in spore concentration. Thus it is seen that when the concentration of spore suspension increases 16 fold, the mortality increases hardly 7 fold. Further, a sudden increase in mortality is evidenced after a period of three days.

Symptoms of infection: Infected caterpillars developed a dark brown colour, became lethargic and fell down before death. However, sometimes the cadavers were seen attached to the leaves by the anal end. The cadavers turned dark brown and assumed a flaccid condition. After 24 hours they liquified giving an unpleasant smell.

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DISCUSSION

D I S C U S S I O N.

In the present investigations susceptibility of twentysix species of caterpillars, damaging various cultivated crops, to infection by B.thuringiensis has been determined. Of these twentysix species, susceptibility to infection by this bacterium has been tested for the first time in seventeen species, namely, Dichocrocis punctiferalis, Sylepta derogata, Psara bipunctalis, Psalis securis, Melanitis ismene, Orthaga exvinacea, Prodenia litura, Polyorycta dimidialis, Pyrausta phoenicealis, Diacrisia obliqua, Nacoleia vulgalis, Hymenia fascialis, Argina cribraria, Achoea janata, Euchromia polymena, Nymphula depunctalis and Euproctis fraterna.

Relative susceptibility of the different species of caterpillar pests to infection by B.thuringiensis has been ascertained by two series of experiments. In the first series which are screening tests, lots of caterpillars are fed on food plant parts immersed in a suspension of spores of B.thuringiensis and dried. Of the twentysix caterpillars tested, those of Eupterote mollifera, P.litura and E.fraterna are not infected by the bacterium eventhough the spores are eaten by them. This observation confirms that of Rao et al. (1962). Other insects show varying degrees of susceptibility.

The caterpillars highly susceptible to the organism are S.derogata, Glyphodes marginata, P.phoenicealis, Margaronia indica, N.vulgalis, Papilio demoleus, H.fascialis and A.janata. This observation is in line with those of

Ayyar (1961) and Ramamurthy (1963 a). Cameron (1963) states that the bacterium is not effective against borers and internal feeders. But in the present investigations Dichocrocis punctiferalis has been found to be fairly susceptible. Similar observations on the susceptibility of borers have been made by Husz (1928, 1929, 1930) and Martouret (1959) on Pyrausta nubilalis and Metalnikov and Metalnikov (1933, 1935) on Pectinophora gossypiella.

The bacterium also appears to be promising against the pests Psara bipunctalis on amaranthus, Phytometra (Plusia) peponis on snakegourd, Nymphula depunctalis and Cnaphalocrocis medinalis on paddy and Dichocrocis punctiferalis on castor.

In the second series of experiments dosage mortality relationships between spores of B.thuringiensis and sixteen host insects have been determined. From the actual data of mortality caused by the organism in the different species of insects under test, they may be grouped under three categories viz. those highly susceptible giving 80-100 per cent mortality, those highly resistant giving below 50 per cent mortality and those of medium susceptibility giving 50-80 per cent mortality. Thus 80-100 per cent mortality is observed in G.marginata, A.cribraria, E.polymena, S.derogata, O.exvinacea, N.depunctalis, C.medinalis, H.fascialis, M.indica, P.peponis and S.mauritia by concentrations of the suspension varying from 6.25×10^9 to 50×10^9 spores per 100 cc. These species can thus be considered highly susceptible to infection by B.thuringiensis. On the other hand caterpillars of

P.litura and E.fraterna give below 15 per cent mortality and are highly resistant. The caterpillars of N.serinopa, D.obliqua and P.ricini show intermediate susceptibility giving mortalities between 70 and 80 per cent only even with the highest concentration of 50 x 10^9 spores per 100 cc. These observations are in agreement with those recorded in the screening tests.

With a view to compare more precisely the susceptibility of the different species, ld-p lines (log. dose - probit mortality) have been plotted for each. From these the Median Lethal Doses (MLDs) are found by interpolation and the slope measured by counting the number of probits for a unit increase in dose. Since for some insects MLD can be interpolated at 72 hours and for others it can be done only at 168 hours, lines have been drawn separately for these two sets of insects.

Table XVIII gives the MLDs of the spores for the different species of insects as well as the slopes of the respective ld-p lines. Figures(15) and (16) show the ld-p lines. From the position of the lines in Fig. (15) and from the Table it will be observed that H.fascialis is the most susceptible and P.ricini the least susceptible in the first group. Similarly from Fig.(16) and the Table it is clear that S.mauritia is the most susceptible and N.serinopa the least susceptible in the second group.

TABLE XVIII.

LD_{50} of *B. thuringiensis* spores to different species of caterpillars and the slopes of the ld-p lines.

Sl. No.	Species of insect	LD_{50} at 72 hours	LD_{50} at 168 hrs.	Slope (No. of probits x 1000)
1.	*Hymenia fascialis*	2.51×10^9		675
2.	*Glyphodes marginata*	2.95×10^9		900
3.	*Margaronia indica*	3.16×10^9		413
4.	*Phytometra peponis*	4.12×10^9		550
5.	*Cnaphalocrocis medinalis*	5.25×10^9		675
6.	*Sylepta derogata*	5.95×10^9		550
7.	*Nymphula depunctalis*	7.24×10^9		675
8.	*Pericallia ricini*	20.42×10^9		775
9.	*Spodoptera mauritia*		6.92×10^9	725
10.	*Argina cribraria*		7.85×10^9	563
11.	*Orthaga exvinacea*		10.23×10^9	313
12.	*Diacrisia obliqua*		12.59×10^9	350
13.	*Euchromia polymena*		16.60×10^9	750
14.	*Nephantis serinopa*		28.18×10^9	563

Fig. (15) GRAPH SHOWING THE LOG. DOSAGE - PROBIT MORTALITY LINES OF DIFFERENT SPECIES OF CATERPILLARS AT 72 HOURS.

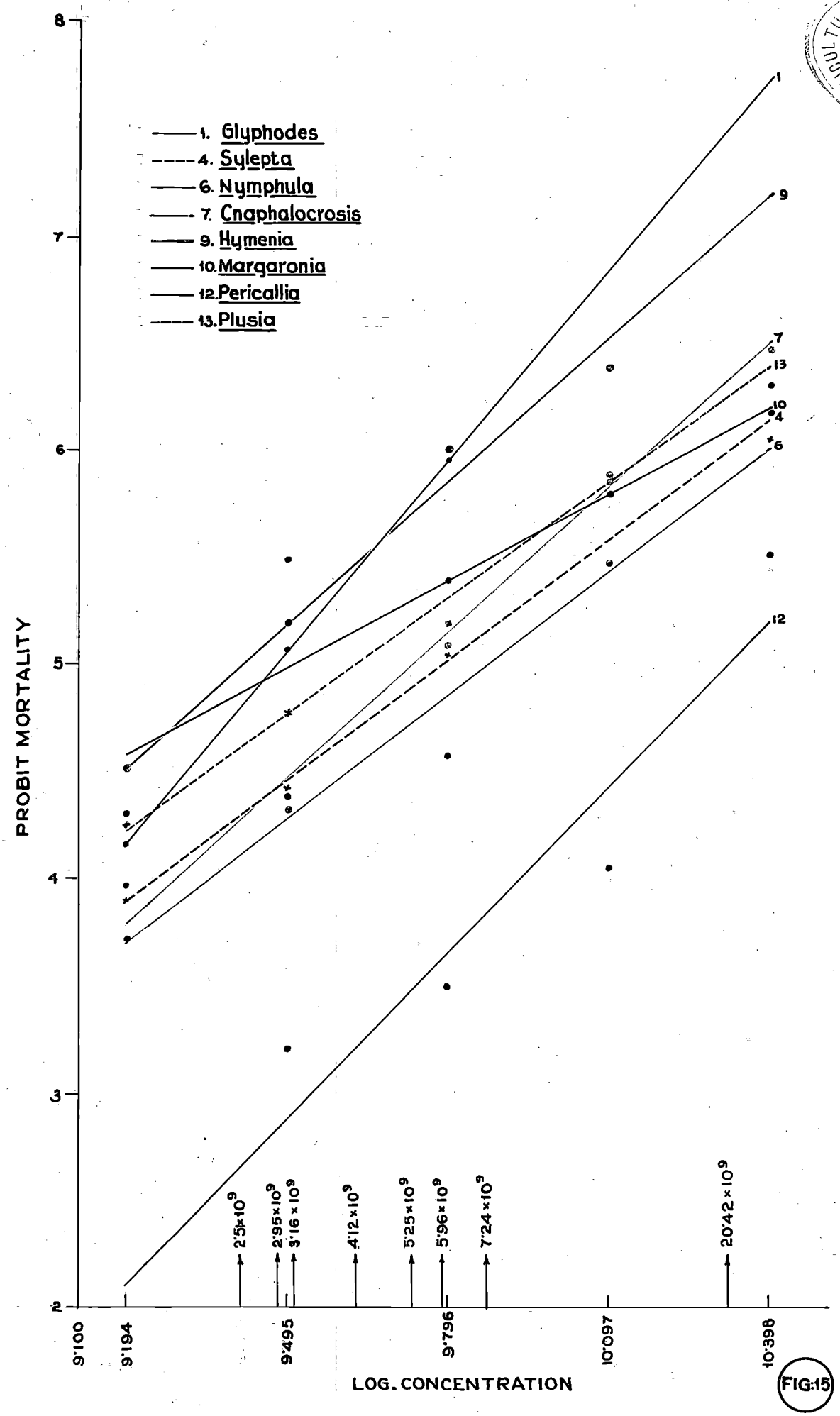

FIG:15

Fig. (16) GRAPH SHOWING THE LOG. DOSAGE - PROBIT MORTALITY LINES OF DIFFERENT SPECIES OF CATERPILLARS AT 168 HOURS.

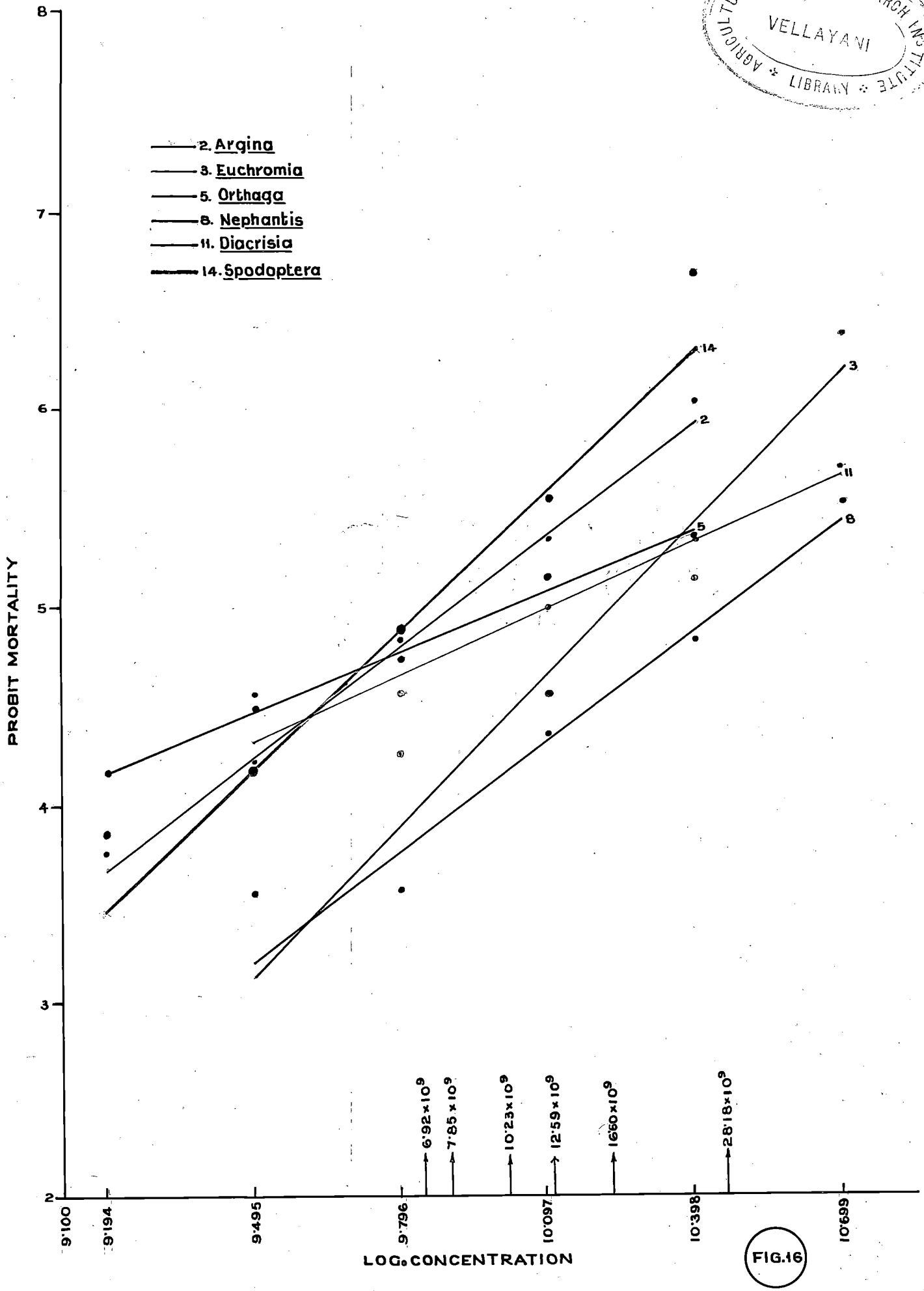

FIG.16

These MLD values are greater than those for Pyrausta nubilalis, Melanoplus bilituratus and Plutella maculipennis worked out by Mc Connel and Cutcomp (1954), Baird (1958) and Menn (1960) respectively. It will be observed from the Table that based on MLDs the insects can be ranked in the order of descending susceptibility as:- H.fascialis, G.marginata, M.indica, P.peponis, C.medinalis, S.derogata, N.depunctalis and P.ricini in the first group (MLD at 72 hours) and as:- S.mauritia, A.cribraria, O.exvinacea, D.obliqua, E.polymena and N.serinopa in the second group (MLD at 168 hours). The observation of high susceptibility of G.marginata, M.indica and P.peponis agrees with the findings of Ayyar (1961). Rao et al.(1962) and Ramamurthy (1963 a) have reported negative effects of the bacterium on P.ricini and C.medinalis respectively. But in the present investigations both these insects are found susceptible, the susceptibility of C.medinalis being fairly high. The lack of effects reported by the earlier workers may be due to the low dosages used. (Rao et al. have used an unspecified number of spores and Ramamurthy a spore concentration of 15 x 10^7 spores per 100 cc.) In the present tests also, mortality of these insects is very low in lower spore concentrations.

The ineffectiveness of the bacterium on P.litura and its comparatively low effect at the earlier hours on S.mauritia strengthens the views of Tanada and Reiner (1960)

that cutworms are not very susceptible to the bacterium.

The slope of the ld-p lines is a measure of the response of the insects to changes in dose of the stimulant. The values of the slopes of the different ld-p lines given in Table XVIII will show that the highest response to dosage changes of the spores is manifested by G.marginata which is also highly susceptible to the organism. On the other hand, M.indica which is also almost as susceptible as G.marginata shows very low response to increased doses of the spores. Further, P.ricini which is relatively highly resistant to the bacterium, shows a high degree of response to the increased doses of the organism. Thus there appears to be no correspondence between extent of susceptibility to infection by spores and response to their dosage changes. With reference to the response of the caterpillars to increased changes in doses of the spores the different species of caterpillars can be ranked in the descending order as follows:- G.marginata, P.ricini, E.polymena, S.mauritia, H.fascialis, C.medinalis, N.depunctalis, N.serinopa, A.cribraria, P.neponis, S.derogata, M.indica, D.obliqua and O.exvinacea.

The symptoms of infection noticed in different larvae are in general agreement with those described by Steinhaus (1949), Sekhar and Gopinath, (1962) and Moore (1962). These authors have recorded the development of a brown colour in the caterpillars before death. During the

present investigations it has been observed that in most cases the colour develops first in the thoracic region or at the anterior region of the abdomen. The stunting effect of the bacterial infection on Pericallia ricini is an interesting phenomenon. (Plate 2) Similar observations have been recorded by Jaques (1961) on Malacosoma americana. The reversal of the toxic effect of the bacterium on this insect after the seventh day is also peculiar.

Larvae of Euchromia polymena show increased movements and restlessness as a result of the infection. Similar symptoms have been recorded by Steinhaus (1949) for bacterial infections in general. Another interesting phenomenon observed in E.polymena is the great increase in mortality observed at the time of moulting. The same phenomenon has been observed by Kushner and Harvey (1962) on larch sawfly in which according to Heimpel (1955 a) the midgut in the moulting larva is usually devoid of food and rather fragile. Kushner and Harvey's explanation to the phenomenon is thus:- "It may be that bacteria are better able to grow in the gut contents of such larvae than in those of feeding larvae so that if they can enter the insect's gut when it is not feeding, or can remain after food is gone, their chances of growth are much greater". The same explanation seems to be applicable in the present observation also. This throws some light on the toxic mechanism of the

bacterium and strengthens the views of Heimpel (1955 b), Lipa (1962) and Smirnoff (1963) that the bacterium should multiply in the gut for causing mortality.

The quicker action of the bacterium as evidenced by high initial mortality and a drastic reduction in feeding are seen in G.marginata, H.fascialis, M.indica and P.peponis. Angus (1953) showed this as the typical toxic effect of the crystal associated with the bacterium. These obeervations support the view that the crystal has a major role in causing mortality in insects as propounded by Vankova (1957), Bonnefoi and Beguin (1959) and Patel and Cuteomp (1961).

In the case of P.peponis a large number of pupae of infected caterpillars die in the cocoons as a result of septicemia. This suggests that the bacterium can be used as a long range control measure against this pest. Similar observations have been made by Legner and Oatman (1962) on Spilonota ocellana and by Lipa (1962) on Pieris brassicae.

Though mortality among caterpillars of Nephantis serinopa is only 70.3 per cent after 8 days of feeding, it appears possible that the bacterium can be used in conjunction with other biological agents such as the parasites, so that, the latter can multiply and maintain their population density on the surviving caterpillars. Further, the reduction in feeding as a result of infection by the bacterium, well manifested among caterpillars of O.exvinacea, S.derogata,

C.medinalis, N.depunctalis and N.serinopa, eventhough their initial mortality is low, suggests that application of the organism can prevent economic loss. Observations in the same line have been made by Creighton et al. (1964) on Trichoplusia ni.

As a whole the effect of the bacterium is more pronounced in the case of caterpillars which scrape the leaves, such as H.fascialis, G.marginata, M.indica, C.medinalis and S.derogata than inthose eating whole leaves such as S.mauritia, A.cribraria, D.obliqua, P.ricini and E.polymena. This is only natural as the surface scrapers are able to ingest relatively more number of spores than the bulk feeders.

From the results discussed above the following conclusions may be drawn regarding the use of B.thringiensis in the control of some crop pests:-

(a) The caterpillars of D.punctiferalis and A.janata on castor; S.derogata on bhindi; P.bipunctalis and H.fascialis on amaranthus; P.securis, C.medinalis, M.ismene, N.depunctalis and S.mauritia on paddy; M.indica and P.peponis on gourds; P.demoleus on citrus; O.exvinacea on mango; A.cribraria on sunnhemp; E.polymena on sweetpotato; G.marginata and P.phoeniceallis on garden plants; and N.vulgalis on greengram may be effectively controlled by spraying the crop with spore suspensions of B.thuringiensis

containing 6.25 x 10^9 to 50 x 10^9 spores per 100 cc.

(b) The bacterium appears to be not very effective against the caterpillars of N.serinopa on coconut, D.obliqua on pea and P.ricini on banana. However, since all these caterpillars are subject to natural control to some extent by parasitic insects, use of the bacterial preparation will help in augmenting the efficiency of control of these pests without upsetting the 'balance' as this bacterium is harmless to parasites.

(c) B.thuringiensis is ineffective in the control of P.litura, E.fraterna and E.mollifera as they are very resistant to the bacterial infection.

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SUMMARY

S U M M A R Y.

Literature on Bacillus thuringiensis Berliner in relation to insect pest control has been reviewed.

Susceptibility of twentysix different species of caterpillar pests, commonly occurring in Kerala, to infection by B.thuringiensis spores has been ascertained. Susceptibility of seventeen of these caterpillars to the bacterium has been tested for the first time.

In a screening experiment all the twentysix species of caterpillars have been infected by feeding them with food materials dipped in the spore suspension of B.thuringiensis containing 12.5 x 10^9 spores per 100 cc. Results show that caterpillars of Dichocrocis punctiferalis, Sylepta derogata, Psara bipunctalis, Glyphodes marginata, Psalis securis, Melanitis ismene, Cnaphalocrocis medinalis, Pyrausta phoenicealis, Margaronia indica, Phytometra peponis, Papilio demoleus, Nacoleia vulgalis, Hymenia fascialis, Achoea janata and Nymphula depunctalis are highly susceptible showing 80-100 per cent mortality in 2 to 6 days. Caterpillars of Prodenia litura, Eupterote mollifera, Nephantis serinopa, Diacrisia obliqua, Pericallia ricini and Euproctis fraterna are highly resistant giving zero to fortyfive per cent mortality in one to six days.

In a second series of experiments dosage mortality relationships between spores of B.thuringiensis and sixteen host caterpillars have been determined in full. Caterpillars of Glyphodes marginata, Argina cribraria, Euchromia polymena, Sylepta derogata, Orthaga exvinacea, Nymphula depunctalis, Cnaphalocrocis medinalis, Hymenia fascialis, Margaronia indica, Phytometra peponis and Spodoptera mauritia are highly susceptible giving 80-100 per cent mortality with spore concentrations of 6.25×10^9 to 50×10^9 spores per 100 cc. suspension. Caterpillars of Prodenia litura and Euproctis fraterna are highly resistant giving only less than 15 per cent mortality. Caterpillars of Nephantis serinopa, Diacrisia obliqua and Pericallia ricini show intermediate susceptibility.

Based on MLDs the insects under test fall under two groups:- those which give 50 per cent mortality and above in 3 days and those which give similar mortality in 7 days. The relative susceptibility of insects in the first group in the descending order is: Hymenia fascialis, Glyphodes marginata, Margaronia indica, Phytometra peponis, Cnaphalocrocis medinalis, Sylepta derogata, Nymphula depunctalis and Pericallia ricini; and that of the insects in the second group is: Spodoptera mauritia, Argina cribraria, Orthaga exvinacea, Diacrisia obliqua, Euchromia

polymena and Nephantis serinopa.

The slopes of the ld-p lines taken as a measure of the response of the insects to changes in dose of the bacterial spores, indicate that there is no definite relation between the extent of susceptibility of the different species of insects to infection by the spores and the response of these insects to changes in the dose of the spores.

The general external symptoms on the caterpillars caused by the bacterial infection are discolouration, sluggishness and cessation of feeding. Stunted growth and reversal of the effects of infection are seen in caterpillars of Pericallia ricini. Larvae of Euchromia polymena become restless and show increased mortality at the time of moulting.

Effect of the bacterium is more pronounced in the case of those caterpillars which scrape the leaves than on those which feed on whole leaves.

It is concluded that spore suspensions containing 6.25×10^9 to 50×10^9 spores of B.thuringiensis per 100 cc. can be used with advantage to control the caterpillar pests Dichocrocis punctiferalis and Achoea janata on castor; Sylepta derogata on bhindi; Psara bipunctalis and Hymenia

fascialis on amaranthus; Cnaphalocrocis medinalis, Nymphula depunctalis, Psalis securis, Melanitis ismene and Spodoptera mauritia on paddy; Margaronia indica and Phytometra peponis on gourds grounds; Papilio demoleus on citrus; Orthaga exvinacea on mango; Argina cribraria on sunnhemp; Euchromia polymena on sweet potato; Glyphodes marginata and Pyrausta phoenicealis on garden plants; and Nacoleia vulgalis on green gram.

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APPENDIX

APPENDIX

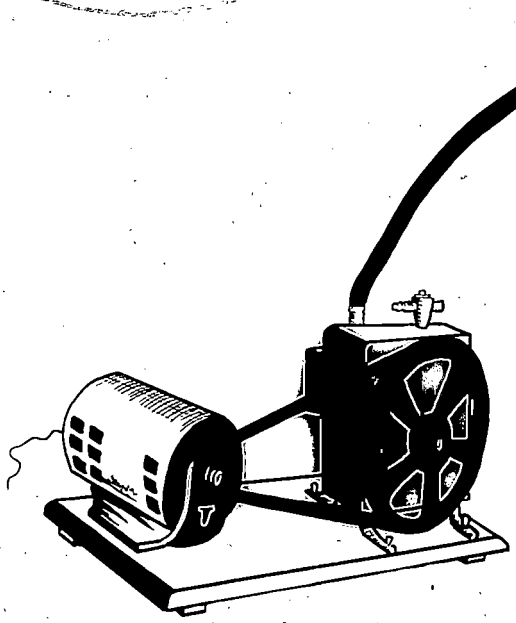
'Record of Temperature and Humidity (August 1963 - March 1964)

Month	Week	Minimum temperature		Maximum temperature.		Humidity	
		From	To	From	To	From	To
August 1963	1	76	82	82	84	90	95
	2	80	82	82	84	90	95
	3	76	81	80	84	81	95
	4	76	80	78	82	89	95
September 1963	1	76	82	80	84	76	92
	2	78	82	82	86	90	92
	3	78	82	84	86	90	91
	4	78	82	82	86	90	91
October 1963	1	78	82	82	86	90	92
	2	78	82	82	86	90	92
	3	76	82	80	86	90	92
	4	76	80	78	84	90	92
November 1963	1	76	80	78	84	85	95
	2	78	82	84	86	80	95
	3	78	80	80	84	76	95
	4	78	80	82	86	89	95
December 1963	1	76	84	82	86	85	95
	2	78	80	81	84	90	95
	3	76	80	82	84	90	95
	4	76	80	82	86	89	95
January 1964	1	78	80	82	86	90	95
	2	76	80	82	85	89	95
	3	78	78	82	84	89	90
	4	76	78	82	84	89	90
February 1964	1	82	82	86	88	90	95
	2	80	82	78	86	71	95
	3	80	82	82	84	85	92
	4	80	82	83	88	84	96
March 1964	1	81	82	84	88	77	91
	2	78	81	87	90	82	91
	3	78	82	88	90	83	88
	4	79	84	86	90	83	89

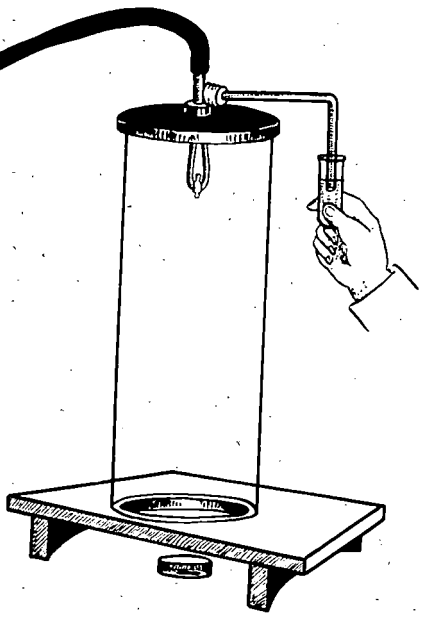
PLATES

PLATE I

Spraying tower



AIR COMPRESSION PUMP



SPRAYING TOWER

PLATE I

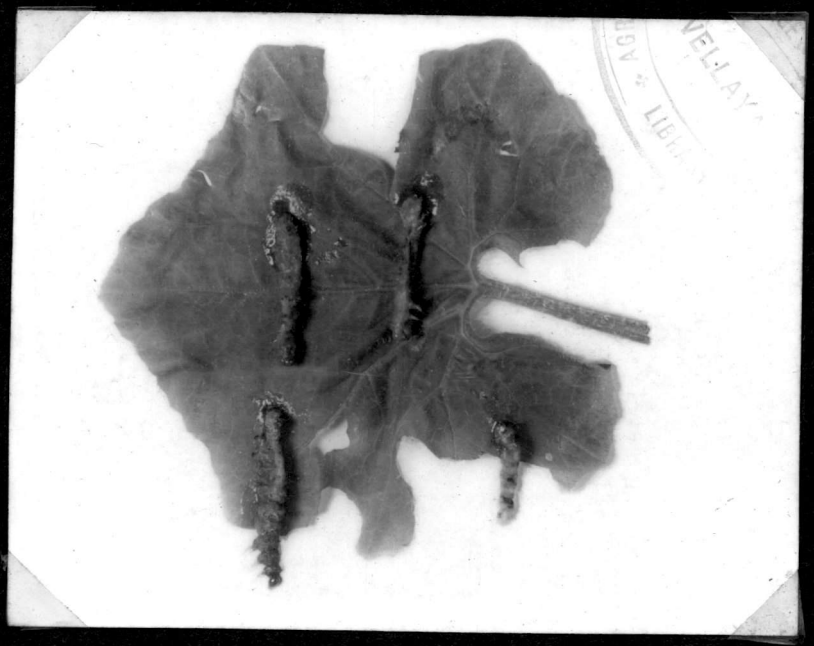
PLATE II

Caterpillars of *Pericallia ricini* normal (left) and stunted (right) due to infection by *B. thuringiensis*. Note the reduction in size due to the bacterial infection.

PLATE III

Caterpillars of *Phytometra peponis* dead due to infection by *B. thuringiensis*, hanging with the posterior end attached to the host leaf.

PLATE II



PIATE III

PLATE IV

Caterpillars of *Diacrisia obliqua*
dead due to infection by *B.thuringiensis*.

PLATE V

Caterpillars of *Argina cribraria*
dead due to infection by *B.thuringiensis*.

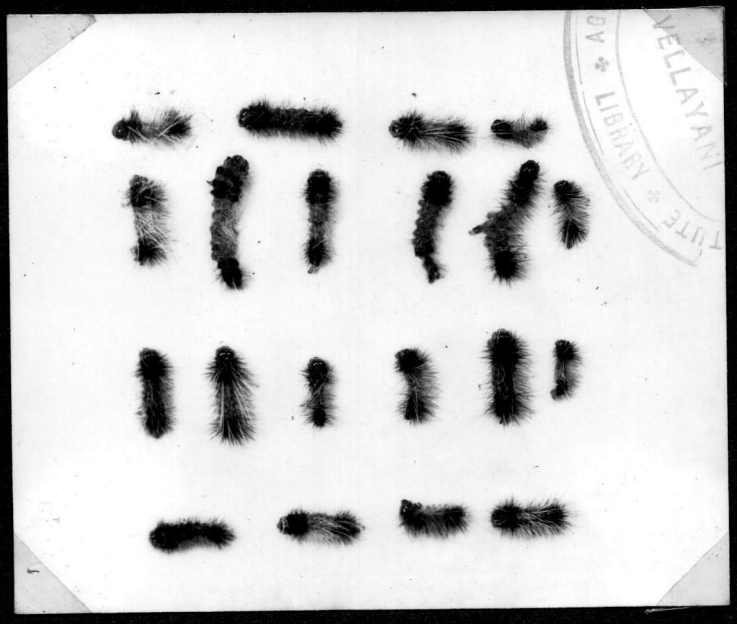


PLATE IV

PLATE V

PLATE VI

Caterpillars of *Pericallia ricini*
dead due to infection by *B.thuringiensis*.
Note the Shrunken size of the caterpillars.

PLATE VII

Caterpillars of *Euchromia polymena*
dead due to infection by *B.thuringiensis*.

PLATE VI

PLATE VII