THE LABORATORY AND FIELD EFFICACY OF *Bacillus thuringiensis* (Berliner) AGAINST TROPICAL CEREAL STEM BORERS (Chilo partellus (Swinhoe) AND *Busseola fusca* (Fuller) AND LEGUME POD BORER (*Maruca testulalis* (Geyer).

BY

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D E C L A R A T I O N

This thesis is my original work and has not been presented for a degree in any other University.

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Bacillus thuringiensis (B.t) (Berliner) strain HD-1 was found to be highly pathogenic to all larval instars of Chilo partellus (Swinhoe) and Maruca testulalis (Geyer) and also 5th and 6th instar-larvae of Busseola fusca (Fuller) in the laboratory. The percentage mortality in 72 hours varied from one instar to another; the early instars being significantly more suceptible than the late instars . The percentage mortality was also found to vary with the concentration of B.t. suspension applied to feed materials . High concentrations, for example $1x10^9$ spores/ml, caused 100% mortality in all larval instars of these pests in less than 30 hours, while a concentration of $4.16x10^6$ spores/ml caused mortality ranging between 7-80% in all instars after 72 hours.

B.t commercial formulations (Dipel, Thuricide and Certan) were also found to be highly pathogenic to these pests both in the laboratory and in the field. These formulations could also reduce the level of damage in sorghum crop caused by other lepidopteran pests such as Sesamia calamistis (Hampson) and Eldana saccharina (Walker) in the field. However, no significant difference was shown to exist in the three formulations in the field on application for pests control . The efficacy of B.t.

commercial formulations in controlling C. partellus in the screenhouse was quite spectacular. Significantly higher yields of sorghum and cowpea in pure stands and also in an intercrop planting pattern was realised in the field in B.t protected stands than in unprotected stands . However, there was no difference in the pure stand and mixed stand as far as efficacy of B.t in control of pests was concerned.

The performance of B.t (HD-1) laboratory culture was found to be influenced by Ultraviolet light from a U.V. lamp and solar radiation. In contrast variation in temperature in the range of 5°C to 85°C had very little effect on B.t.(HD-1). Exposure of B.t(HD-1) to U.V light from a lamp was found to be more deleterious than exposure to sunlight . This indicates U.V light is the most deleterious component of solar radiation to B.t.(HD-1). B.t.(HD-1) sprayed on sorghum plants in the screenhouse was also adversely affected by solar radiation, although not as much as B.t. exposed directly in petri dishes. After three days of exposure of B.t to solar radiation on sorghum leaves and in petri dishes, the B.t then gave 30% and 10% mortalities respectively after 72 hours when assayed with third instar larvae of C.partellus. The time taken for 50% loss in pathogenicity of B.t to C. Partellus was found to be 0.62 hr. and 9.3 hr. after exposure to U.V and sunlight, respectively.However, loss of 50%

pathogenicity due to the effect of solar radiation on B.t.(HD-1) sprayed on sorghum in screenhouse was 6.3 hours. The extreme temperatures of 5°C and 85°C had very little effect on B.t.(HD-1). A larval mortality greater than 80% (after 72hr.exposure) was recorded with all heat-treated samples.

It was concluded from the results that B.t (HD-1) is pathogenic to local lepidopteran pests of cereal and legume crop bores in the laboratory; and commercial preparations of this bacterium would provide a pest management tool against these pests under field conditions in Kenya.

CHAPTER ONE

INTRODUCTION

1.1.0 The Sorghum Crop

Sorghum (Sorghum bicolor (L Moench)) is the most important cereal crop in the semi-arid parts of Asia and Eastern Africa (Brhane, 1985). Of the total 47 million hectares of sorghum grown in the world, Eastern Africa cultivates nearly 14% (Seshu Reddy and Omolo, 1985). Although sorghum is grown mainly for animal feed in developed countries, production in Africa and Asia is chiefly for human consumption (Leuschner, 1985). In some areas of Africa, the stem is used as building materials and plant remains (after the head is harvested) may be used for fuel.

In developing countries, where sorghum is a significant food crop, particularly in semi arid areas, the yields are very low being 500-700kg/ha (Davies, 1982; Leuschner,1985). The major production constraints are many; and insect pest damage is one of them (Seshu Reddy, 1981; Leuschner,1985).

1.1.1. Pests of Sorghum

The most important field pests of sorghum in growing areas are shootflies and a range of lepidopterous stem borers. Lepidopteran stem borers consitute the most widely distributed and serious group of insect pests of sorghum in the world, the most damaging species in Africa being Chilo partellus (Swinhoe) Busseola fusca (Fuller), Eldana saccharina (Walker) and Sesamia calamistis (Hampson). C. partellus is one of the most widespread pests in East, Central and Southern Africa (Young and Teetes, 1977; Van Hamburg, 1980). At Mbita Point Field Station of the International Centre of Insect Physiology and Ecology (ICIPE) it consititutes 99% of the stem borer population (Seshu Reddy, 1982). Assessment of yield losses caused by these pests is often quite difficult but according to Jepson (1954) 40 to 100% plants were infested by B. fusca in Tanzania. In Uganda 56% loss of grain resulted when sorghum was infested with C. partellus 20 days after plant emergence (Starks, 1969). Stem borers cause damage by feeding on leaves and by stem tunnelling leading to "dead hearts" and "chaffy heads" (Seshu Reddy, 1983; Young and Teetes , 1977; Dabrowski and Kidiavai, 1983).

In Kenya the bulk of sorghum is grown in the semi-arid parts by small scale farmers on small plots (Ogwaro, 1983). In 1983, Kenya's production was 2% of the total Africa production. Africa's production in the same year was 14.4% of World production (FAO,1983). A preliminary survey carried out in Kenya showed that, the stem borer infestation on sorghum may range between 8 to 100 % (Seshu Reddy, 1983).

1.2.0. The Cowpea Crop

Cowpea, Vigna unguiculata (L) Walp., is the most important grain legume crop throughout the tropical belt covering Asia, the Far East, Africa, Central and Southern America and in the Southern United States. It provides a major source of protein in human diets (Singh, Jackai, and Shoyinka, 1985). Cowpea, which probably originated in Africa, is now grown wherever the climate is suitable around the world. It is the most important food legume grown south of the Sahara, with 2.7 million hectares planted comprising 90% of the world's production (McKelvey, 1974). West Africa grows about 75% of the world's cowpeas (Singh et al.,1985).

The most important use of cowpea in African growing regions is in the form of dried beans as human food, with the residue used as stock feed. It is also eaten in the green seed and green pod form, as sprouted

seedlings; and the tender green leaves are cooked as a green vegetable.

Cowpea yields from Africa are below 400kg/ha (Singh, 1977). The major production problems being poor crop husbandry, plant diseases and severe insect damage.

In Kenya, cowpea ranks first in drier areas and comes second in bean growing areas. As one of the few crops of arid and semi arid lands of Kenya, cowpea is grown in about 271,000 hectares of land mostly as a mixed crop with other crops such as pigeon pea, maize, and sorghum (Muruli, Pathak, Mukunya, Karel, Keya and Ssali, 1980). About 85% of the total areas under cowpea lies in the marginal rainfall areas of Eastern Province while the remaining 15% is distributed among three Provinces: Coast, Nyanza and Western (Muruli, et al., 1980).

1.2.1. Pests of Cowpea.

Cowpea is attacked by several insects which may reduce yield by up to 80% (Singh, et al.,1985). The most damaging pests include the pod borer Maruca testulalis(Geyer), thrips and pod sucking bugs.

The legume pod borer M.testulalis is one of the most important pests which limit production of cowpea in the tropical world (Okeyo-Owuor, Agwaro and Simbi, 1983). According to Taylor (1979), M. testulalis is the most

important cowpea pest in most of Africa because its damage is severe, it occurs perennially and dominates control practices and pest populations are such as to render cultivation of cowpea uneconomic. The larvae of this pest destroy the major growing stages of the crop, especially flowers and developing seeds, which results in a major limitation in cowpea production. Okeyo-Owuor and Ochieng (1981) reported the yield loss due to M. testulalis on an indigenous cowpea variety (Nyar-Milambo) as being upto 80% in Mbita, South Nyanza (Kenya).

1.3.0. Pest Control Measures

Control of the insect pests of these crops is accomplished by using one of several methods including cultural control and chemical insecticide applications. Cultural practices involving manipulation of the environment to make it favourable for crop growth and to encourage the natural enemies of pest insects are being promoted. However, some of the methods are impractical for farmers as they do not always cause appreciable economic control. Presently, studies are being done in some countries in East Africa on host-plant resistance to insect pests with emphasis on the identification of sources of resistant plant cultivars and breeding for resistance to major insect pests, especially those of

sorghum (Gebrekidan, 1982; Seshu Reddy, 1983). The most convenient approach at times of crisis is the use of chemical insecticides. When chemical insecticides are used the results are visible within a short time of application. This method is not always used by the subsistence farmers in eastern African countries because of being expensive, because of the unavailability of the appropriate chemical insecticides or because of a lack of knowledge (Seshu Reddy and Omolo, 1985). Moreover the governments are initiating some moves in banning the use of some of these chemicals especially DDT. Application of chemical insectides to control M. testulalis, for instance may not be acceptable in East Africa to cowpea growers who grow the crop for both its green leaves (as vegetables) and for grain (Otieno, Odindo and Okeyo-Owuor, 1983). The growing awareness of problems caused by the use of chemical insecticides, such as the destruction of useful parasites and predators of pest insects and environmental pollution and possible harmful effects on human health have emphasized the immediate need for effective control measures which are environmentally sound. So far no single control approach has proved effective in replacing chemical insecticides. Other approaches among them the use of microbial insecticides, are being investigated.

1.4.0. Microbial Insecticides

In recent years, microbial insecticides have emerged as a significant pest management component (Norris, 1978). Rapid development has taken place in terms of research and commercialization. Several insect pathogens including bacteria, viruses, fungi, protozoa, rickettsia and nematodes have been described and many of them have been tested for their ability to control field infestations.

1.4.1 Bacterial Pathogens

Bacterial insect diseases have been described arising from both sporeforming or nonsporeforming bacteria (Falcon, 1971). Sporeforming bacteria of the genus Bacillus have been popular for biological control of insect pests because of the ease of economical mass-production by fermentation, good survival of endospore in nature, specificity and the lack of harmful effects on the environment (Burges and Hussey, 1971). Of these, Bacillus popilliae and certain varieties of crystal forming B. thuringiensis (which affect a wide range of lepidoptera) have been developed for commercial control.

1.4.1(1) Bacillus thuringiensis General Introduction

Bacillus thuringiensis Berliner (hereafter, B.t.) is a widely distributed, rod-shaped, sporeforming aerobic gram-positive bacterium. The vegetative cells of B.t. are peritrichously flagellated rods which often occur as filaments of four or more cells (Luthy et al., 1982).

B.t is at present the most widely used pathogen for microbial control of insect pests (Krieg and Langenbruch, 1981). It has been successfully and extensively used for the control of agricultural pests (Falcon, 1971) and has been tested against a wide spectrum of insects (including Lepidoptera and Diptera) in both laboratory and field (Angus, 1968). It is a complex species divisible in more than 20 varieties (or H - serotypes) by serological and biochemical tests, which produce several insecticidal toxins two of which (delta-endotoxin and beta-exotoxin) are used in agriculture and forestry (Dulmage, 1981; Fast and Regniere, 1984).

B.t. is reported to produce proteinaceous parasporal body a delta-endotoxin or crystal toxin (Hanny, 1953) which is toxic to target insects. It is the principal insecticidal component of present commercial preparations (Fast, 1981).

Susceptible caterpillars such as Heliothis spp., Trichoplusia ni, Ostrinia nubilalis etc. have the right combination of pH, salts and enzyme in their digestive system necessary to break down and activate the highly insoluble B.t. crystals (Dulmage, 1981; Couch and Ross, 1980; Bulla, 1975). The alkaline pH of the gut and enzymes dissolutes the crystals into toxic components which attack the lining of the caterpillars mid gut (Percy and Fast,1983; Faust, 1974). Osmotic balance is disrupted and the gut lining is abraded, permitting leakage of the alkaline gut contents into the insect haemocoel. The leakage from the lesions may be severe enough to kill the larvae and may cause changes within the larvae which allow growth of B.t. or other organisms resulting into septicaemia (Fast,1981, Bull, Rhodes and St. Julian, 1975; Couch et al., 1980). Damage to the larval digestive tract also causes it to stop feeding 15 min to 1 hr. after B.t. ingestion (Angus, 1956; Burgerjon,1962), and combinations of the gut leakage, lack of feeding and septicaemia usually kills the insect within one to several days (Fast et.al.,1984) depending on the dose, species and environmental conditions. The lack of feeding results in lower crop damage even if the caterpillar does not die quickly.

Because of its mode of action, B.t. is highly pathogenic and specific, being quite harmless to man,

other mammals, beneficial insects and fish and so it can safely be used in catchments and near water reservoirs (Kuteer, Lyashenko, Zurabora and Chekanov, 1983; Kumar, 1982; Krieg,et al., 1981). The major disadvantage of B.t. is the rapid disappearance of its activity in the field and its inability to spread in insect populations. The activity of B.t. sprayed on plants is rapidly destroyed by solar radiation (Burges, 1982; Norris, 1978; Burges, 1973; Pruett, Burges and Wyborn, 1980). It must therefore be applied as soon as new pests appear because it does not persist. It is thus suitable for the areas in which intense cultivation can be maintained.

Many successful laboratory and field trials have shown that preparation of B.t. containing the spore-crystal complex can effectively control lepidopterous pests in agriculture and forestry (Taylor, 1968; Berger,1981; Roehrich,1964; Daoust and Roome, 1974; Bullock and Dulmage, 1969; Schesser, 1976; Dulmuge, 1970; Waikwa and Mathenge, 1977; Creighton, McFadden and Cuthbert 1971; McGaughey, 1978). Today B.t. forms the corner stone upon which integrated pest management (IPM) is based (Couch et al., 1980).

According to a list of lepidopteran pests by Kreig et al.(1981) that have been tested for susceptibility to B.t., Maruca testulalis was reported to be susceptible to B.t. var. thuringiensis in the laboratory by Taylor

(1968). It is apparent from that list that no tests on the susceptibility of Busseola fusca and Chilo partellus was carried out. However three species of genus Chilo (C.auricilius, C. sacchariphagus indicus, and C. suppressalis) were reported to be susceptible to varieties of B.t. C. auricilius was reported to be susceptible to B.t. var.thuringiensis in the laboratory and in the field. C. sacchariphagus indicus and C.suppressalis were found to be susceptible to B.t. var thuringiensis in the field and in the laboratory respectively. C.suppressalis was also found to be susceptible to B.t. var thuringiensis and B.t. var. aizawai in the field. Berger (1982) reported the field efficacy of Thuricide commercial preparation of B.t. var. kurstaki in controlling C. partellus in maize crop. In his laboratory work with M. testulalis, Taylor (1968) found that early instars of this pest were more susceptible than mature instars.

1.4.1(2) The Discovery of B.t.

The discovery of B.t. is credited to Ishiwata in Japan, who described a severe flacherie or "sotto disease" in larvae of the silkworm (Bombyx mori) and also isolated the causative organism (Dulmage and Aizawa, 1982). However, he did not give a complete description of his isolate and the first microbiologically valid description of B.t. was made in Germany by Berliner who isolated the

Bacillus in 1911 from diseased larvae of the mediterranean flour moth (Anagasta kuehniella) and in 1915 he identified it as a new species and proposed the name Bacillus thuringiensis after the province of Thuringen, in which it had been discovered.

This bacterium was viewed as a potential tool for the control of insect pests and it was hoped that its release in areas infested by damaging insects would initiate epidemics that would reduce the insect population (Dulmage et al 1982). Early trials met with sporadic success, and interest was lost until Mattes (1927) reisolated the organism found by Berliner, again from A. kuehniella. The concept of the use of B.t. for insect control was revived, and Mattes strain was eventually adopted in France for commercial production of B.t.

1.4.1(3) The B.t. Varieties

B.t. is closely related to Bacillus cereus, and the only distinctive characteristic is the presence of a parasporal inclusion that exhibits insecticidal activity (Luthy et al., 1982; Kurstak and Tijssen, 1982). Some investigators had proposed B.t. to be considered as a variety of B. cereus (Dulmage, et al.,1982) but then Heimpel and Angus (1958 and 1959) suggested that although the various isolates did appear to be closely related to

B. cereus, the difference - particularly their pathogenicity to insects and the presence of a crystalline inclusion body were significant enough to warrant placing them in a separate species. Heimpel and Angus also proposed that B.t. be accepted as a species and also proposed a series of biochemical tests to identify varieties and suggested that Berliner's isolate be selected as the type culture for the species and be named B.t. var. thuringiensis (Dulamage et al., 1982). So Ishiwata's isolate was classified as B.t. var. sotto (Luthy et al.,1982).

The B.t. varieties can now be distinguished by comparison of the serological characterisitics of the flagellar protein (Kurstak et al., 1982) or by the electrophoretic patterns of the esterases produced in the vegetative cells (Norris, 1964). With this test 20 groups (variously referred to as varieties, strains, serovars) have been recognized as distinct varieties. Perhaps the most important isolate for the control of crop pests is B.t. var. alesti reported by Dulmage (1970) and designated HD-1. He found it to be pathogenic to pink boll worm (Pectinophora gossypiella) in the laboratory. De Barjac and Le Mille (1970) assigned the HD-1 isolate to var. kurstaki. Because of its activity, most commercial

B.t. preparations are based on variety kurstaki (Luthy et al., 1982; Dulmage et al., 1982)

1.4.1(4) The Laboratory and the Field Efficacy of B.t.

Since the discovery of B.t. several infectivity tests have been conducted in the laboratory to find out the susceptibility of different pests and also to compare the pathogenicity of different strains of B.t. (Frye, 1957; Splittstoesser, McEwen, 1961; Smirnoff, 1965; Ignoffo, Graham, 1967). Most B.t. serotypes have been found to be pathogenic for larvae of Lepidoptera. Krieg et al (1981) have outlined arthropod species that have been found to be susceptible to B.t. and also the variety of B.t. involved, in the laboratory or in the field.

Since B.t. products have to be ingested in order to have an effect, leaf feeding pests have been found to be the easiest to control. B.t. is particularly important for vegetable crops where no preharvest period has to be observed as is the case with other pesticides because of their chemical toxicity.

Cotton pests Heliothis virescens and H. zea have been shown to offer the biggest market for B.t. field use (Luthy et al., 1982). However, their control in the field has been very difficult (Pfrimmer, 1979) despite the fact

that both are susceptible to B.t. Cotton leaves have been shown to contain an antagonistic factor which interferes with the action of B.t. and satisfactory control is obtained only if B.t. is combined with chlorodimeform (Clift, 1979; Durant, 1972) insecticide.

Combination of B.t. with a low dosage of other chemical insecticides and other additives such as attractants and feeding stimulants has been shown to increase its activity (Sneh and Gross, 1983; Sorenson, 1977).

Other successful field results with B.t. control have been reported in the control of several pests of tobacco (e.g Trichoplusia ni), corn (e.g. Ostrinia nubilalis) and pine forest (e.g. Malacosoma disstria) (Luthy et al., 1982). B.t. has seldom been applied in fruit tree plantations. Although some pests e.g. Laspeyresia pomonella (codling moth) are highly susceptible in laboratory tests, their control under field conditions is not successful (Luthy et al, 1982).

B.t. has been good in controlling pests of stored grain e.g. Plodia interpunctella, Indian meal moth and Anagasta kuehniella, Mediterranean flour moth (McGaughey, 1978).

1.4.1.(5) Standardization of B.t.

Prior to 1954 there was no reliable method of establishing the effectiveness of B.t. except spore count related to the mortality achieved . However the discovery of crystal toxin (Hannay, 1953) which was demonstrated to be the toxic materials (Angus, 1954) solved the problem for a reliable index of potency. Since no chemical method could measure this potency, insect assay was the only alternative. A bioassay method was developed by Splittstosser et al., (1961) using Trichoplusia ni as a test organism which was fed on an artificial diet. A Colloquium on Insect Pathology and Microbial Control in Wageningen in 1966 recommended a formulation of B.t. from the Institute Pasteur, Paris France be adopted as the international standard and designated as being 1000 International Units/mg (IU/mg) (Dulmage et al., 1971). Although B.t. manufacturers have adopted secondary standards for their most important target insects, the activity is still based on a comparison with the international standard and expressed in the original IU (Luthy et al., 1982).

1.4.1(6) The Persistence of B.t. in Nature

Because of its high pathogenicity many

investigators have tried to establish whether B.t. is transmissable within the insect population. A little information about the presence of B.t. in nature and its transmission and behaviour under natural conditions is known (Dulmage et al., 1982). B.t. has been found to act like a chemical and not like an infective agent. It is a very inefficient infective agent but it is a very effective narrow - spectrum insecticide. It is commonly found in insects, but almost never causes epizootics in nature (Burges,1982).

Normally, B.t. commercial formulation is a mixture of viable spores and delta endotoxin. The spore has been shown to be sensitive to sunlight while the toxin is not (Cantwell and Franklin,1966; Frye, School, Scholz and Funke, 1973; Burges, Hillyer and Chanter, 1975). Although the spore plays a secondary role in pathogenicity of B.t. the knowledge of its persistence may help in evaluating the effect of adding B.t. to the ecosystem. Morris and Hilderbrand (1974) reported that there was a 93% reduction in the viability of spores after 24 hours upon direct exposure to sunlight; and yet spores of B.t. could persist for several months in shade areas of Pine forest (Dulmage et al., 1982).

Spores of B.t. have been found to persist for a considerable time in soil. Sahel Harris and Allen (1969,

1970a and 1970b) showed that B.t. can successfully survive in many types of soil although it failed to survive in acid soil. However B.t. can survive in soil but in a smaller population than other Bacilli naturally present (Dulmage, et al.,1982). However unlike the spore, the toxin does not persist in the soil (Pruett, Burges and Wyborn, 1980).

1.4.1(7) Commercial Formulations

B.t. is an ideal organism for large scale commercial production because it grows easily in submerged cultures using conventional fermentation equipment (Luthy et al., 1982) Stock cultures are best preserved as freeze dried samples. In addition, spores also retain their viability on agar slants for a long period of time.

The first commercial product containing B.t., Sporein, was produced in France before 1938 (Burges, 1971). Present worldwide production of B.t. is in the order of 10 million tons/ year (Otieno W.A., personal communication). In the United States, the two leading manufacturers, Abbott Laboratories (Dipel) and Sandoz, Inc. (Thuricide) produce about 1.5 million lb/year. The French company, Biochem Products S.A. (Bactospeine), produce more than 100,000 lb annually (Luthy et al., 1982).

Because of the stable nature of the insecticidal component of B.t., it has a long shelf life (Burges, 1982; Norris, 1978).

1.4.1(8) Safety of B.t.

Test on the safety of B.t. to mammals were reviewed by Heimpel (1971). Heimpel described the results of extensive test by many scientists on the safety of B.t. to mice, rats, guinea pigs, and human volunteers. Only in guinea pigs injected with extremely high concentrations of vegetative rods scraped from agar slants was there significant signs of toxicity. Dulmage et al. (1982) did an extensive review on the toxicity and pathogenicity of B.t. to a wide variety of wild and domestic birds, fish and mammals. He reported that there was no evidence of toxicity in any formulation (free of Beta-exotoxin) to any species except target insects.

Although B.t. apppears to have little direct effect on non-target insects, some workers have warned that indiscriminate use of formulations of B.t. could indirectly affect populations of beneficial parasites or predators by destroying the lepidopteran pests on which they normally prey (Dulmage et al., 1982).

1.4.2. Other Microbial Pathogens

More work has been done on fungi than other insect pathogen because of their easy growth in artificial medium (Stockdale and Priston, 1981). Fungal species of the genus Beauveria have often been isolated from diseased insects and frequently used in microbial control tests (Robert, 1981).

Growing interest has been placed by many investigators on protozoans for microbial control but although some are highly pathogenic and could effect short-term control, most species lack virulence . For control purposes, the microsporidian Nosema sp. has been much studied (Henry and Oma,1982).

In recent decades interests in nematodes as biological control has grown immensely and according to Finney (1981), Neoplectana spp. have been used widely to control forest, soil and plant pests primarily because of their wide host range and ease of mass propagation.

Much research has been done on viruses as possible control agents, and in particular, those of occluded type. Much work has been done on the production of nuclear polyhedrosis viruses (NPV) in lepidopteran insects (in_vivo production (Stockdale and Priston,1981).

1.4.3. The Modes of Action of Microbial Insecticides.

B. popilliae and viruses control their hosts by virtue of their ability to multiply extensively in the infected bodies (Norris, 1978). Because of its ability to recycle in the environment within the host pest (*Popillia japonica*) population, *B.popilliae* is persistent and therefore maintains the pest population at low level. Studies have shown the presence of milky disease in residual pest populations, 25 to 30 years after original application (Klein, 1981; Burges, 1982).

The efficacy of *B. thuringiensis* is concerned very largely with toxin synthesized during the growth of the bacterium and the multiplication of the microorganism in the target insect (Cooksey, 1971; Norris, 1978; Dulmage, 1981; Burges, 1982). The effectiveness of this bacterium does not persist and therefore calls for repeated applications.

Many entomogenous fungi overcome hosts presumably by toxin production (Roberts, 1981) while protozoans, for instance, *Nosema* kill their hosts by rapid germination and multiplication in the host tissue (Henry *et al.*, 1981).

1.4.4. The Role of Microbial Insecticides in Pest Control

Because of the ability of the above microbial pathogens to kill insects considered to be harmful, they have undergone fast development for the purpose of use in the field. Today, they are being used in integrated pest management (IPM) programmes for short term or long term control of pests. In short term control, the pathogen or its product is short-lived in the environment and therefore it has to be applied repeatedly like conventional chemical insecticides (Burges, 1982; Norris, 1978). Microbial insecticides based on B. thuringiensis and occluded viruses are used for short term control of a wide range of lepidopterous defoliators in agricultural crops and forests. B. popilliae, has however been used in long term control of Japanese beetle, Popillia japonica in grass fields in climatically and ecologically suitable areas (Klein, 1981).

1.4.5 The Advantages and Disadvantages of Microbial Insecticides.

1.4.5(1) Advantages

The advantage of microbial insecticides is the

presence of a resistant stage in majority of them which are very resistant to adverse enviromental conditions. For instance spores, in the case of sporulating bacteria, have a long shelflife as dusts, wettable powders and stabilized emulsions (Burges, 1982). Because of their high specificity and pathogenicity to target pests, they have proved to be remarkably safe for man, other mammals and non-target fauna and flora (Norris, 1978, Burges, 1981, 1982). Although the cost of production was found to be higher than chemical insecticides currently accepted safety-test requirements are much less expensive than chemical insecticides; hence development costs are correspondingly lower (Norris, 1978), . The possibility of using microbial insecticides with chemical insecticides may help in increasing their rate of action (Fast and Angus, 1965; Benz, 1971; Jacques and Morris, 1981; Salama, Foda, Zaki, Moawad,1984). The chemical insecticide dosage could also be reduced to levels which would minimize enviromental pollution. Also, synergistic combinations involving microbial agents could partially offset their disadvantage of being too specific when pest complexes must be controlled in a crop (Richter and Fuxa, 1984)

1.4.5(2)Disadvantages:

The necessity to determine the exact time of

treatment because of the incubation period of disease and the possibility of controlling only one or few species of insect sensitive to the pathogen are some of the major disadvantages of B.t. Also there is a need to maintain the pathogen or the preparation in a state of high viability before it becomes into contact with noxious insect. The influence of climatic conditions on the effectiveness of microbial insecticides is considerable. This is so particularly when entomogenous fungi are applied (Lipa,1975). Majority of microbial insecticide attack only via the oral route and act after ingestion and thus crop damage may occur before the pest is cleared (Burges,1982). Also there is great variability in microorganism and virulence may be lost (Lipa 1975).

O B J E C T I V E S

In Africa and in some developing countries elsewhere in the world there has been very little use of B.t. for pest control and correspondingly few laboratory or field tests conducted to evaluate their importance. It is against this background that this study was undertaken with objectives of evaluating the pathogenicity of B.t. against local cereal stem borers C. partellus and B. fusca and legume podborer M. testulalis in the laboratory and in the field under local enviromental conditions .

To accomplish these studies bioassay tests were carried out in the laboratory using B.t. var. kustaki strain HD_1 cultured in Nutrient Agar medium. Field trials were conducted using B.t. commercial formulations to evaluate the efficacy of control of these pests in their respective host crops.

C H A P T E R T W O

M A T E R I A L S A N D M E T H O D S

MATERIALS

The materials used in this study are as described in the method section. However, some special materials and equipment used included the following:-

Refrigerator - Electrolux Mod. TR 1171 9243526-10 Sweden.

Incubator - Heraeus Typ. BT 6042 E Hanau

Haemacytometer chamber - Tiefe Improved Neubauer Superior West Germany.

Vortex shaker - Heidolph No. 54113, Typ. REAX 1R West Germany.

Microscope (phase contrast) - Leitz Dialux 20 EB West Germany

Balance - Sartorius 1700 Typ.1712 West Germany.

Ultraviolet lamp - General Electric G30 T8 Germicidal Lamp U.S.A.

Chemicals used beside reagents and those chemicals commonly used in the laboratory include:-

Nutrient Agar - GIBCO EUROPE No. 152-3560

Nutrient Agar-Oxoid Code CM3

Nutrient Broth-Oxoid Code CM1

Triton X-100 BDH Chemicals Ltd.

Methods

The starter culture of B.t. was obtained from Dr. W. A. Otieno (Biological Control Unit ICIPE, Mbita Point Field Station). The bacterium was cultured on Nutrient Agar (NA) medium (Oxoid) at 30°C.

2.1.0. Media Preparation

The methods used in the preparation of the medium are as described by Poinar and Thomas (1978). A quantity (14g) of Nutrient Agar medium was weighed and put into a one litre conical flask. To the medium was added 500 ml. of distilled water and then the mouth of the conical flask was firmly secured with cotton wool rolled into a ball. The medium was then autoclaved for 15 min at 15lb/sq. in

pressure. The medium was then allowed to cool down to 45°C for easy handling and then poured aseptically in sterile plastic petri dishes, about 20ml per plate. The plates were preserved inverted after the medium had solidified and stored in the refrigerator at 5°C .

Nutrient broth medium for preserving B.t. was prepared according to the makers instructions. After mixing the dry ingredients of Nutrient broth with distilled water, it was dispensed into 25 ml universal bottles which were then fitted with screw tops and then autoclaved as indicated above.

2.1.1 Inoculation of the Medium with B.t.(HD_1)

The method adopted in this excercise was the streak plate method as outlined by Cantwell(1974), Poinar and Thomas (1978) and Baker and Silverton (1978). The inoculating loop was flamed along its entire length until red hot, then the holder was passed quickly through the flame a few times. The loop was cooled by dipping it into 70% alcohol and flaming off the excess. A loopful of inoculum was placed close to the edge of the agar in a petri dish, and spread at several angles. The plates were then incubated while inverted at 30°C for 24 hours.

Unless otherwise stated, the methods outlined above were used in all the subculturing excercises in this study.

2.1.2 Microscopy and Staining Procedures.

After 24 hours, the culture was examined with a dissection microscope and colony characteristics were noted, as outlined by Cantwell (1974).

A wet mount was done by picking up bacteria with a sterile inoculating loop and then placing them on a drop of distilled water on a slide. The suspension was then covered with a cover slip and then observed under the microscope.

Gram stain procedures were carried out as described by Cantwell (1974), Poinar and Thomson (1978) and Baker and Silverton (1978) to ascertain the Gram reaction of the culture.

2.1.3 Spore and Crystal Delta-endotoxin Staining.

A B.t.(HD-1) culture that was more than 72 hours old was used. The procedures of staining were as

described by Lipa (1975). The reagents used were 5%(w/v) water solution of malachite green and 0.2%(w/v) water solution of safranin.

A dried smear of B.t. was fixed over a flame and the slide was then flooded with malachite green, then heated until steam rose from the surface of the solution. The preparation was then rinsed for 30 sec in water, blotted dry and counterstained for 15 min in a 0.2% water solution of safranin. The slide was then rinsed, blotted dry and studied under the microscope.

2.2.0. Preliminary Tests on the Pathogenicity of B.t.(HD-1) to C. partellus, M. testulalis and B.fusca .

2.2.1 Recovery of B.t. Culture

A B.t. culture grown on nutrient agar and incubated for 72hours was used. Growth was removed from the medium surface into a sterile conical flask by washing with sterile water from a wash bottle. A strong L-shaped glass rod was used to assist in detachment of growth from medium surface. The bacterial suspension was vigorously shaken until it appeared homogeneous. Further mixing was done by use of Heidolf vortex shaker for 2 min.

2.2.2 Spore Count.

Several methods can be used in establishing the activity of microbial insecticides as described by Burges and Thomas (1971). Among them is the spore count. Spore counts were carried out using an Improved Neubauer haemocytometer following the procedures described by Cantwell (1974) and Baker and Silverton (1980). The concentration of the B.t.(HD-1) suspension was found to be 1×10^9 spores/ml.

2.2.3 Pests used

Larval instars of C. partellus, M. testulalis and B. fusca were obtained from the Insect Mass Rearing and Technology Section, ICIPE. C. partellus was reared on artificial diet while M.testulalis and B.fusca were reared on a natural diet of cowpea (flowers and pods) and sorghum (leaves and stems) respectively at room temperature (27 ± 2C).

2.2.4 Food Materials used in the tests.

In the bioassay the food materials used were the natural food materials for the pests. Thus sorghum (var. serena) plant was used in case of C. partellus and B.

fusca and cowpea plant (ex-Luanda) was used in case of M.testulalis. First, second and third instar larvae of C. partellus and M. testulalis were fed on leaves and flowers respectively; while 4th and 5th instars were fed on stems and mixture of flowers and pods. B. fusca larvae were fed on stem only.

2.2.5 Inoculation of Food Materials.

The stems and leaves were previously cut into sizes which would fit in the petri dishes.The food materials were contaminated with B.t. by complete immersion in B.t.(HD-1) suspension (Frye, 1967; Hall and Dunn, 1958; and Sneh, Silvia Schuster and Broza 1981), in a 100 ml. beaker. To contaminate the stems, they were split open by making a single radial cut through the entire length of the stem prior to immersion. The contaminated food was then put in the petri dishes holding the test larvae. A control experiment was set as described above but using food materials dipped in sterile distilled water.

2.2.6 Bioassay

The bioassay was conducted at room temperature ($27 \pm 2^{o}C$). For every instar ,50 larvae were used. A test using 10 larvae in a petri dish was conducted and

replicated four times. In the case of B.fusca, only 5th and 6th larval instars were available for test.

The time of setting the experiment was observed and noted. The number of dead larvae was noted and recorded every 12 hours until all the larvae in the test experiment were dead. A control experiment was set using ten larvae for each instar being fed on uncontaminated food.

2.2.7 Recovery of B.t. from Dead Larvae

Some of the dead larvae were dissected on glass slides using a dissecting needle. Using a sterilized inoculating loop, the haemolymph was streaked onto an agar plate and incubated at $30^{o}C$. Some dead larvae were also mounted directly on a Nutrient agar plate and the growth of B.t. observed after 24 hours.

2.3.0 The Effect of B.t. on Starved and Non-starved Larvae

A culture of B.t.(HD-1) (incubated for 3 days) previously harvested and made into suspension was used. Its spore count had been determined to be 8.8×10^{7}/ml. Serial 1 in 2 dilution to give further theoretical concentrations of 4.4×10^{7}/ml, 2.2×10^{7}/ml and 1.1×10^{7}/ml, of spore was done. These B.t suspensions of different spore concentrations were used in inoculating the test-larvae food material.

Portions of sorghum leaves and cowpea flowers were cut (prior to contamination) into circular discs of 10mm in diameter using a cork-borer and used for feeding C.partellus and M. testulalis respectively. Sorghum stems cut into small pieces (2cm) were used in the case of B. fusca. Inoculation of these food materials was accomplished by immersing them in the required concentration of B.t. suspension and then placed in a 20ml thunderbolt cup whilst still moist .

Contaminated food was offered to the larvae by placing the pieces on the floor of a thunderbolt cup. One piece of leaf, flower or stem was used per cup. The leaf and flower pieces were prevented from rapid drying by laying a moist filter paper on the floor of the thunderbolt cup which was cut in such a manner that it was not in contact with the food materials. Each cup's cover was then replaced after putting in the test larva.

The available fourth instar larvae of C. partellus and M. testulalis were used while sixth larval instars were used in the case of B. fusca because of availability. A number of 50 larvae of each pest-type was used for each assay. To starve the larvae, they were isolated from the rearing diet and placed in empty petri dishes (10 per petri dish) and left to starve for 12 hours (Broersma, Buxton, 1967; Sneh, Silvia, Schuster and Broza,

1981). The non-starved larvae were left in the rearing diet until the time of experimentation. Each larva was fed in an individual thunderbolt cup.

The recording of the dead larvae was done after every 6 hours during which moisture and fresh food materials were added if necessary. The percentage mortality achieved after three days was calculated. The final percentage mortality was corrected in relation to the control by Abbots formula(Busvine, 1977).

$$P_T = \frac{P_o - P_c}{100 - P_c} \times 100$$

where, P_T = Corrected mortality,

P_o = Observed mortality,

P_c = Control mortality.

2.4.0 Dose Response Mortality of C. partellus and M. testulalis 2nd to 4th Instar-larvae Exposed to Various Concentrations of B.t. (HD-1).

B.t. colonies were recovered, prepared into suspension and spore number determined as before. The spore count was determined to be 8.2×10^7/ml.

The original B.t. suspension (8.2×10^7/ml spores) was diluted so that five different concentrations were obtained. Serial 1 in 2 dilution in sterile water to give theoretical concentration of 4.1×10^7/ml, 2.05×10^7/ml, 1.025×10^7/ml and 5.0125×10^6/ml. was carried out. The concentrations were checked by spore count and true concentrations were determined and were found to be 4.28×10^7/ml, 1.96×10^7/ml, 1.04×10^7/ml and 4.16×10^6/ml spores. The B.t. suspensions were preserved in the refrigerator ($5^{\circ}C$) for later use.

2.4.1 Bioassay.

Second to fourth instar-larvae of C. partellus and M. testulalis were used after starving them for 12 hours. The food materials used were sorghum leaves and cowpea flowers cut into small circular discs, 6mm (so that only a small amount of B.t. was consumed) in diameter, using a

cork-borer as described earlier. Each larva was fed individually in a thunderbolt cup containing inoculated food. A batch of 50 larvae per test was used. In a control experiment, leaf and flower discs of the same size were used after dipping in sterile distilled water. Ten larvae of each pest were used in the control. The readings were taken after six hours during which the number of dead larvae were recorded. The final mortality was corrected as mentioned earlier by Abbot's formula as before. The LD_{50} for every instar was estimated after plotting mortality transformed into probits against log of concentration of inoculating B.t. suspension (Finney,1952).

2.5.0 Evaluating the Effect of Some Relevant Physical Factors on the Virulence of B.t.(HD-1) in the Laboratory

The effect of various enviromental factors on a B.t. suspension (8.8×10^7/ml spores) was assessed using third larval instars of C. partellus and M.testulalis for assay. Each larva was fed individually in a thunderbolt cup on contaminated natural food materials (cut into circular discs of 10mm diameter) as described earlier. In a single experiment the total number of larvae used for tests and controls was 50.

2.5.1 Temperature

Six 5ml samples of B.t. suspension in sterile universal bottles were used in the experiment. Each of the samples was exposed to a different temperature viz, $5^{o}C$, $25^{o}C$, $45^{o}C$, $65^{o}C$, $85^{o}C$ and room temperature ($27 \pm 2^{o}C$) for 8 hours. For $5^{o}C$, a refrigerator set at this temperature was used. while an incubator adjusted to the required temperature was used for the rest.

2.5.2 Ultraviolet Radiation(U.V)

A B.t. suspension was divided into nine samples of 15ml each and placed in separate sterile petri dishes . The samples were then placed within a Steril Gard Hood ^{R} 70 cm. beneath an electric Germicidal Lamp G30 T8 0.7 Amp . All the samples were irradiated for a different period, the times of exposure being 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes. The water loss due to evaporation was replenished by maintaining the original volume with sterile distilled water . A control experiment was set up in which samples of the same B.t. suspension were not exposed to U.V.

Bioassay of the B.t. suspension was then conducted using third larval instar of C. partellus as described in the previous section.

2.5.3 Solar radiation

The B.t. suspension was divided into 15 samples of 4 ml each and put in Universal Glass bottles. The fifteen samples were divided into 3 groups and each group subjected to one of the following regimes:-

i) Sunlight between 10.00 a.m and 4.00p.m. for different periods of 5, 25, 50, 100 and 150 hours.

ii) As (i) above outside in the shade under a piece of hardboard sufficiently high above the bottles to allow normal ventilation. The shaded samples were placed adjacent to the sunlight exposed B.t. samples.

iii) The other 5 samples were kept in darkness at room temperature for the same periods of time as above.

Each of the B.t. suspension samples was then bioassayed using third larval instars of C. partellus and M. testulalis as detailed earlier.

2.5.4 Persistence of B.t. on Sorghum Leaves in the Screenhouse

A suspension of B.t. with spore count of 7.4 x 10^7/ml was prepared as described earlier. A drop of Triton x - 100 detergent was added to the suspension to

ensure an even coverage of leaf surface (Sneh et al., 1981). The suspension was then put in a 1 litre compressed - air hand sprayer (Baygon^{R}). The spraying was done once on potted sorghum plants (3weeks old) in the screenhouse. Spraying was done from above covering the upper side of the leaves. Sorghum leaves were picked after 24, 48, 72, 96, 120 and 144 hours and taken to the laboratory for use in the bioassay with C. partellus third instar larvae as described earlier. In the control, the larvae were fed on sorghum leaves sprayed with distilled water alone.

2.6.0 Larvicidal Activity of B.t. Commercial Formulation .

B.t. commercial formulations -"Dipel" (Abbot Laboratories), "Thuricide" (Sandoz Ltd) and "Certan" (Biochem Ltd) were evaluated against C. partellus and M. testulalis third instar larvae in the laboratory.

The physical properties of the formulations were described by the manufacturers. Thus, Dipel was reported to contain 32,000 International Units (I.U.) per mg based on bioassay with cabbage looper Trichoplusia ni (Hubner); Thuricide containted 16,000 I.U. per mg based also on a bioassay with T.ni. No details were given about Certan except the batch No. Sandoz SAN 415 I WG 03 Lot 228-1.

An aqueous suspension of each test material was prepared at desired concentration and used for inoculating the food materials. B.t. concentrations of each formulation equivalent to a field application rate of 0.75 kg/ha were made by weighing 16mg powder and dissolving in 10ml of sterile distilled water in a 100 ml conical flask. The mixture was thoroughly mixed with a vortex shaker (for 2 min). Three further dilutions were prepared (0.16mg/ml, 0.016mg/ml and 0.0016 mg/ml) by serial dilution and used in the bioassay.

Bioassay was then conducted as previously described using third instar larvae of C. partellus and M. testulalis and mortality recorded over a period of three days.

2.7.0 Mortality of C. partellus third instar larvae due to Dipel, Thuricide and Certan in the screenhouse.

Sorghum (var. serena) was planted in the screenhouse in four small blocks measuring 2 x 2m. There were five rows 50cm. apart and 15 cm from plant to plant in a row. When the plants were 41 days old, ten laboratory reared third instar-larvae of C.partellus were released on each plant by putting in the leaf funnel using fine

forceps. The first four rows of each block were infested with the larvae leaving the fifth row uninfested.

Three of the blocks were sprayed with Dipel, Thuricide and Certan respectively at a concentration of 0.75 kg/ha using a compressed-air hand sprayer 8 hours after infestation. During spraying, a polythene sheet was put around each block to prevent contamination with drifting spray droplets.

Sampling was done one day after infestation by randomly picking one plant per row. Each plant was dissected to recover the borers. This was done daily for six days. Each row represented one replicate.

The percentage mortality due to each biocide was then calculated

2.8.0 Efficacy of B.t. Commercial formulations in field trials.

The studies were conducted at the Mbita Point Field Station (MPFS) of the ICIPE on the shores of Lake Victoria. The Station is located at latitude 0^{o} 25'S and Longitude 34^{o} 10'E at an altitude of about 1000m above sea level. The experiment was carried during the Long Rains Season (February - July, 1986).

2.8.1 Agronomy

Sorghum (S. bicolor var. serena) and an early maturing variety of cowpea (Ex-luanda),V. unguiculata (L. Walp) were planted in pure and intercrop stands. Each block measured 19 x 4 m and was subdivided into four 4 x 4 m plots, leaving a space of 1m between adjacent plots as guard rows. The experiment was a complete randomized block design, replicated four times. The spacing of sorghum was 75 cm between rows and 30 cm between hills (equivalent to 44,375 plants/hectare) and the spacing of cowpea pure stand was 50cm between rows and 30 cm between hills (equivalent to 66,668 plants/hectare).

Monophosphate fertilizer was applied at the rate of 100 kg/ha before planting and ammonium nitrate fertilizer was applied at the same rate 21 days after plant germination (DAG). Cowpea was not top dressed. Plants were thinned to one per hole after emergence and one (original) was maintained per hill during the time of experimentation until the time of harvest by constantly removing the tillers in the case of sorghum.

Other normal management practices were followed during the course of the trials.

2.8.2 Microbial Applications

In each planting pattern (i.e. pure sorghum, pure cowpea and sorghum/cowpea intercrop) there were four treatments distributed in the four plots per block. The treatments consisted of 3 types of commercial formulations of B.t. viz, Dipel (Abbot Laboratory), Thuricide (Sandoz Ltd) and Certan (Biochem Ltd), and a control where there was no B.t. application. The treatments were randomized within the block. Each B.t. formulation (wettable powder) was dissolved in water and then sprayed on sorghum and cowpea using a compressed-air hand sprayer (Baygon^{R}) (plate 1). The concentration of B.t. was maintained at 0.75 kg/ha, a dosage falling within the manufacturers recommendation for the control of caterpillar larvae.

Cowpea was sprayed every week starting with the onset of flowering (Okeyo-Owuor et al., 1983) from about 35 days after germination (DAG) until the time of harvest. Sorghum was sprayed 3 days after artificial infestation (15 DAG); then every week for five weeks, and thereafter fortnightly until the time of harvest.

2.8.3 Pests

Damage caused by two major pests C. partellus and M. testulalis found in sorghum and cowpea respectively,

Plate 1: Spraying of cowpea and sorghum in the field using B.t. commercial formulations; using a cheap locally available compressed-air hand sprayer (Baygon^{R}).

was assessed. However, the presence of other stem borers in the sorghum crop such as B. fusca, E. saccharina and S. calamistis was observed and recorded. Sorghum plants were artificially infested with 10 first larval instar of C. partellus per plant 12 DAG. It has been reported that natural infestation of sorghum by C. partellus occurs between the first and the third week after plant emergence (Seshu Reddy, 1983). Artificial infestation was done by picking the larvae from hatching eggs in petri plates using a fine hair brush, and placing them in the whorls of each plant. The exercise was carried out early in the morning before the sun was too hot to damage the fragile larvae. No artificial infestation was carried out in the case of cowpea since natural infestation is high at the station.

2.8.4 Sampling

Sampling in sorghum commenced 3 weeks after germination, i.e. approximately one week after treatment, and continued weekly until the time of harvest thus covering seedling, vegetative and reproductive stages of the crop. Two plants were sampled at random from each treatment per week giving a total of 64 sampled plants each week. The first outermost row on either side of the plot and the first three plants from each end of the row

were used as guard plants and were not sampled. When sampling, the following parameters were used in assessing the borer damage; plant height, number of larvae recovered from each plant and stem tunnelling. Addition sampling was done during the ninth week after germination to assess the amount of leaf injury and the number of "dead Hearts" in plants selected at random.

Sampling was done by uprooting the whole sorghum plant. The total plant height was measured and recorded. The damage on the leaf (assessed by the amount of eating or feeding on the leaf) and the number of "dead hearts" were recorded at flag leaf when all leaves were formed (9 weeks after germination in the case of Serena variety).

A grading of leaf injury was as follows :-

1-3 - a low intensity of leaf injury,

4-6 - a medium(intermediate) of leaf injury

7-9 - high intensity of leaf injury

as used by Guthrie , Dicke and Neswander (1960), Kishore (1984) and revised by Saxena and Kibuka (in press) (plate 2).

"Dead hearts" (plants with partially or entirely dried shoot) were recorded by taking a random sample of ten plants and then counting the number of "dead hearts" (plate 3).

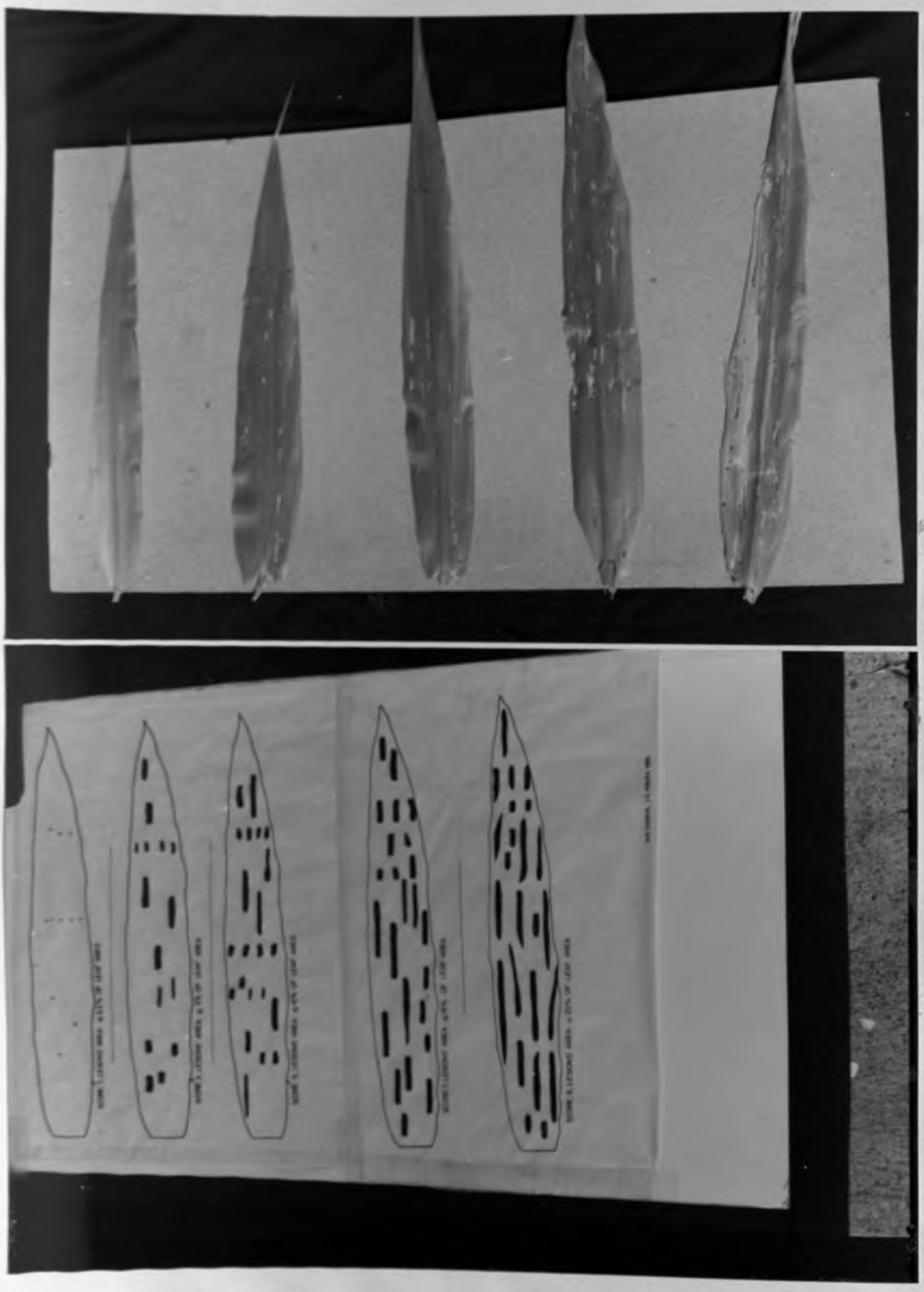


Plate 2: FOLIAR DAMAGE: Leaf damage was assessed and graded using the chart given.

Plate 3: "DEAD HEARTS": In the control sorghum stands where B.t. was not applied most plants infested by stem borers did not mature but died early, thus reducing the plant population in that stand.

The stem of sorghum plants were split open and observation made on the number of larvae, pupae and pupal cases of stem borers; both live and dead larvae and pupae were noted . For measuring stem tunnelling, plants were split into halves lengthwise to record the total stem length tunnelled by the borer larvae(plate 4). The percentage stem length tunnelled was thus worked out for each treatment. The percentages were converted to "arcsin" values for statistical analysis (Kishore, 1984). Harvesting was done 15 weeks after germination when the sorghum panicles of 5 plants (randomly selected from each plot) were put into individual paper bags, and dried in the sun for one week, after which they were thrashed and the grain yield weighed.

Sampling in cowpea started 5 weeks after germination and involved uprooting five plants from each treatment using the following parameters for assessing the damage by pod borer:- the total number of flowers per plant, the number of damaged flowers per plant, the number of larvae in the flowers, total number of pods per plant, number of damaged pods per plant and the number of larvae in pods. After harvesting the total number of seeds per plant, the number of damaged seeds per plant and seed yield in tons/ha were recorded. The percentage of damaged

Plate 4: STEM TUNNELLING: It was used as one of the parameters to assess damage by stem borers. Infestation by borers early in the growing season corres ponded with tunnel, appearing in the lower side of the stem while late infestation corressponded with tunnels appearing on the upper side of the stem, in most of the plants.

flowers and pods were calculated since the number of flowers and pods sampled per plant was not constant. The percentages were converted to "arcsin values" for statistical analysis as required. All data were subjected to two way Analysis of Variance and means separated at P = 0.05 by Duncan's Multiple Range test (DMRT).

2.8.5 Disease Diagnosis

Dead larvae were carried to the laboratory to determine the cause of death. Where B.t. symptoms were obvious (brown/black cadavers) the larvae were not examined further. A simple method of diagnosis was conducted as described by Poinar and Thomas (1978). This involved examining a wet mount of the insects haemolymph under the phase contrast microscope.

A record of the parasites of the stem borers was made by collecting the parasitized larvae and parasites that emerged from the collected dead larvae.

CHAPTER THREE

RESULTS

3.1.0 Growth of B.t. (HD-1) on Nutrient Agar.

B.t. growing on the surface of Nutrient Agar appeared whitish-cream in colour (plate 5). The growth was very fast and within 24 hrs at 30°c , a single colony had attained a diameter of more than 1 mm. The colonies were circular in shape but became irregular and non uniform as they grew. The margin of an individual colony was undulating . The colonies were flat, thin and raised little above the agar surface; the surface of the colonies was smooth and even.

Wet mount examination under the microscope showed motile rod-shaped B.t. cells with an average dimension of 3-5x1-1.5 µm . They appeared as single rods, pairs and short chains.

3.1.1 Gram Reaction.

The Gram stain revealed the Gram positive property

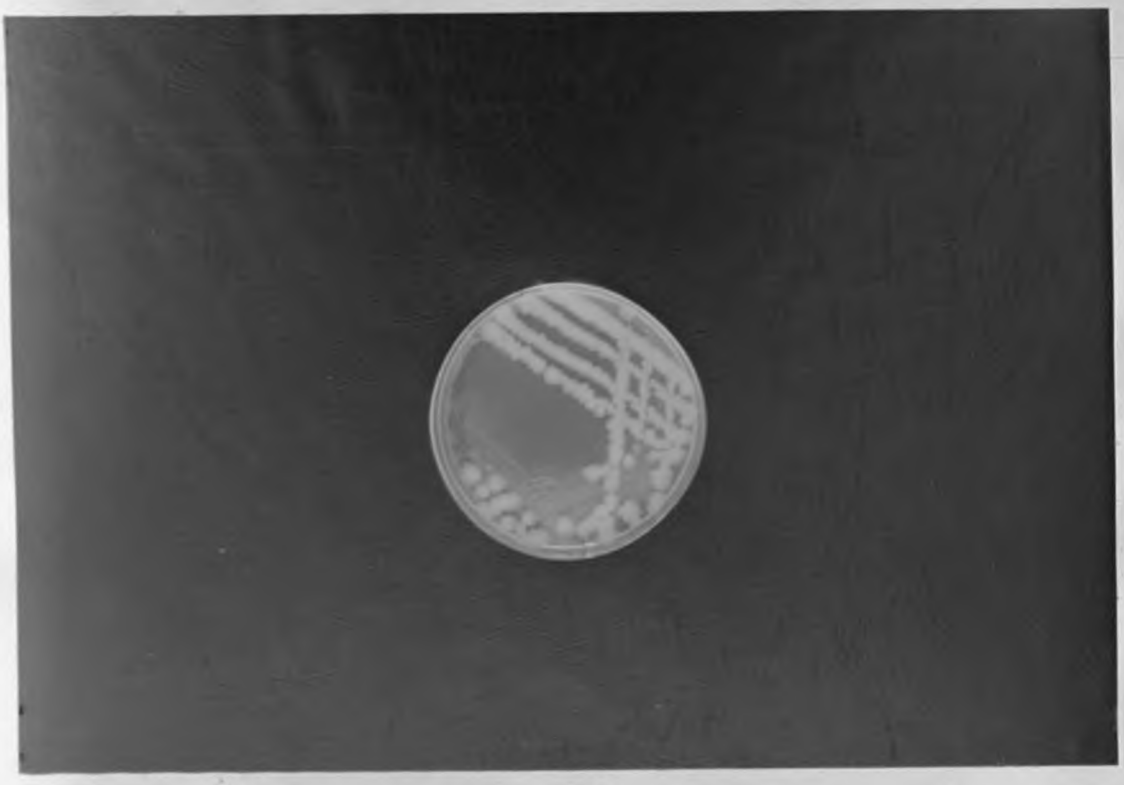


Plate 5: IN-VITRO GROWTH: B.t. (HD-1) colonies on the Nutrient Agar plate.

of this bacterium (Plate 6). Stain procedures for spores by Lipa (1975) showed green, oval-shaped spores and reddish pink, diamond shaped delta endotoxin crystals (Plate 7).

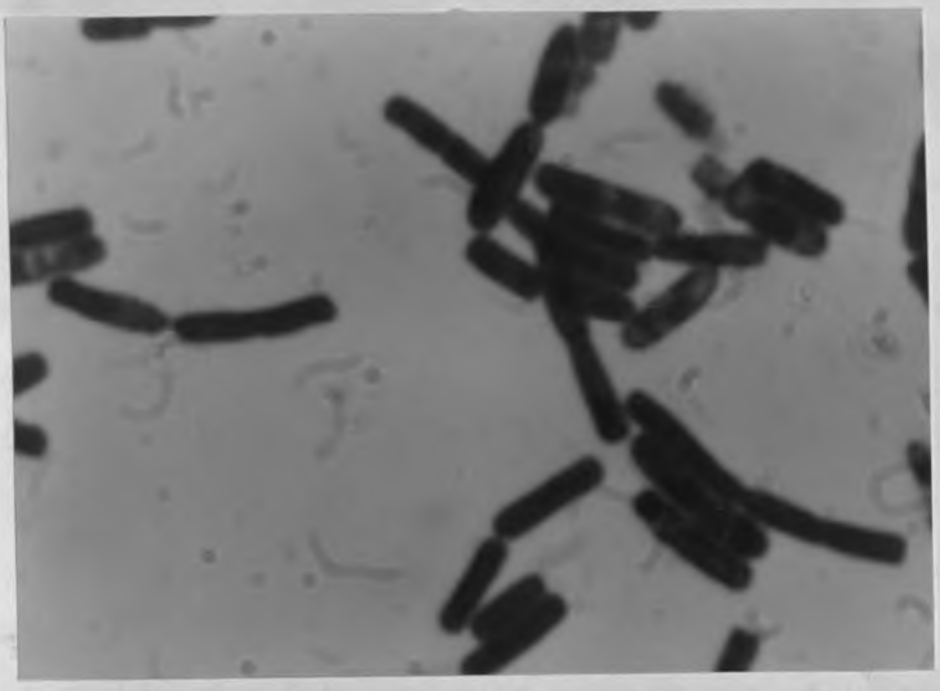
3.2.0 Pathogenicity of B.t. to the Test Larvae.

Soon after setting the larvae in the petri plates containing food materials, they moved around before settling to feed.

After about 30 min. most larvae were settled and were feeding on the materials provided especially in the control experiment. After about 2hr. visible damage to the food material was evident in the control experiment.

By the 6th hour, larvae in the control had fed on a larger proportion of the food than larvae in the tests which showed only slight signs of feeding. Most larvae in the test experiment at this time had hidden under the food materials provided .Most of the younger instar larvae in the test experiment were dead while the mature larvae appeared sluggish and paralysed, later the larvae became flaccid and moribund and death followed. Larvae that ingested

(6)



(7)

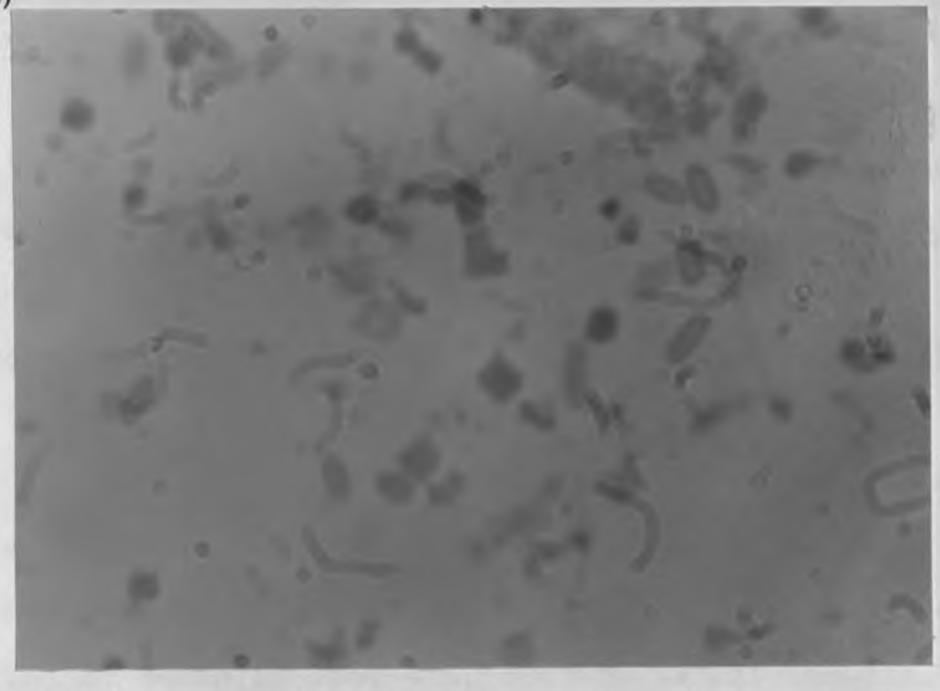


Plate 6: VEGETATIVE CELLS: Gram positive rod shaped cells of B.t. (HD-1)

Plate 7: CRYSTAL AND SPORES OF BACTERIA: Green oval-shaped spores and reddish pink, diamond shaped delta endotoxin crystals.

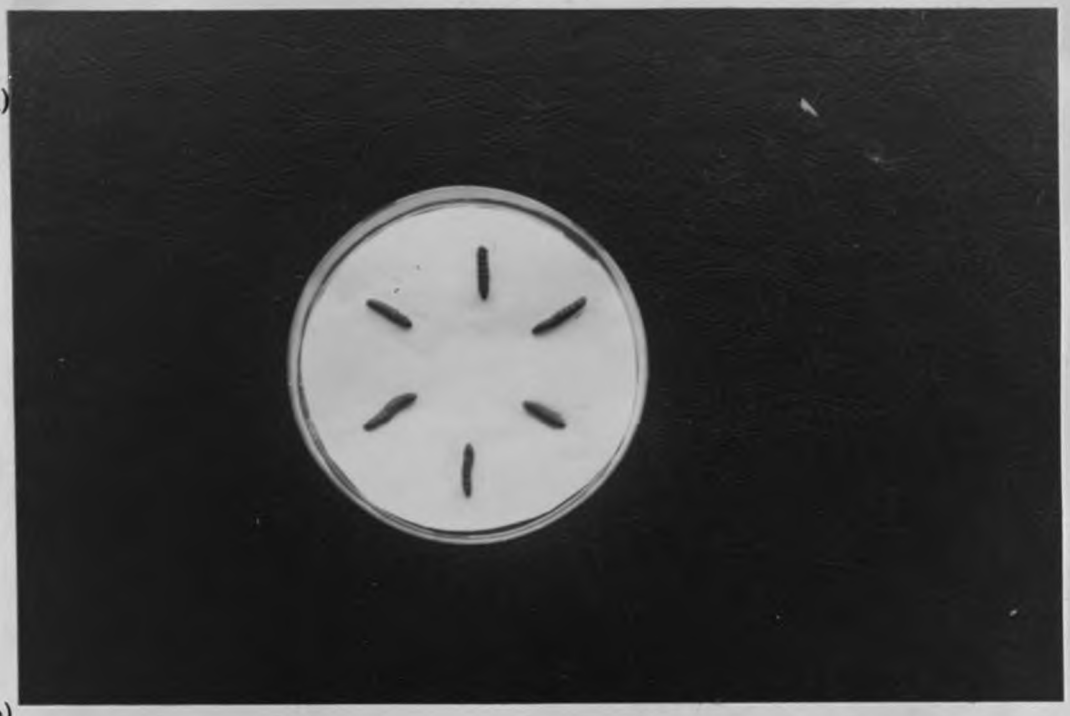
B.t. at even the lowest concentration were slow and weak in their feeding behaviour before death.

One day after the start of bioassay the majority of the larvae in the test trials were dead. The dead larvae soon changed colour to brown or black 30 min after death. This was mostly noticeable in C. partellus which turned black immediately after death (Plate 8). Dead larvae were quite soft and often ruptured when touched releasing haemolymph . B.t. killed larvae mounted on a Nutrient Agar plate resulted in the formation of characteristic B.t. colonies growing around it after incubation at 30 ^{o}c for 24 hours (Plate 9).

The time of death varied from one larval instar to the next (Table 1). The young instars of M. testulalis and C. partellus (1st and 2nd) were significantly ($p=0.05$) more susceptible than the mature instars (4th and 5th).

For C. partellus, the duration before death of the 1st and 2nd instars were significantly different. However, there was no significant difference between the time of death of 3rd, 4th, and 5th larval instars. In the case of M. testulalis, the time of death of 1st instars was significantly different from that for the 2nd and 3rd instars which was significantly different, from that

(a)



(b)

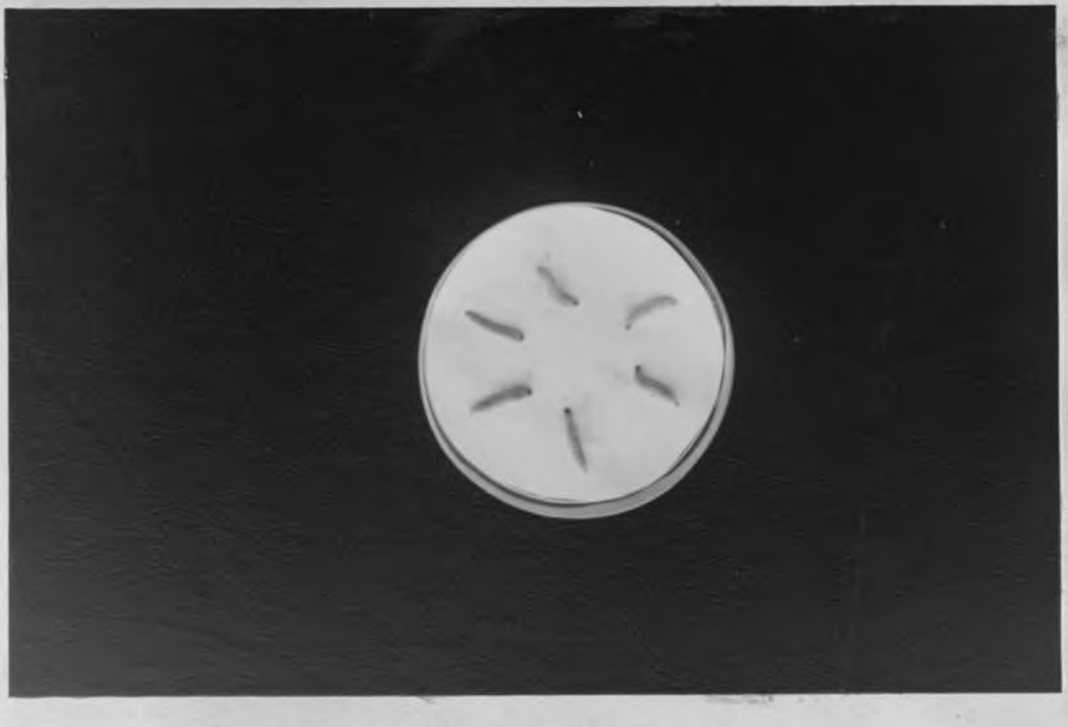


Plate 8: SYMPTOMS: Larvae that ingested B.t. turned black/brown soon after death(a). This was in contrast to larvae killed with chloroform which retained there original colour (b).

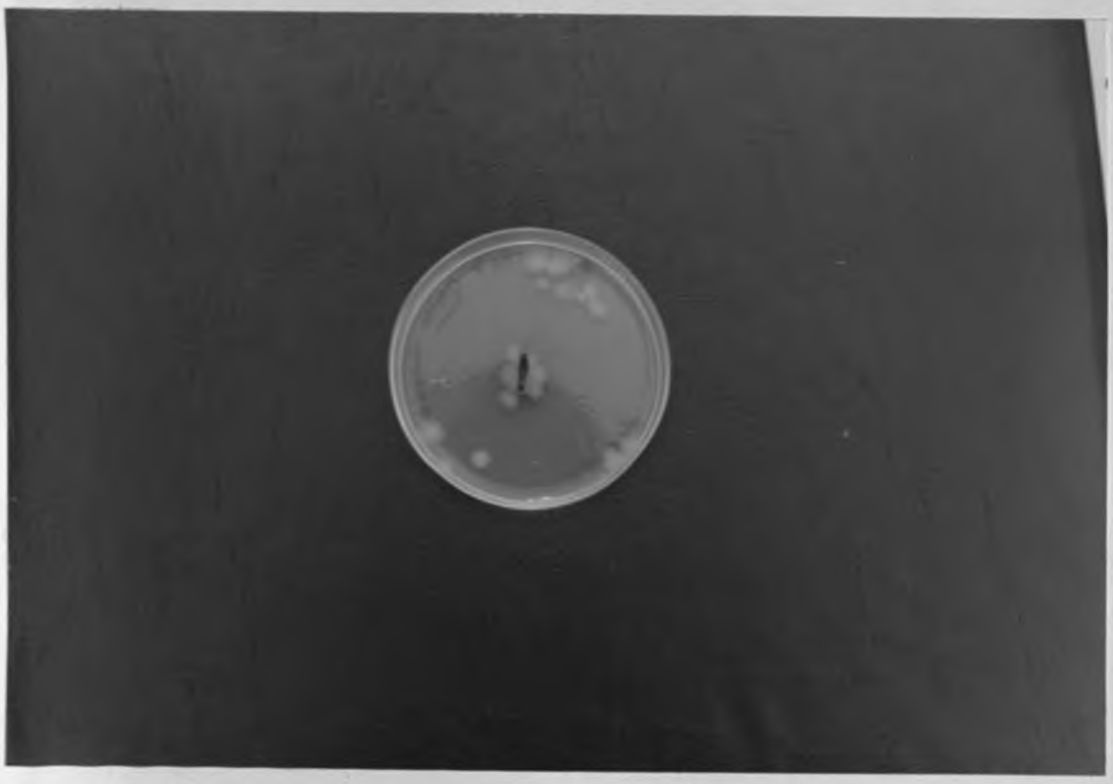


Plate 9: BACTERIA PATHOGENICITY: B.t. (HD-1) colonies growing around a dead larva that had been fed on food materials contaminated with B.t.

Table 1: Time of death of different larval instars of C. partellus exposed to Bacillus thuringiensis (HD-1) spore count 1 x 10^9/ml for three days.

(a) C. partellus

INSTARS	REPLICATES 1	2	3	4	TOTAL	MEAN (No.hr.)	±S.E
1.	18.6	16.8	15.8	15.0	66.2	16.45^{b}	0.8
2.	24.6	19.8	19.8	19.8	84	21.00^{ab}	1.2
3.	16.8	27.0	24.0	25.8	93.6	23.4^{a}	2.3
4.	19.2	26.8	20.2	25.2	91.4	22.85^{a}	1.9
5.	23.4	27.0	25	23.4	98.8	24.75^{a}	0.9

F- value at d.f. (3,12) is 4.42 (Appendix Ia).
Means followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.

N.B. Duncan multiple range test is used to separate means where calculated F - value has turned out to be significant.

for the 4th and 5th instars (Table 2). The mean time of death of 5th and 6th instars of B. fusca was lower than that of 5th instars of C. partellus and M. testulalis. It was 19.50 and 22.70 hours for 5th and 6th instars respectively. 1st 2nd and 3rd instars of M. testulalis were more susceptible than the corresponding instars of C. partellus .

3.3.0 Effect of B.t.(HD-1) on Starved and Non-starved Larvae of C. partellus and M. testulalis.

Exposure of the larvae to stavation (12 hours before bioassay) increased their susceptibility to B.t except with B. fusca at concentration of 2.2×10^7/ml.

before bioassay) increased their susceptibility to B.t. (Fig. 1). The mortality of the larvae in 72 hours increased as the B.t. concentration increased.

Starved larvae were therefore used in subsequent experiments because they showed a linear response in mortality with concentration of applied B.t.

3.4.0 Larval Instars Dose-response.

From the results obtained it was quite clear that the cumulative mortality of each of the larval instars of C. partellus and M. testulalis increased as the time of exposure to B.t to a given concentration increased ex e t with 3rd and 4th instars of C. partellus (Fig. 2,3 and 4). At each B.t. concentration, 2nd instars died faster than 3rd and 4th instars (in that order) second and

Table 2. Mortality of different larval instars of *M. testulalis* exposed to *Bacillus thuringiensis* (HD-1) spore count $1x10^9$/ml for three days.

INSTAR	REPLICATES 1	2	3	4	TOTAL	MEAN (No.hr.)	± S.E
1.	13.2	10.8	10.8	13.8	48.6	12.15^c	0.8
2.	16.6	19.8	20.0	21.6	78.0	19.50^b	0.5
3.	20.0	19.8	20.0	19.2	79.0	19.75^b	0.2
4.	19.2	28.8	26.6	26.6	100.8	25.20^a	2.1
5.	24.0	28.8	29.6	24.0	106.4	26.60^a	1.5

F-value at d.f. (3,12) is 21.39 (Appendix Ib)
Means followed by the same letter are not significantly different (P=0.05) by Duncan's multiple range test.

Figure 1: Percentage mortality of starved and non-starved crop pests after 72 hours of exposure to B.t. (HD-1). Fourth instar larvae were used except with B. fusca where sixth instar was used.

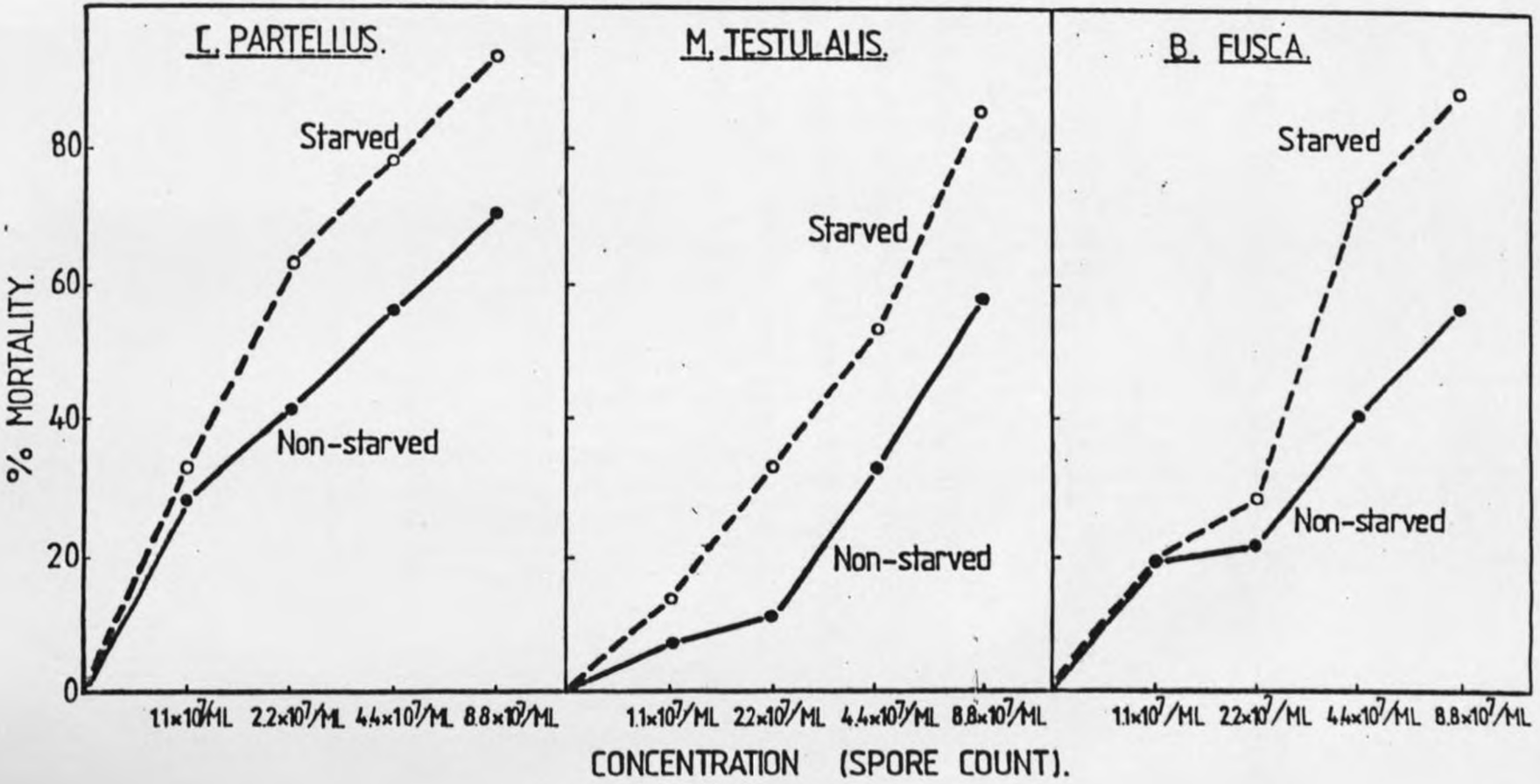

C. PARTELLUS.
M. TESTULALIS.
B. FUSCA.
Starved
Non-starved
% MORTALITY.
0
20
40
60
80
1.1×10⁷/ML 2.2×10⁷/ML 4.4×10⁷/ML 8.8×10⁷/ML
CONCENTRATION (SPORE COUNT).

Figure 2: Cumulative percentage mortality of different larval instars of Chilo partellus and Maruca testulalis after exposure to 4.16×10^6 spores/ml. of B.t. (HD-1) for three days.

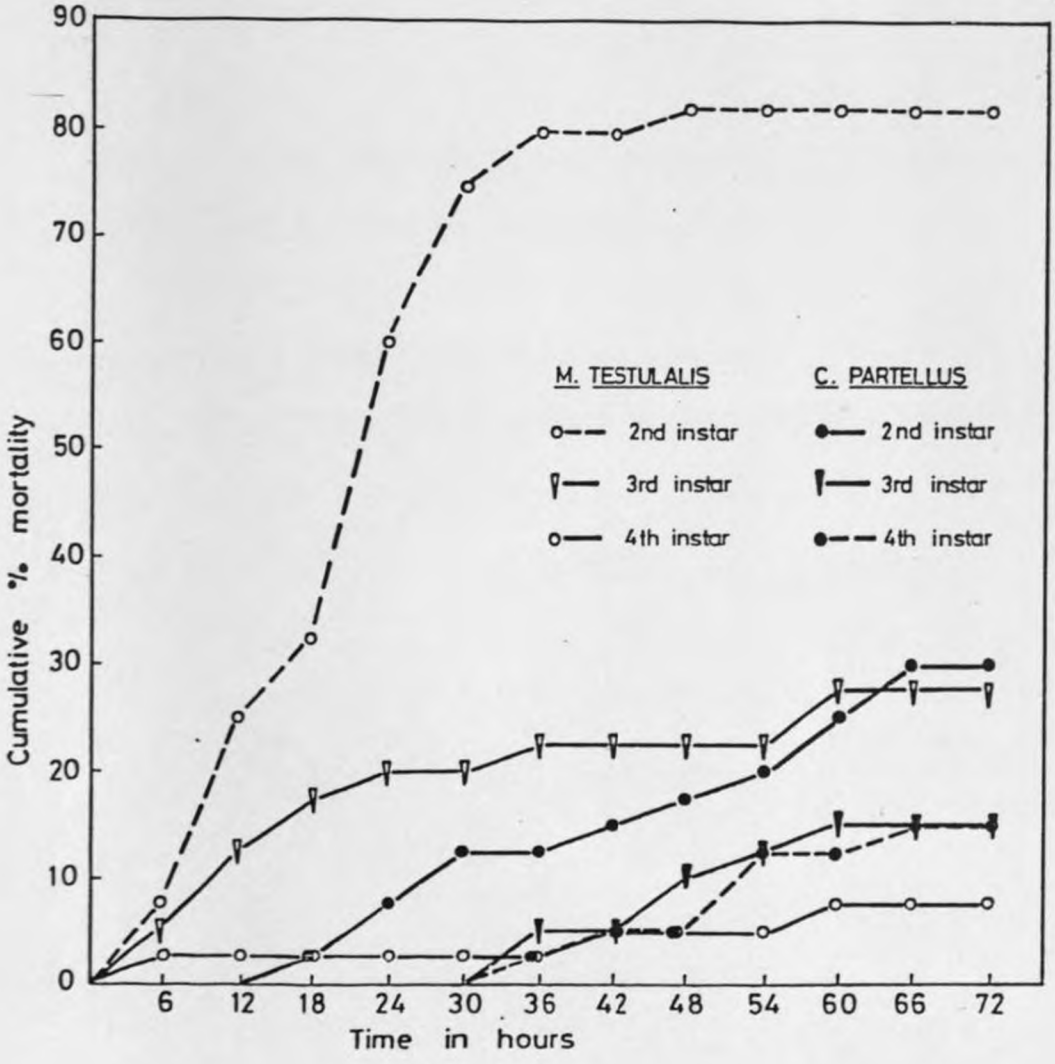

90
80
70
60
50
40
30
20
10
0
Cumulative % mortality
6
12
18
24
30
36
42
48
54
60
66
72
Time in hours
M. TESTULALIS
2nd instar
3rd instar
4th instar
C. PARTELLUS
2nd instar
3rd instar
4th instar

Figure 3: Cumulative percentage mortality of different larval instars of *Chilo partellus* and *Maruca testulalis* after exposure to 1.96×10^7 spores ml. of *B.t.* (HD-1) for three days.

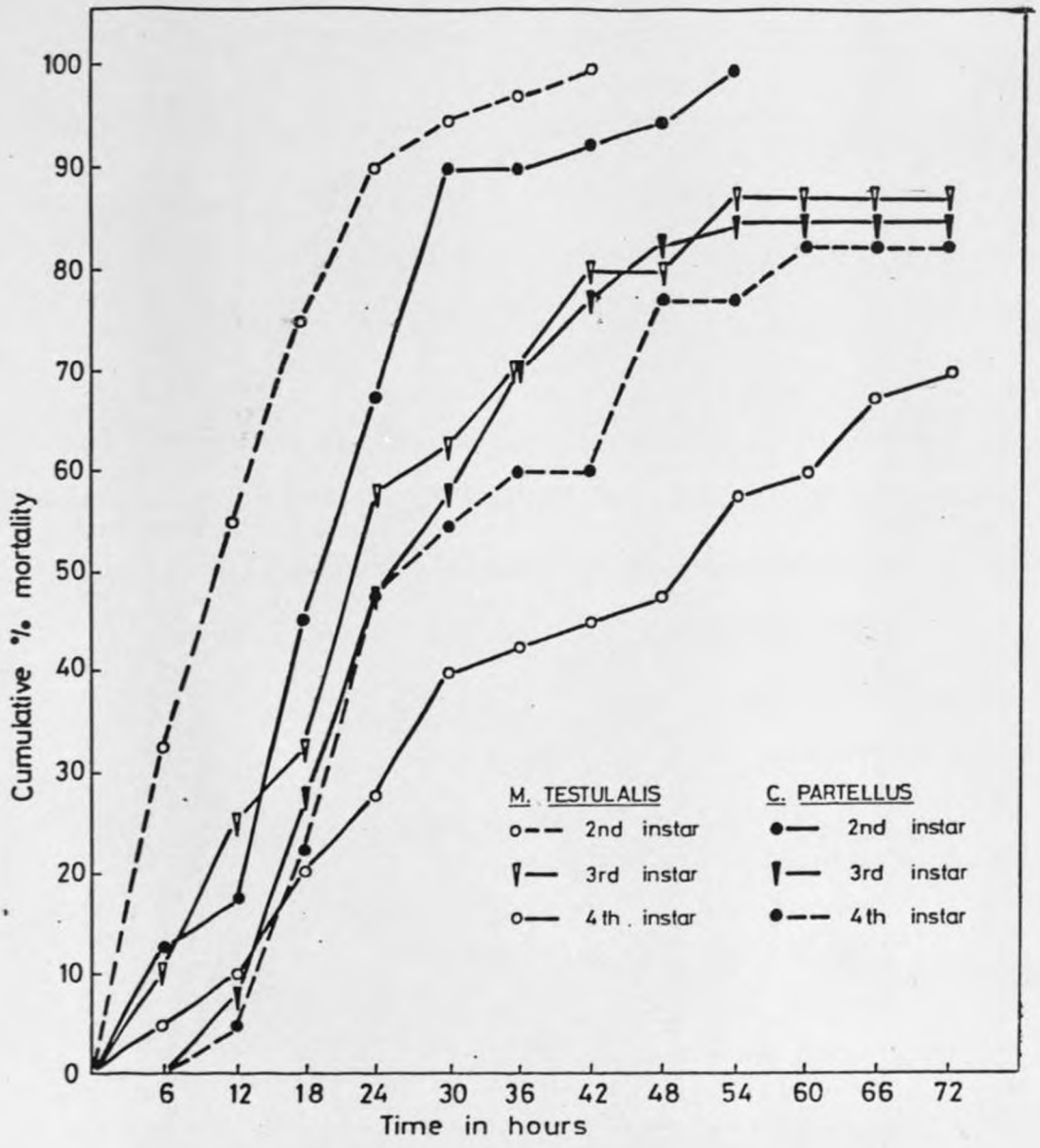
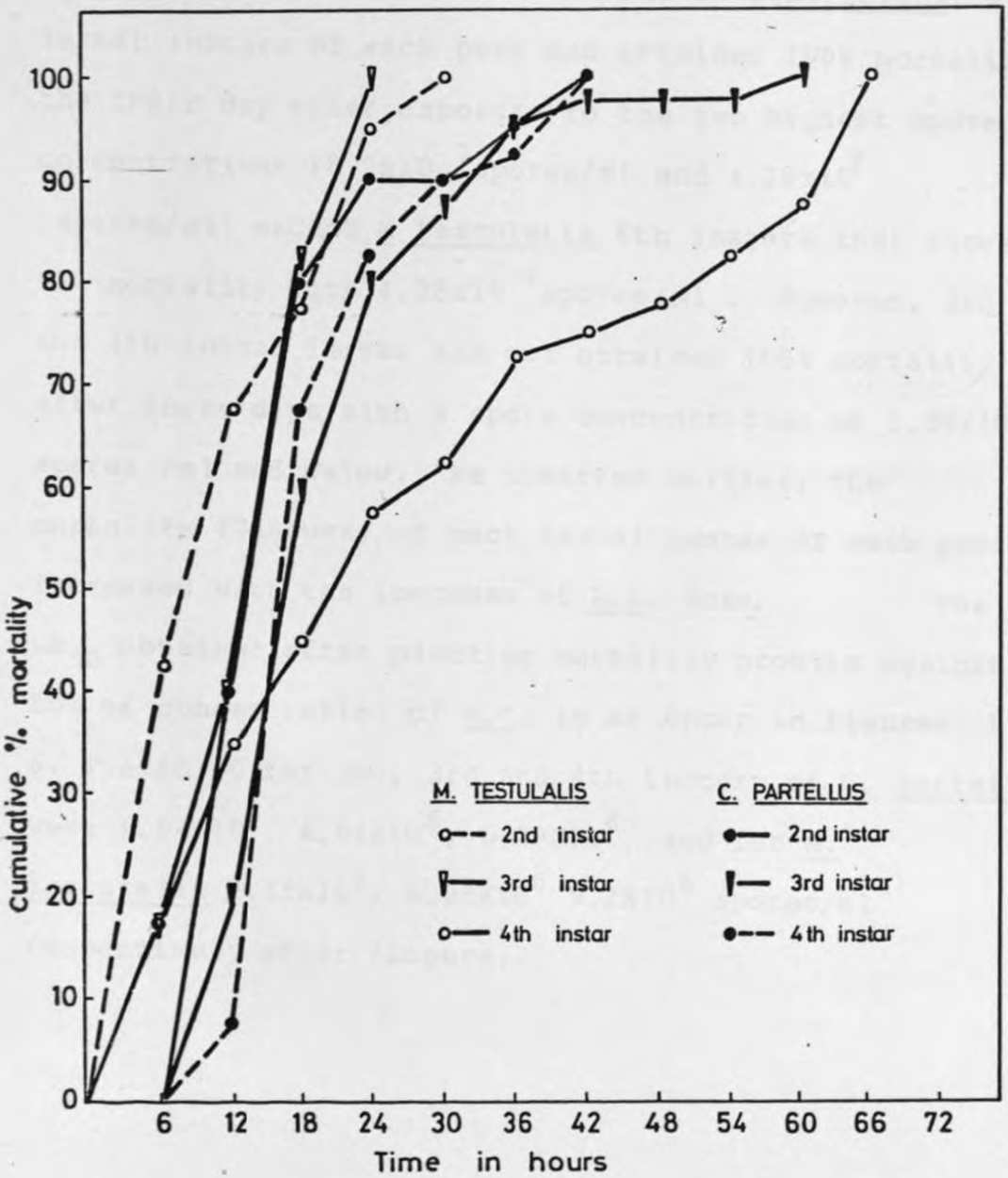

Cumulative % mortality
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
42
48
54
60
66
72
Time in hours
M. TESTULALIS
2nd instar
3rd instar
4th instar
C. PARTELLUS
2nd instar
3rd instar
4th instar

Figure 4: Cumulative percentage mortality of different larval instars of Chilo partellus and Maruca testulalis after exposure to 8.2×10^7 spores/ml. of B.t. (HD-1) for three days.


Cumulative % mortality
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
42
48
54
60
66
72
Time in hours
M. TESTULALIS
2nd instar
3rd instar
4th instar
C. PARTELLUS
2nd instar
3rd instar
4th instar

third instars of M.testulalis were more sensitive than 2nd and 3rd instar larvae of C.partellus at low concentrations but at higher concentrations it was difficult to differentiate. However, M.testulalis 4th instar were in fact less sensitive than 4th instar of C.partellus. All larval instars of each pest had attained 100% mortality by the third day after exposure to the two highest spore concentrations (8.2×10^{7} spores/ml and 4.28×10^{7} spores/ml) except M testulalis 4th instars that showed 90% mortality with 4.28×10^{7} spores/ml . However, 3rd and 4th instar larvae had not attained 100% mortality after three days with a spore concentration of 1.96×10^{7} spores /ml and below. As observed earlier, the mortality (72hours) of each larval instar of each pest increased with the increase of B.t. dose. The LD_{50} obtained after plotting mortality probits against Log of concentration of B.t. is as shown in figures 5 and 6. The LD 50 for 2nd, 3rd and 4th instars of C. partellus were 1.94×10^{6}, 4.04×10^{6}, 6.39×10^{6}, and for M. testulalis 2.13×10^{5}, 6.25×10^{6} 9.2×10^{6} spores/ml respectively after 72hours.

Figure 5: Dose-mortality regression lines of Chilo partellus after 72 hours exposure to B.t. (HD-1). The equations given were used to calculate the corresponding LD_{50}s.

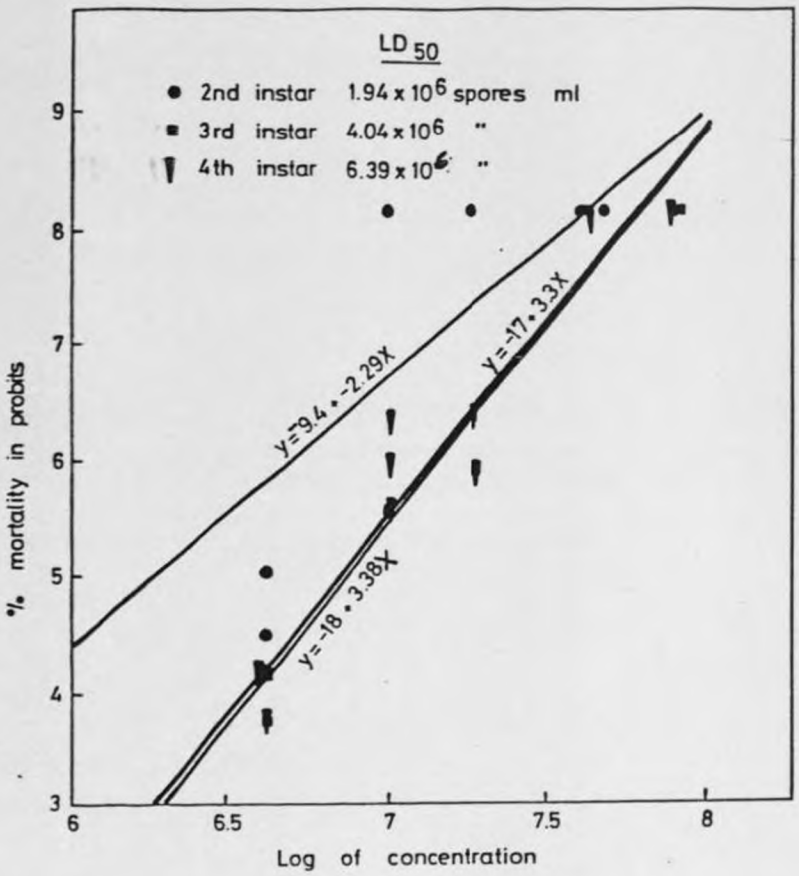
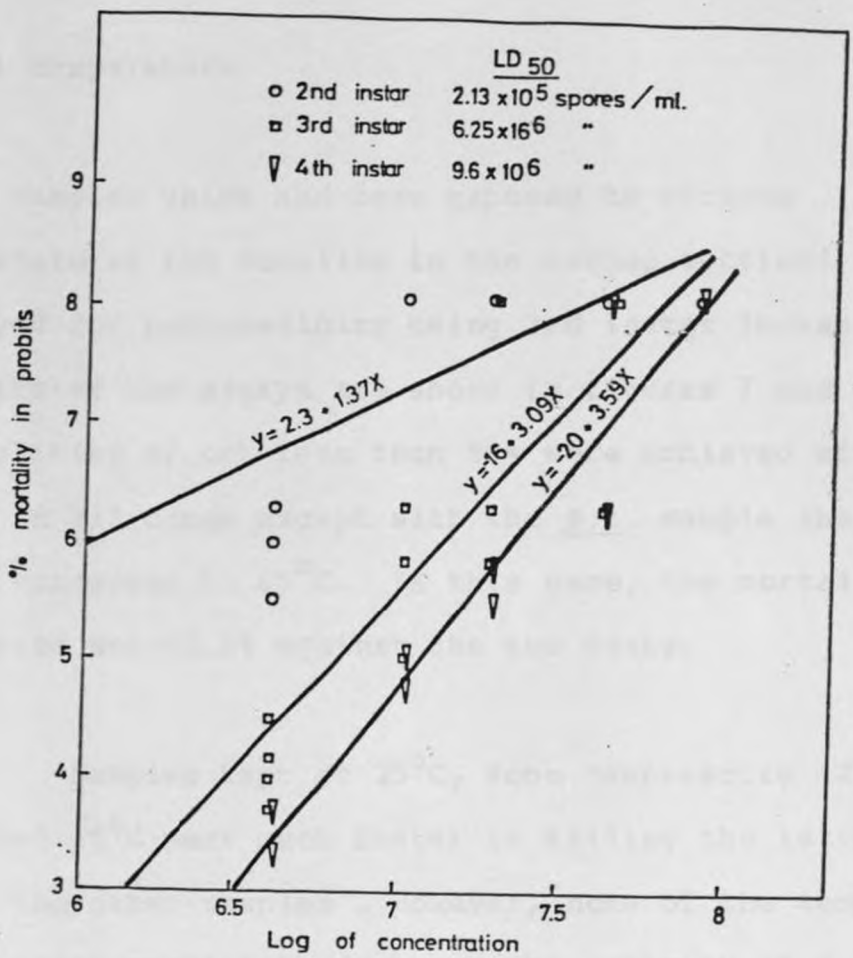

LD 50
2nd instar 1.94 x 10^6 spores ml
3rd instar 4.04 x 10^6 "
4th instar 6.39 x 10^6 "
y=9.4 • -2.29X
y=-17 • 3.3X
y=-18 • 3.38X
% mortality in probits
Log of concentration
3
4
5
6
7
8
9
6
6.5
7
7.5
8

Figure 6: Dose-mortality regression lines of Maruca testulalis after 72 hours exposure to B.t. (HD-1). The equations given were used to calculate the corresponding LD_{50}s.


LD 50
o 2nd instar 2.13 x10^5 spores / ml.
▫ 3rd instar 6.25x16^6 "
▽ 4th instar 9.6 x 10^6 "
y= 2.3 • 1.37X
y=-16 • 3.09X
y=-20 • 3.59X
% mortality in probits
Log of concentration
9
8
7
6
5
4
3
6
6.5
7
7.5
8

3.5.0 Effect of Environmental Factors on Performance of B.t. (HD-1).

3.5.1 Temperature

B.t. samples which had been exposed to various temperatures (as detailed in the method section) were assayed for pathogenicity using 3rd instar larvae. The results of the assays are shown in figures 7 and 8. Mortalities of not less than 90% were achieved after three days in all cases except with the B.t. sample that had been subjected to 85°C. In this case, the mortality achieved was 82.5% against the two pests.

Samples kept at 25°C, Room temperature (27±2), 5° and 45°C were much faster in killing the larvae than the other samples . However, none of the temperature treatments completely destoyed the activity of B.t.

After treatment at these temperatures M. testulalis appeared to be more susceptible than C. partellus to all concetrations of the B.t. preparation.

Figure 7: The effect of temperature on larvicidal activity of B.t. (HD-1) against 3rd larval instars of Chilo partellus.

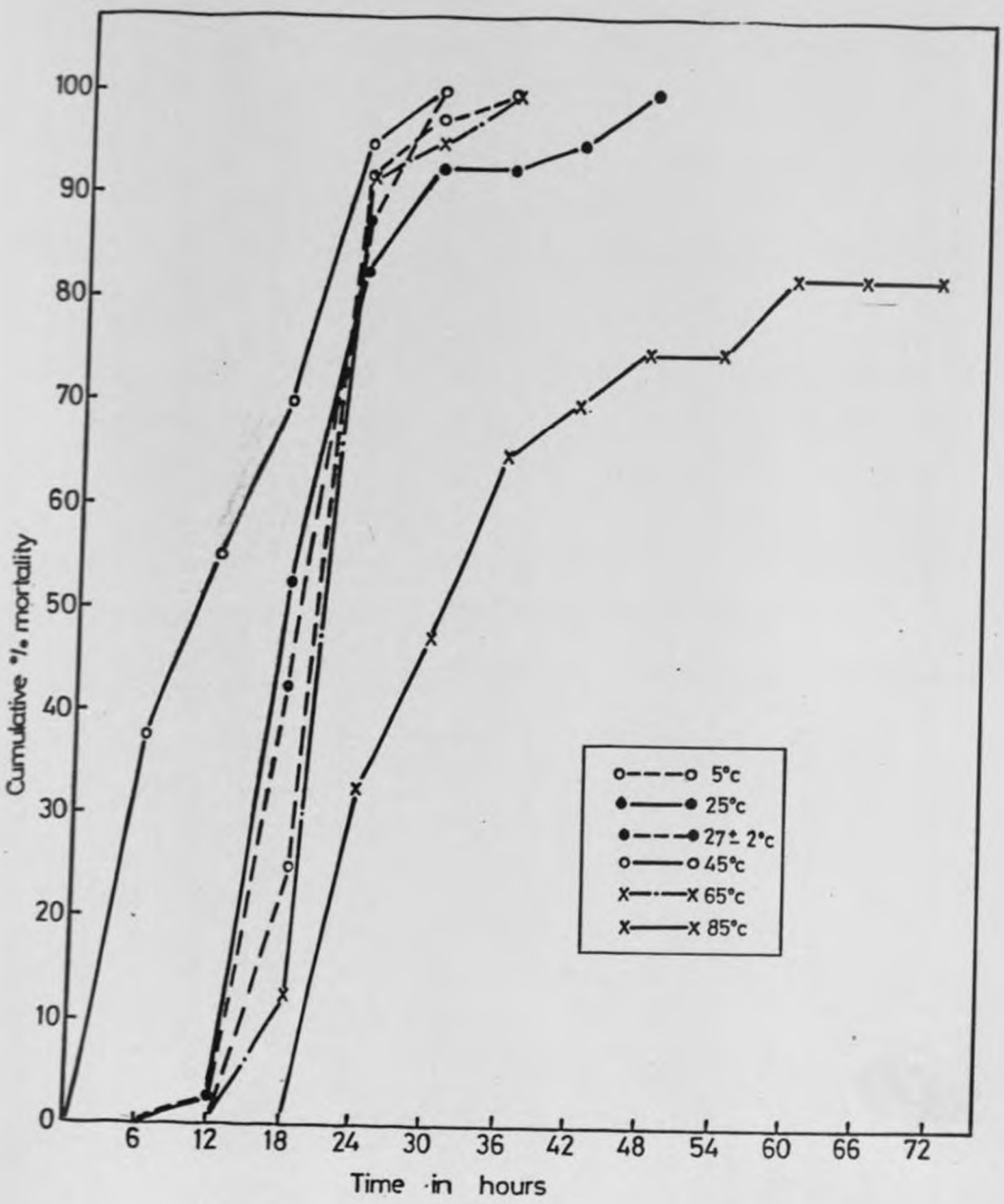
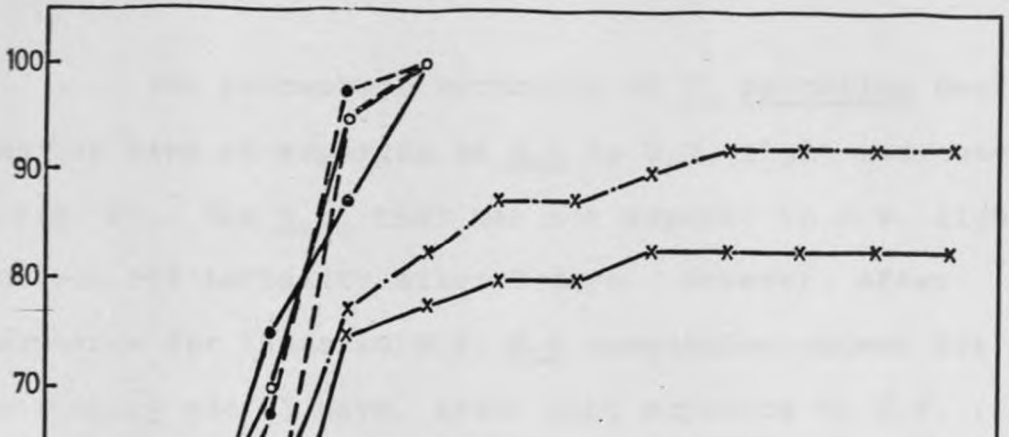

Cumulative % mortality
100
90
80
70
60
50
40
30
20
10
0
6
12
18
24
30
36
42
48
54
60
66
72
Time in hours
5°c
25°c
27 ± 2°c
45°c
65°c
85°c

Figure 8: The effect of temperature on larvicidal activity of B.t. (HD-1) against 3rd larval instars of Maruca testulalis.


100
90
80
70

3.5.2 Ultraviolet Radiation.(U.V)

The percentage mortality of C. partellus decreased as the time of exposure of B.t to U.V. light increased (Fig. 9). The B.t. that was not exposed to U.V. light caused 90% mortality after 3 days. However, after exposure for 15min to U.V, B.t suspension caused 82% mortality after 3 days. After long exposure to U.V. (6hr), B.t caused 16% mortality to C. partellus. The decrease in mortality was found to be correlated (r=coefficient of correlation = 0.75) to the time of U.V exposure.

From the regression line of percentage mortality in probits against log period of exposure of B.t., it was found that, the time of U.V exposure taken to reduce mortality of C. partellus by 50% was 37.2min (Fig. 9).

3.5.3 Solar Radiation.

There exists a clear difference between the percentage mortalities caused by B.t. after exposure to different conditions (Table 3). After exposure for only 5 hours in the sunlight, the efficacy of B.t suspension decreased in comparison to B.t. kept in the shade and darkness. After this period of exposure to sunlight, the mortality recorded after 72 hours was 70% to 85% for

Figure 9: B.t. (HD-1) pathogenicity to 3rd larval instar of Chilo partellus after different period of exposure to U.V light.

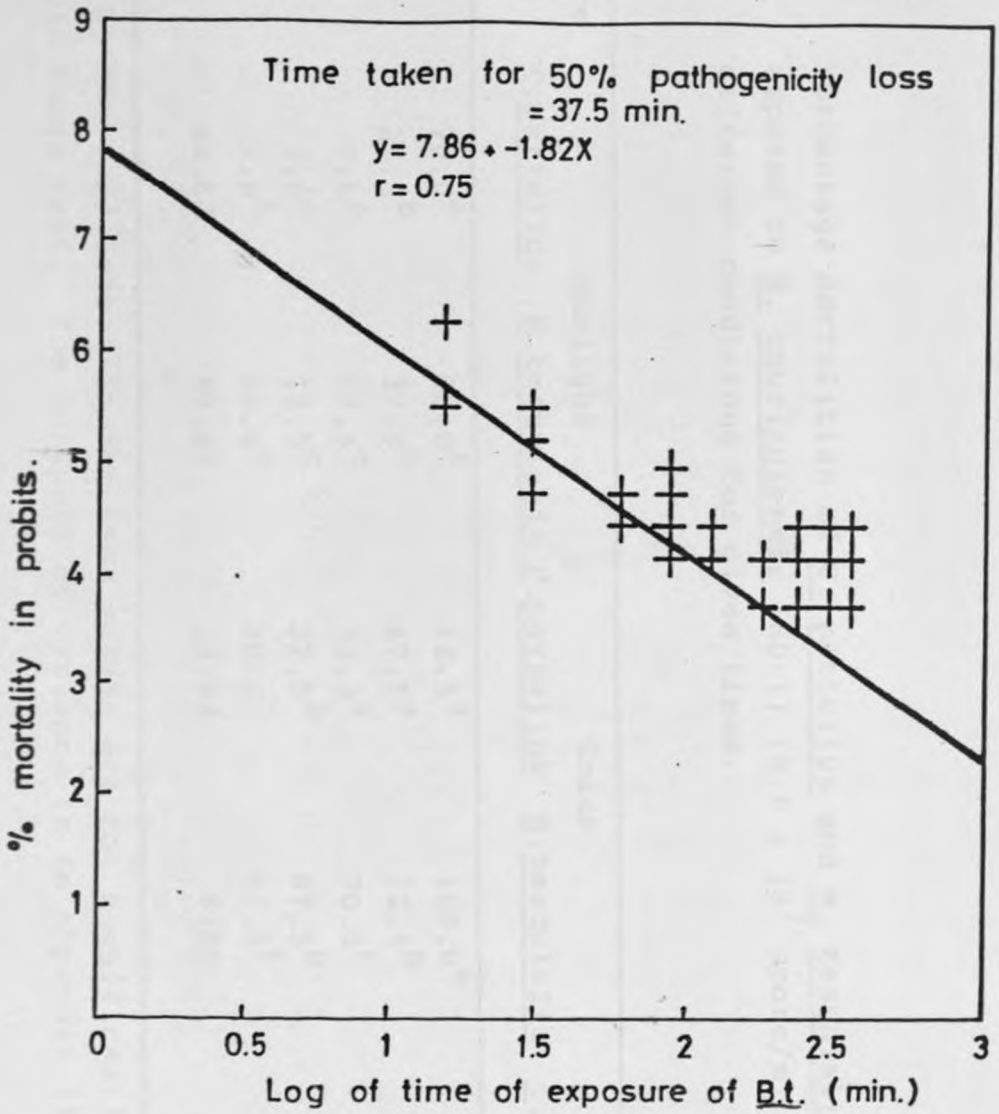

Time taken for 50% pathogenicity loss
= 37.5 min.
y= 7.86 + -1.82X
r = 0.75
% mortality in probits.
9
8
7
6
5
4
3
2
1
0
0.5
1
1.5
2
2.5
3
Log of time of exposure of B.t. (min.)

Table 3. Percentage mortalities of *C. partellus* and *M. testulalis* third larval instars exposed to *B. thuringiensis* (HD-1) (8.8×10^7 spore/ml) after exposure to different conditions for given times.

Time of exposure of *B.t.* (Hours)	Sunlight		Shade		Darkness	
	C.partellus	*M.testulalis*	*C.partellus*	*M.testulalis*	*C.partellus*	*M.testulalis*
5	70.0^a	85.0^a	92.5^a	100.0^a	97.5^a	97.5^a
25	27.5^b	37.5^b	87.5^a	72.5^b	95.0^a	97.5^a
50	7.5^c	17.5^c	72.5^a	70.0^b	97.5^a	95.0^a
100	5.0^c	12.5^c	32.5^b	67.5^b	95.0^a	95.0^a
150	5.0^c	10.0^c	30.0^b	62.5^b	92.5^a	95.0^a
F - value	65.63	45.58	23.84	6.02	0.43	0.57

Means followed by the same letter in the same column are not significantly different (P=0.05) by Duncan's multiple range test. The analysis of variance is in appendix II.

both pests compared to mortalities of 92.5 - 100% caused by B.t. kept in the shade and darkness for the same period. The pathogenicity of B.t. was even more reduced after keeping it in sunlight for 150 hours; after which it caused 5 - 10% mortality in both pests after 72 hours. B.t. kept in the shade for the same period of time caused 30% and 62% mortality in both C. partellus and M. testulalis respectively.

After plotting the percentage mortality transformed into probits against the log period of exposure to sunlight, it was found that, the time taken to reduce mortality of C. partellus and M. testulalis by 50% was a 9.2 hr. and 17.8 hr. respectively (Fig. 10). A negative correlation, -0.69 and -0.79, existed between the time of exposure of B.t. to solar radiation and the mortality of C.partellus and M.testulalis respectively.

The loss of B.t. pathogenicity was very low in the shade and negligible in darkness.

3.5.4 Persistence of B.t.

Separate assays conducted at different periods using sorghum leaves initially sprayed with B.t. and 3rd instar of C. partellus as the test larva indicated that the % mortality decreased as the time after the original spray increased (Fig. 11). Immediately after spraying, larvae fed with the sprayed leaves showed a mortality of 77.5% after 72 hr. Larvae fed with leaves three days

Figure 10: Log-time mortality lines of Chilo partellus and Maruca testulalis reflecting loss in pathogenicity of B.t. (HD-1) after exposure to sunlight.

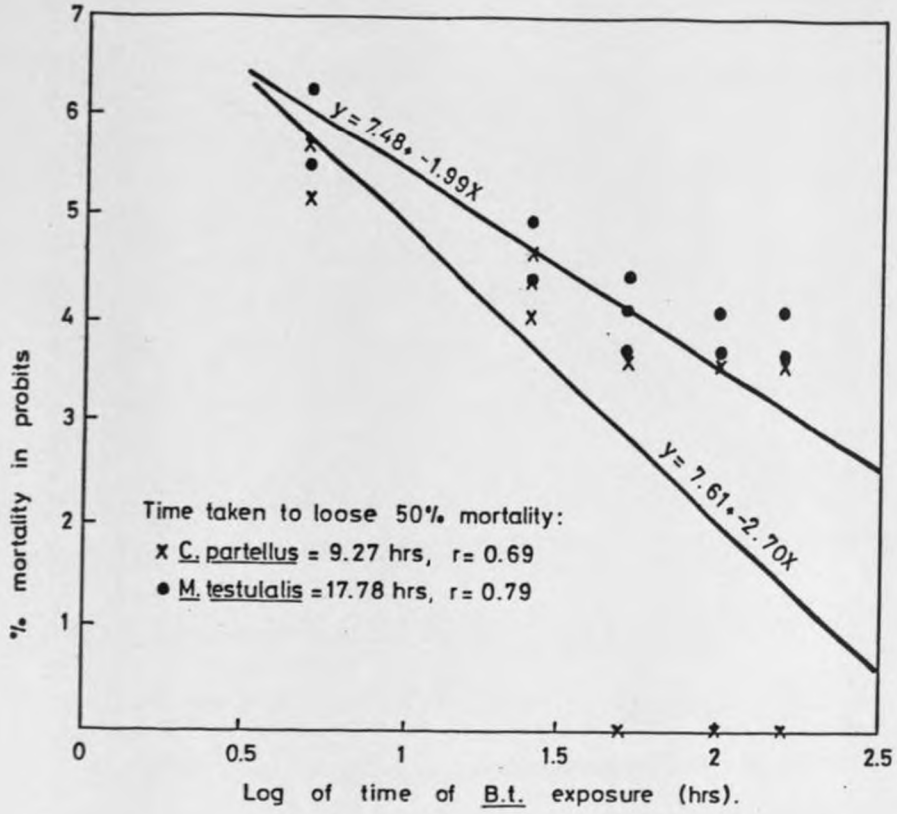
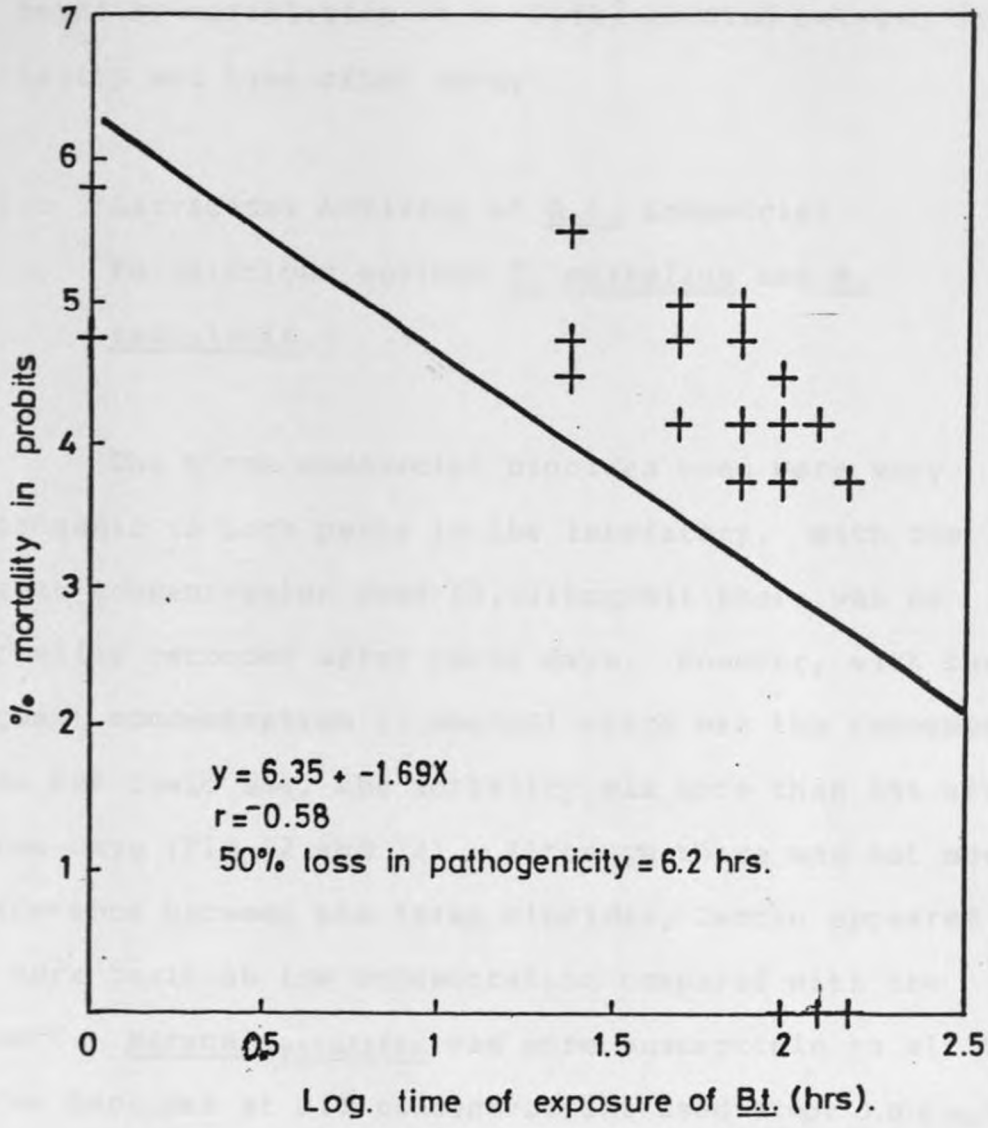

7
6
5
4
3
2
1
0
0.5
1
1.5
2
2.5
% mortality in probits
Log of time of B.t. exposure (hrs).
y = 7.48 - 1.99x
y = 7.61 - 2.70x
Time taken to loose 50% mortality:
x C. partellus = 9.27 hrs, r = 0.69
• M. testulalis = 17.78 hrs, r = 0.79

Figure 11: Persistence in pathogenicity of B.t. (HD-1) to Chilo partellus (3rd instar) larvae with varying time of exposure to solar radiation on the sorghum.


% mortality in probits
7
6
5
4
3
2
1
0
0.5
1
1.5
2
2.5
y = 6.35 + -1.69X
r = -0.58
50% loss in pathogenicity = 6.2 hrs.
Log. time of exposure of B.t. (hrs).

after spraying showed 30% mortality. The time taken for a 50% reduction in mortality of C. partellus was 6.3 hr . A negative correlation (r = -0.58) existed between the mortality and time after spray.

3.6.0 Larvicidal Activity of B.t. Commercial Formulations against C. partellus and M. testulalis.

The three commercial biocides used were very pathogenic to both pests in the laboratory. With the lowest concentration used (0.0016mg/ml) there was no mortality recorded after three days. However, with the highest concentration (1.6mg/ml) which was the recommended dose for field use, the mortality was more than 94% after three days (Fig 12 and 13). Although there was not much difference between the three biocides, Certan appeared to be more toxic at low concentration compared with the others. Maruca testulalis was more susceptible to all the three biocides at all concentrations used except 0.016 mg/ml, than C.partellus. Contrary to the laboratory results, Certan was found to be less effective than the two other biocides in the screenhouse against C.partellus (Figure 14). Less damage was done to sorghum plants sprayed with B.t. (Plate 10) indicating the potential of commercial B.t preparations for control of pest insects.

Figure 12: Insecticidal activity of B.t. commercial formulations to 3rd larval instars of Chilo partellus in the laboratory.

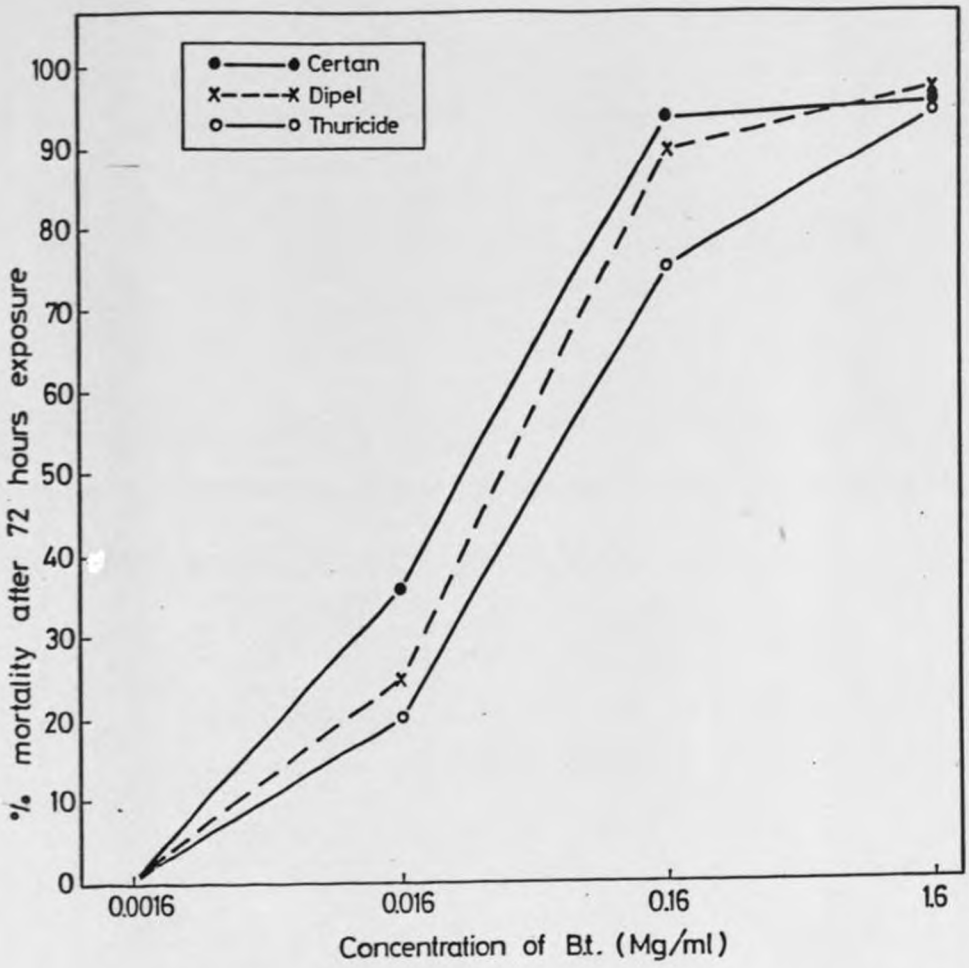

Certan
Dipel
Thuricide
% mortality after 72 hours exposure
100
90
80
70
60
50
40
30
20
10
0
0.0016
0.016
0.16
1.6
Concentration of Bt. (Mg/ml)

Figure 13: Insecticidal activity of B.t. commercial formulations to 3rd larval instars of Maruca testulalis in the laboratory.

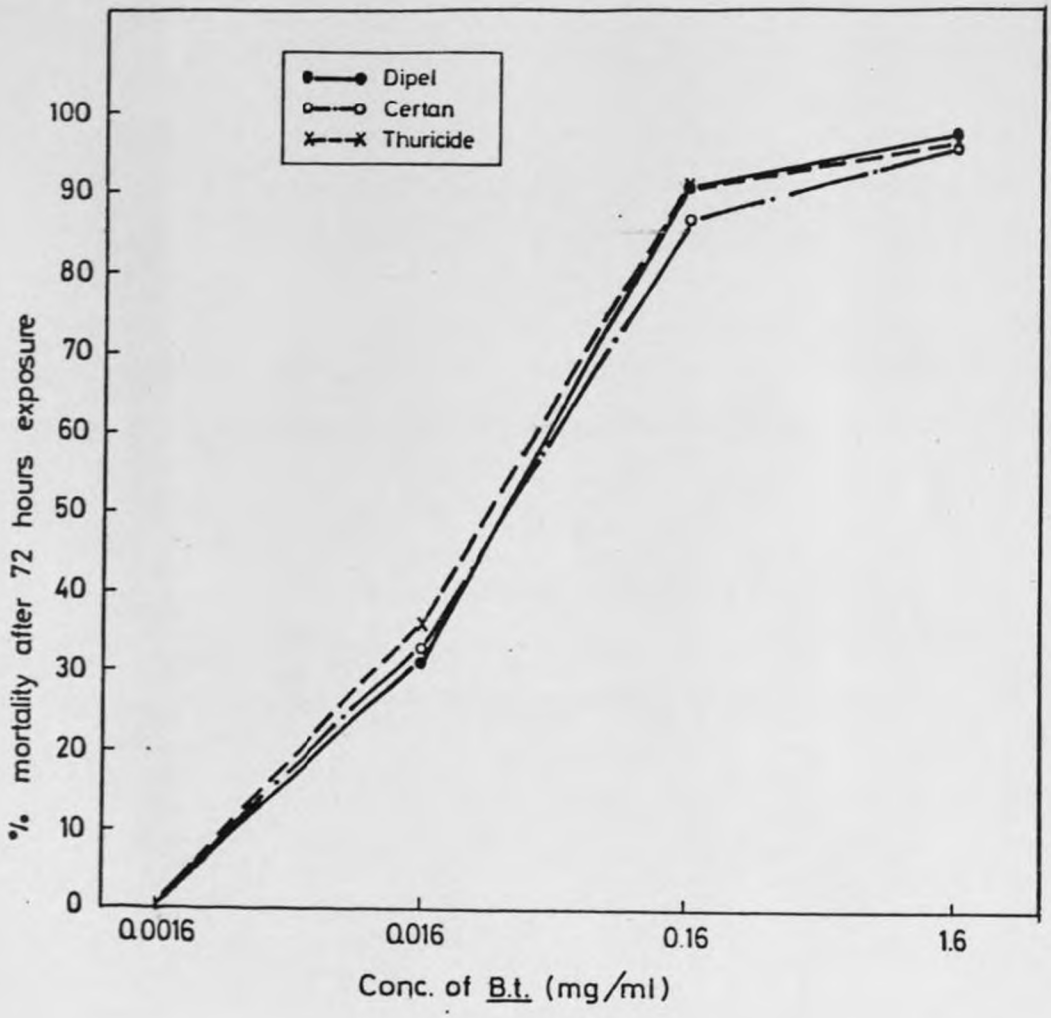

Dipel
Certan
Thuricide
100
90
80
70
60
50
40
30
20
10
0
% mortality after 72 hours exposure
0.0016
0.016
0.16
1.6
Conc. of B.t. (mg/ml)

Figure 14: The insecticidal activity of B.t. commercial formulations to third larval instar of Chilo partellus in the laboratory and Screenhouse after three and six days exposure respectively.

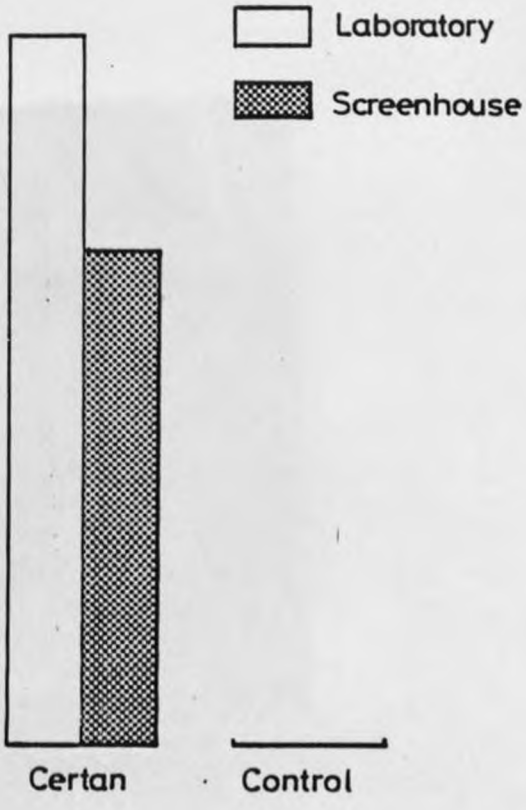

Laboratory
Screenhouse
Certan
Control

Plate 10: SCREENHOUSE EXPERIMENT: Treatment with various B.t. commercial formulations of sorghum infested with C. partellus in the screenhouse helped to reduce damage as compared to the untreated control.

3.7.0 Efficacy of B.t. Commercial Formulations in Field Trials.

3.7.1 Sorghum

3.7.1(1) Stem borers

During the sampling period all the four stem borers mentioned earlier were observed, but in varying proportions. The most dominant pest was C. partellus followed by B. fusca, S. calamistis and E. saccharina in that order, comprising 75.5%, 20.7%, 2.3% and 1.5% respectively. Although sorghum plants were artificially infested with C. partellus initially, natural infestation appeared early in the growing season and infestation continued throughout. Natural infestation with the other pests appeared much later in the growing season (8 weeks after germination).

In general, stem borers were found to be significantly fewer (P=0.05) in plots treated with B.t. commercial formulations than in untreated plots (Table 4). The mean number of stem borers (larvae and pupae) detected in treated plots was [illegible] per plant whilst in the control plots the number was a mean of 5.5 per plant in

Table 4: Mean number of stem borers on sorghum plant in different stages of development in different planting pattern after treatment with B.t. Commercial formulations.

Treatment	Stage of Sorghum Development		
	Seedling	Vegetative	Reproductive
Dipel	0.38[b]	0.81[b]	3.78[b]
Thuricide	0.53[b]	0.75[b]	4.83[b]
Certan	0.44[b]	1.16[b]	4.00[b]
Control	2.75[a]	4.59[a]	8.38[a]
Dipel*	0.47[b]	0.81[b]	3.33[b]
Thuricide*	0.50[b]	0.75[b]	4.20[b]
Certan*	0.34[b]	1.00[b]	4.20[b]
Control*	2.91[a]	4.07[a]	8.35[a]
Mean(x)	1.04	1.78	5.07
± S.E.	0.39	0.56	0.73
LSD (5%)	0.59	1.14	1.32
F-value	30.22	19.56	20.27
(d.f)	(7,21)	(7,21)	(7,28)

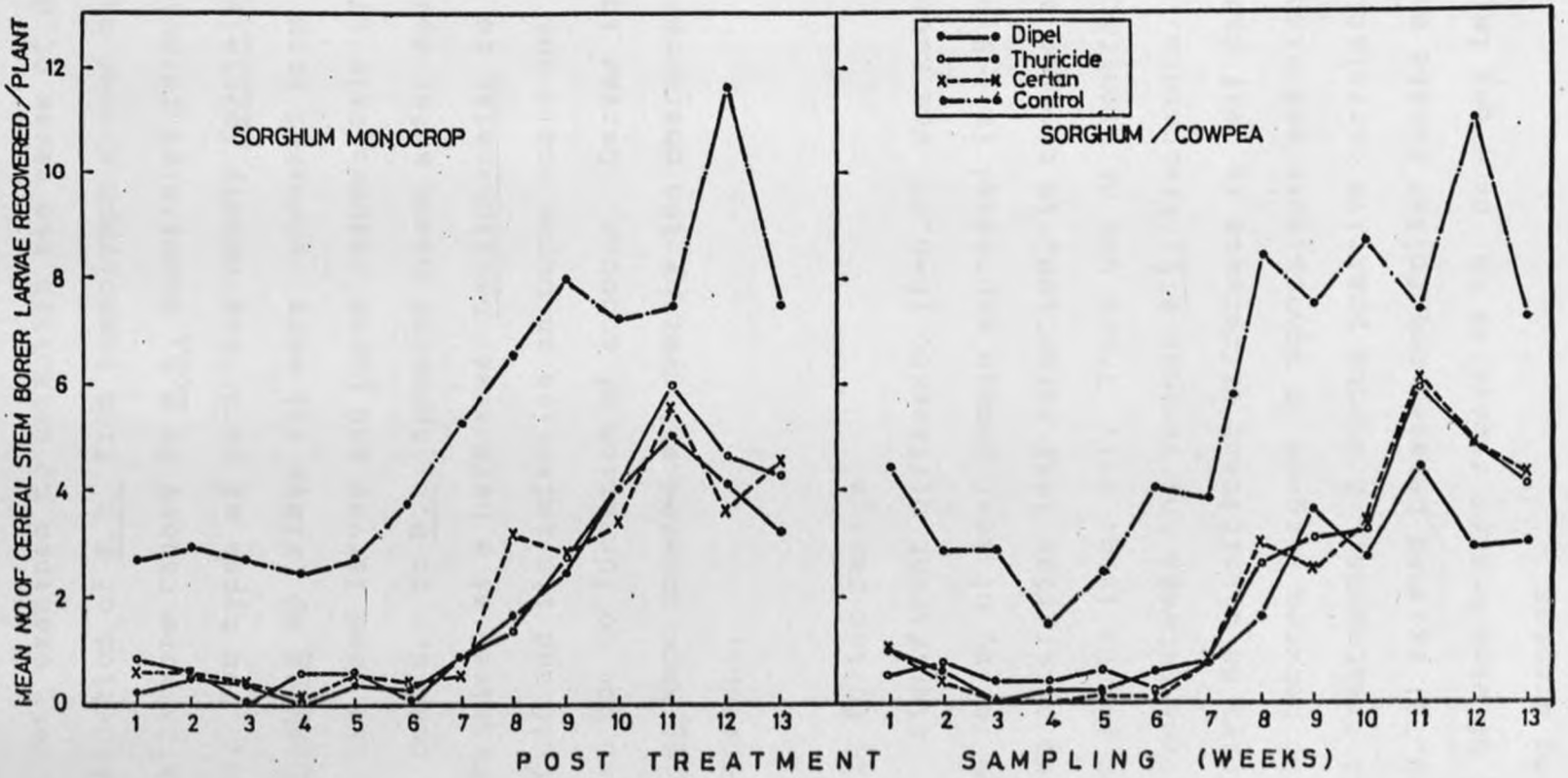
* Sorghum/Cowpea intercrop

Mean followed by the same letter in the same column are not significantly different (P=0.05) by Duncan's multiple range test. Analysis of variance is in Appendix III.

both sorghum pure and sorghum/cowpea intercrop stands. C. partellus was found to be the major pest with an average of 1.4 borers per plant in protected stands and an average of 3.9 borers per plant in the control stands in both planting patterns. There was no significant difference observed between the level of infestation in sorghum monoculture and the level of infestation in a mixed planting pattern. Among the three B.t. commercial formulations used, no significant difference was observed between them as far as borer control efficacy was concerned.

The population of stem borers throughout the growth period was not constant. The pest population was stable during the 3rd and 4th week after germination followed by a downward trend for the following two weeks. The pest population started increasing slowly again in the treated stands but faster in the untreated (control) stands until the 12th week and then dropped slightly during the time of harvest (Fig. 15), both in pure and mixed planting patterns. The stem borer population increased with plant developmental stages. Thus, the seedling stage had the lowest number with increased number during the vegetative stage and maximum numbers in the reproductive stage in both types of planting patterns (Table 4).

Figure 15 The incidence of stem borers in the field after treatment with B.t. commercial formulations. The stem borer recorded included Chilo partellus, Busseola fusca, Eldana saccharina and Sesamia calamistis larvae and pupae.


MEAN NO OF CEREAL STEM BORER LARVAE RECOVERED/PLANT
SORGHUM MONOCROP
SORGHUM / COWPEA
Dipel
Thuricide
Certan
Control
0
2
4
6
8
10
12
1 2 3 4 5 6 7 8 9 10 11 12 13
POST TREATMENT SAMPLING (WEEKS)

During the course of investigation, the dead larvae were examined to establish the cause of death. Identification of B.t. from haemolymph showed that 77 % of the deaths were caused by B.t. commercial formulation applied. Two types of parasites namely Apanteles sp. and Dentichasmias sp (Plate 11) were recovered from 11% and 12 % of the dead larvae and pupae respectively (Table 5). Larvae dead due to B.t. appeared black after death and often remained as a hard mass. Dentichasmias is a pupal parasitoid and its infection in pupae could not be mistaken due to formation of cocoons. Larvae infested by Apanteles were covered all over by its characteristic white cocoons.

3.7.1(2) Foliar Damage

Significant difference (P=0.05) was noted when the average rating of leaf damage expressed in 1-9 scale was compared after flag leaf formation, in treated and control sorghum plants (Fig. 16). There was no significant difference between the various B.t. treatments. Similarly, no significant difference in leaf damage was observed between sorghum in monoculture and mixed culture for all treatments. A strong positive correlation ($r=0.76$ and $r=0.72$) existed between the foliar damage and the number of stem borers recovered in pure and intercrop planting systems.

(a)

(b)

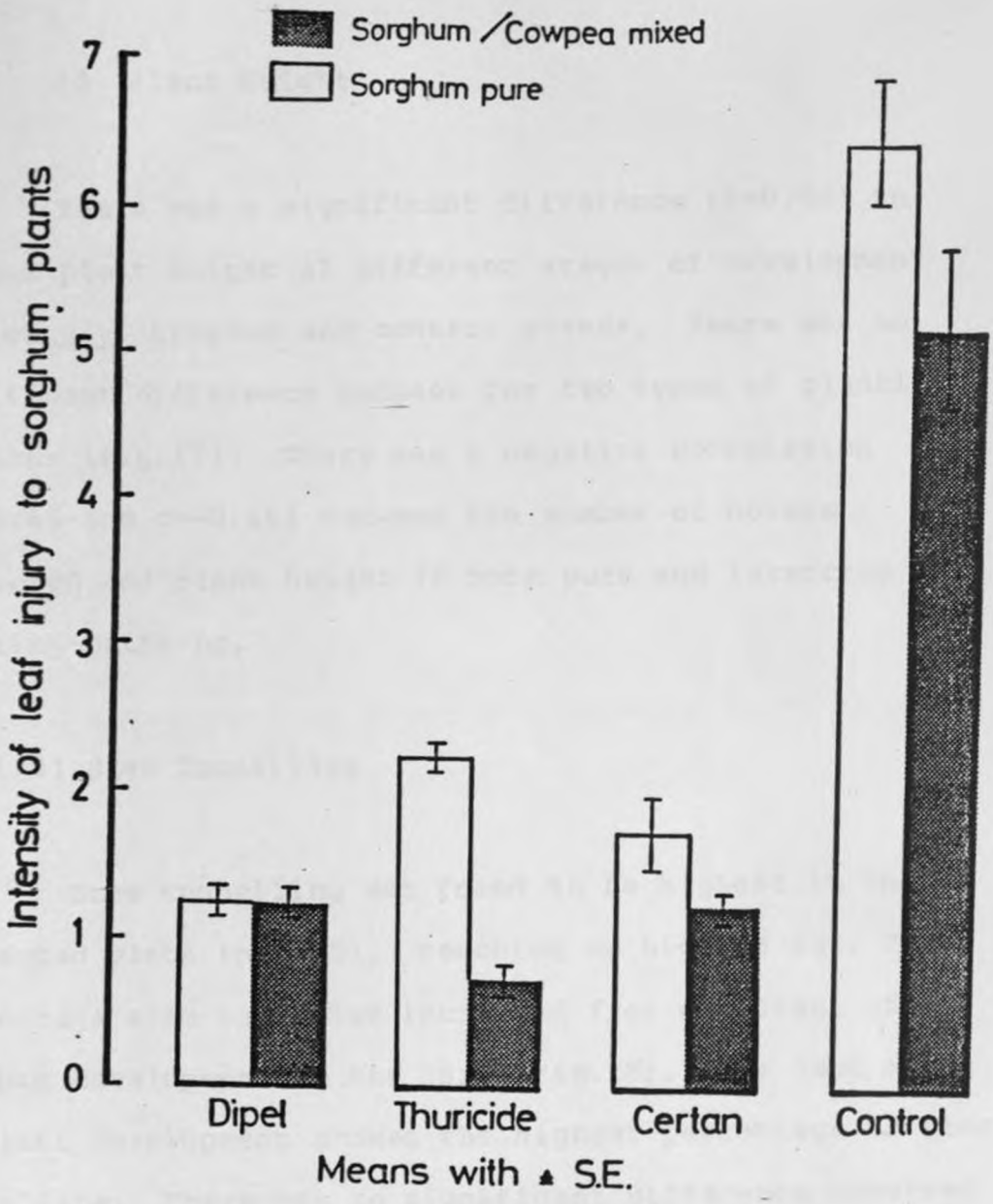
Plate 11: OTHER MORTARITY FACTORS: (a) B. fusca larvae infected by a larval parasitoid, Apanteles sp. and (b) C. partellus infected by a pupae parasitoid, Dentichasmias sp.

Table 5: Evaluation of causes of mortality of stemborers field trials after treatment with B.t. commercial formulations.

Agent responsible for death	No. of dead larvae	% Total No.of dead larvae
B. thuringiensis	50.0	76.92
Dentichasmias	8.0	12.31
Apenteles	7.0	10.77
TOTAL	65. 0	100.00

During the sampling period, dead larvae were collected and kept in the laboratory. Any emerging parasite was noted and a record of parasitized and non-parasitized larvae made.

Figure 16: Foliar damage. Leaf damage to sorghum plants was estimated at flag-leaf stage of the plant (9 weeks after germination).


Sorghum / Cowpea mixed
Sorghum pure
Intensity of leaf injury to sorghum plants
0
1
2
3
4
5
6
7
Dipel
Thuricide
Certan
Control
Means with ▲ S.E.

3.7.1 (3) Plant Height

There was a significant difference (P=0.05) in sorghum plant height at different stages of development between B.t. treated and control stands. There was no significant difference between the two types of planting patterns (Fig.17). There was a negative correlation (r=-0.65 and r=-0.66) between the number of borers recovered and plant height in both pure and intercrop planting patterns.

3.7.1(4) Stem Tunnelling

Stem tunnelling was found to be highest in the untreated plots (p=0.05), reaching as high as 45%. The percentage stem tunnelled increased from one stage of sorghum development to the next (Fig.18). The last stage of plant development showed the highest percentage of stem tunnelling. There was no significant difference observed between the various B.t. treatments. Similarly the type of planting pattern seemed not to influence the percentage stem tunnelled. A positive correlation (r=0.69 and r=0.51) existed between the percentage stem tunnelled and the number of stem borers recovered in both pure and intercrop planting patterns .

Figure 17: Sorghum plant height at different stages of development in different planting patterns after treatment with B.t. commercial formulations.

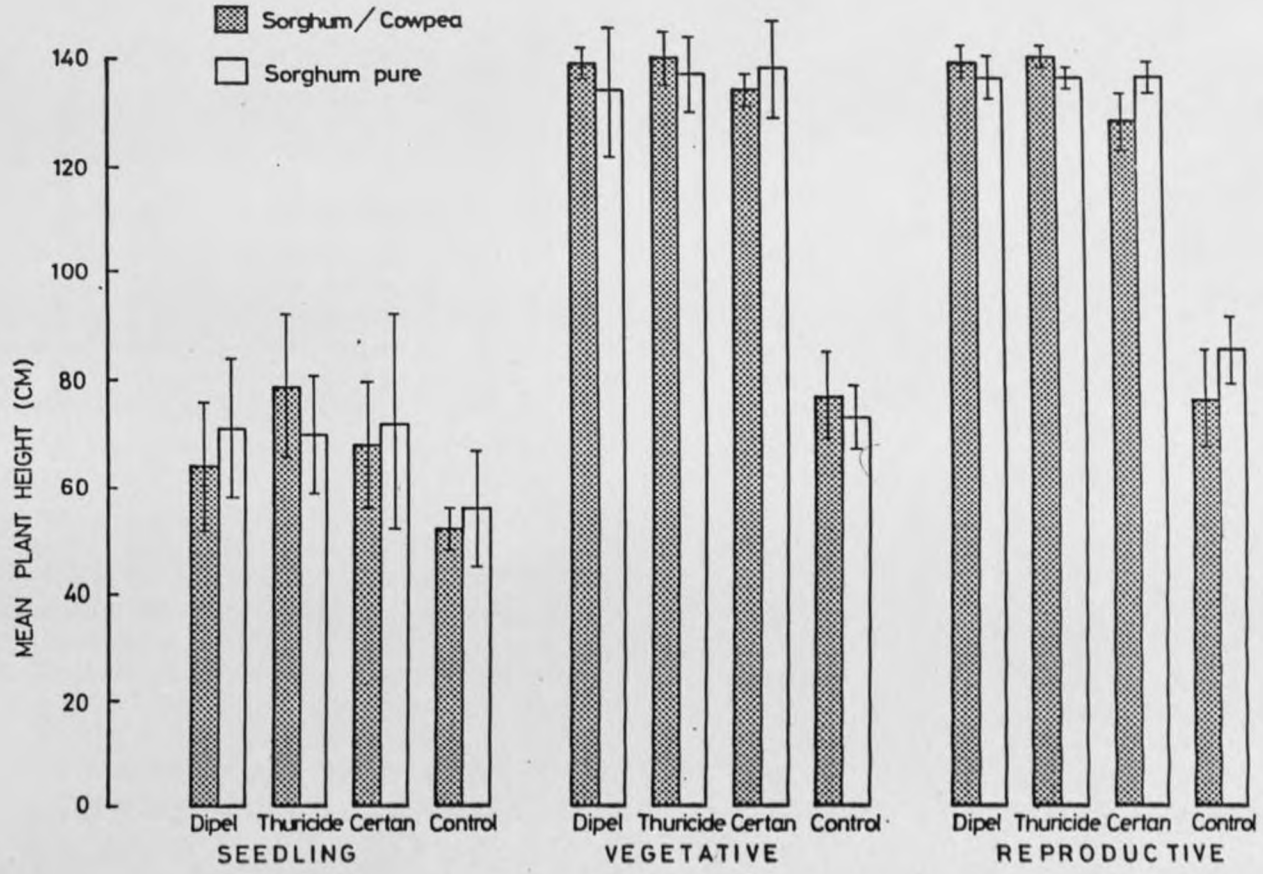
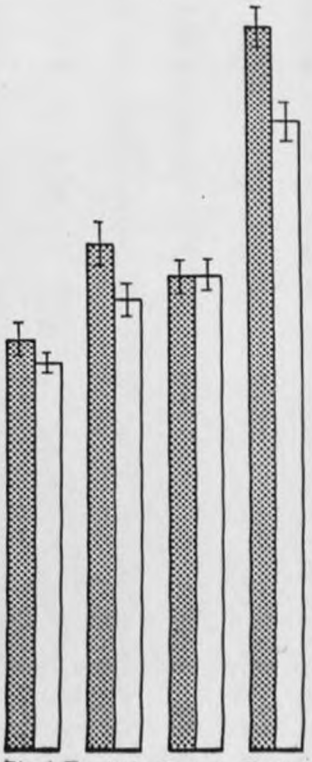

Sorghum / Cowpea
Sorghum pure
MEAN PLANT HEIGHT (CM)
140
120
100
80
60
40
20
0
Dipel
Thuricide
Certan
Control
SEEDLING
VEGETATIVE
REPRODUCTIVE

Figure 18: Percentage stem tunnel of sorghum plant at different stages of development in different planting patterns after treatment with B.t. commercial formulation.


Dipel
Thuricide
Certan
Control
REPRODUCTIVE

3.7.1(5) "Dead hearts"

Significant differences between treated and untreated stands (P=0.05) were noted for the percentage of "dead hearts" caused by the stem borer larvae (Table 6). In the untreated stands, larvae destroyed 85% - 87% of sorghum plants while only 5% - 14% were destroyed in B.t. treated stands. There was no significant difference observed between the various B.t. treatments and also between the various planting patterns (Fig. 19). There was a strong positive correlation (r=0.88) between the number of "dead hearts" observed and the number of stemborers recovered in both planting patterns.

3.7.1(6) Sorghum grain yield.

The different treatments had varying effects on grain yield (Table 7). Grain yield in B.t. treated stands was significantly (P=0.05) higher than in the untreated stands. However, no significant difference was observed among the different B.t treatments. Also no significant difference was observed in grain yield between mono and mixed planting patterns .

Table 6: Estimated amount of foliar damage and number of "dead hearts"(per 10 plants) due to stem borers in different planting pattern of sorghum after treatment with B.t. commercial formulations. A score of 0-9 was used to indicate the intensity of foriar damage.

Treatments	foliar damage	'dead hearts'
Dipel	1.25[b]	0.63[b]
Thuricide	2.25[b]	0.75[b]
Certan	1.75[b]	0.88[b]
Control	6.50[a]	8.50[a]
Dipel*	1.25[b]	0.50[b]
Thuricide*	0.75[b]	1.37[b]
Certan*	1.25[b]	1.13[b]
Control*	5.25[a]	8.25[a]
Mean(x)	2.53	2.75
± S.E.	0.76	0.44
L S D (5%)	1.66	0.81
F - value	14.37	161.47
(d.f)	(7,21)	(7,21)

* Sorghum/Cowpea intercrop

Means followed by the same letter in the same column are not significantly different (P=0.05) by Duncan's multiple range test. Analysis of variance was done as shown in Appendix IV and VII

Figure 19: "Dead hearts" count in different planting patterns of sorghum after treatment with B.t. commercial formulations.

Table 7: Sorghum yield in different planting patterns after treatment with B.t. commercial formulations.

Treatments	Grain yield in ton/ha	
	Sorghum pure	Sorghum/cowpea mixed
Dipel	2.685[a]	2.603[a]
Thuricide	2.070[a]	2.205[a]
Certan	2.438[a]	2.169[a]
Control	0.585[b]	0.762[b]
Mean ($\bar{x}$)	1.927	
± S.E.	0.285	
F - value	12.92	
L.S.D. (5%)	0.66	
D.f	7.21	

Mean followed by the same letter are not significantly different (p = 0.05) Duncan's multiple range test

Analysis of variance is as shown in Appendix viii

Comparison of the grain yield with the percentage 'deadhearts', percentage stem tunnelling, foliar damage and number of stem borer recovered showed that there is direct correlation between all of them and yield reduction. There was a negative correlation for sorghum yield and percentage stem tunnelling (r=-0.78) foliar damage (r= -089), "deadhearts",(r = -0.9), and number of stem borer/plant (r = -0.88) in sorghum monoculture and respective correlation of r = -0.74, r = -0.56, r = -0.77 and r = -0.56 in sorghum/cowpea intercrop. However, a positive correlation was observed between mean grain yield and the plant height (r = 0.63) and (r = 0.48) in pure and intercrop sorghum stands respectively.

3.7.2 Cowpea.

Maruca testulalis larvae were the only lepidopteran pests that were found associated with cowpea in the field. Another pest found to cause considerable damage to cowpea was thrips especially on the cowpea flower buds and flowers. Damage due to thrips was not assessed.

3.7.2(1) Larvae

The mean number of larvae per flower was greater than the mean number of larvae per pod (Table 8). The number of larvae collected from flowers and pods was

Table 8: Distribution of larvae of Maruca testulalis on different parts of Cowpea plant in different planting patterns after treatment with B.t. commercial formulations.

Treatment	Mean no. of larvae/plant	Distribution of larvae	
		flower	Pod
Dipel	0.54[b]	0.28[b]	0.26[b]
Thuricide	0.56[b]	0.35[b]	0.21[b]
Certan	0.43[b]	0.26[b]	0.17[b]
Control	2.00[a]	1.19[a]	0.81[ab]
Dipel*	0.62[b]	0.28[b]	0.34[ab]
Thuricide*	0.60[b]	0.37[b]	0.23[b]
Certan*	0.73[b]	0.33[b]	0.40[b]
Control*	2.32[a]	1.43[a]	0.89[a]
Mean (x)	0.96	0.56	0.41
± S.E.	0.25	0.17	0.10
L S D (5%)	0.91	0.61	0.58
F - value	4.31	4.91	2.0
(d.f)	(7,35)	(7,35)	(7,35)

* Sorghum/Cowpea intercrop.

Means followed by the same letter in the same column are not significantly different (P =0.05) by Duncan's multiple range test.

Analysis of variance is shown in Appendix IX.

significantly lower (P = 0.05) in plots sprayed with B.t. commercial formulations than in unsprayed plots (Fig.20). No significant difference was observed between the number of pod borers infesting cowpea in monoculture and cowpea in a mixed planting pattern.

3.7.2.(2) Flowers

Flower damage by larvae of M. testulalis was highest in the control plots in both pure cowpea and intercrop stands (Fig.21). The damage in B.t treated plots was significantly lower (p = 0.05) with 11 - 18% in B.t. treated stands and 29-31% in untreated plots in both planting patterns (Table 9). There was no significant difference between the percentage flower damage in the B.t. treated stands in either of the planting patterns. The flower damage in plots treated with different B.t. commercial formulations was not significantly different in either of the planting pattern.

3.7.2(3) Pods

Pod infestation by M. testulalis both in number and percentage of pods damaged was significantly lower (p=0.05) in plots treated with the three B.t. formulations than in the control in both planting patterns (Fig. 22). Pod damage in both pure and intercrop cowpea stands ranged

Figure 20: The incidence of Maruca testulalis in cowpea after treatment with different B.t. commercial formulations in different planting patterns.

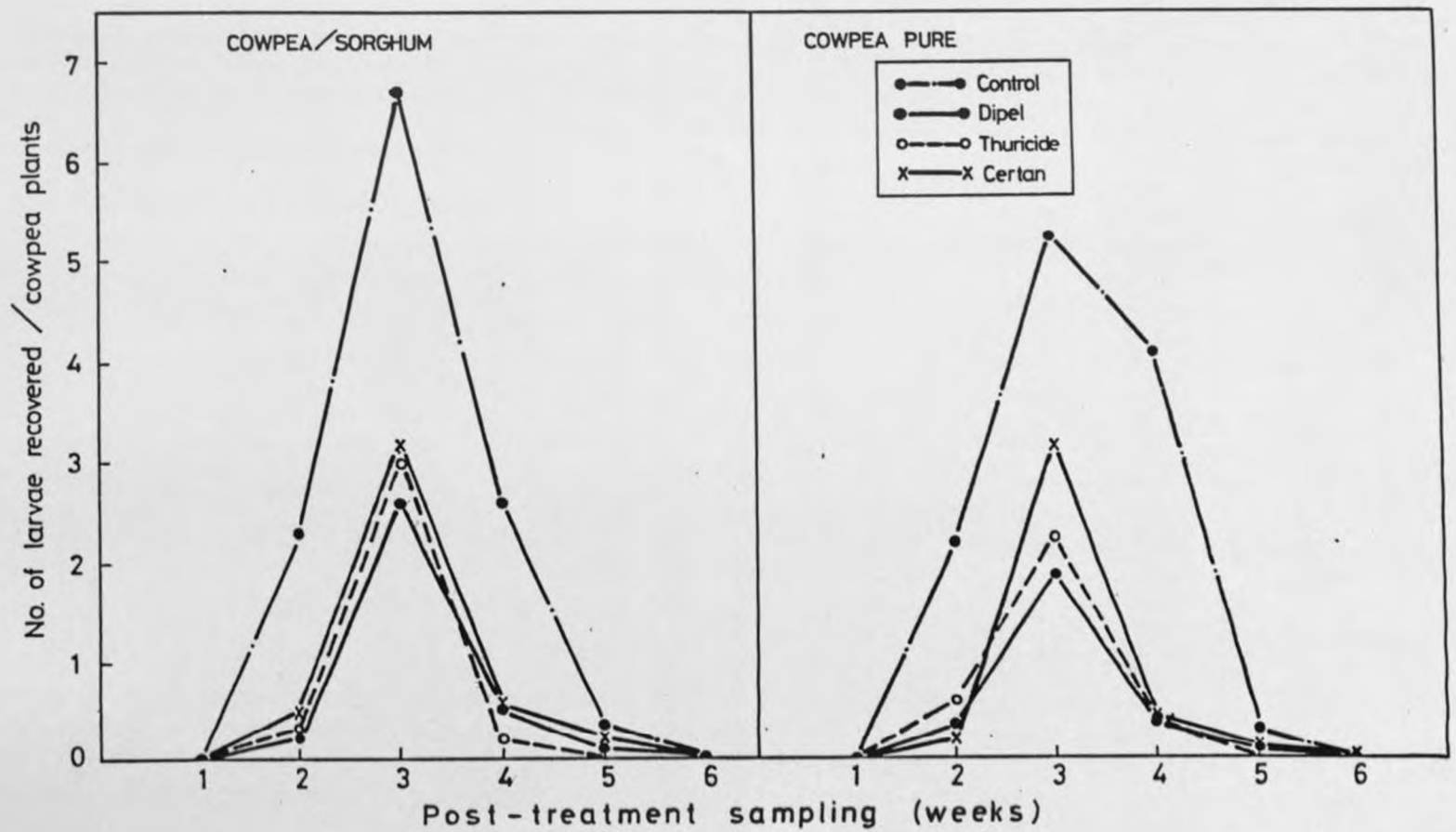

COWPEA / SORGHUM
COWPEA PURE
Control
Dipel
Thuricide
Certan
No. of larvae recovered / cowpea plants
0
1
2
3
4
5
6
7
1
2
3
4
5
6
Post-treatment sampling (weeks)

Figure 21: The effect of various B.t. commercial formulations on cowpea flower damage by M. testulalis in different planting patterns.

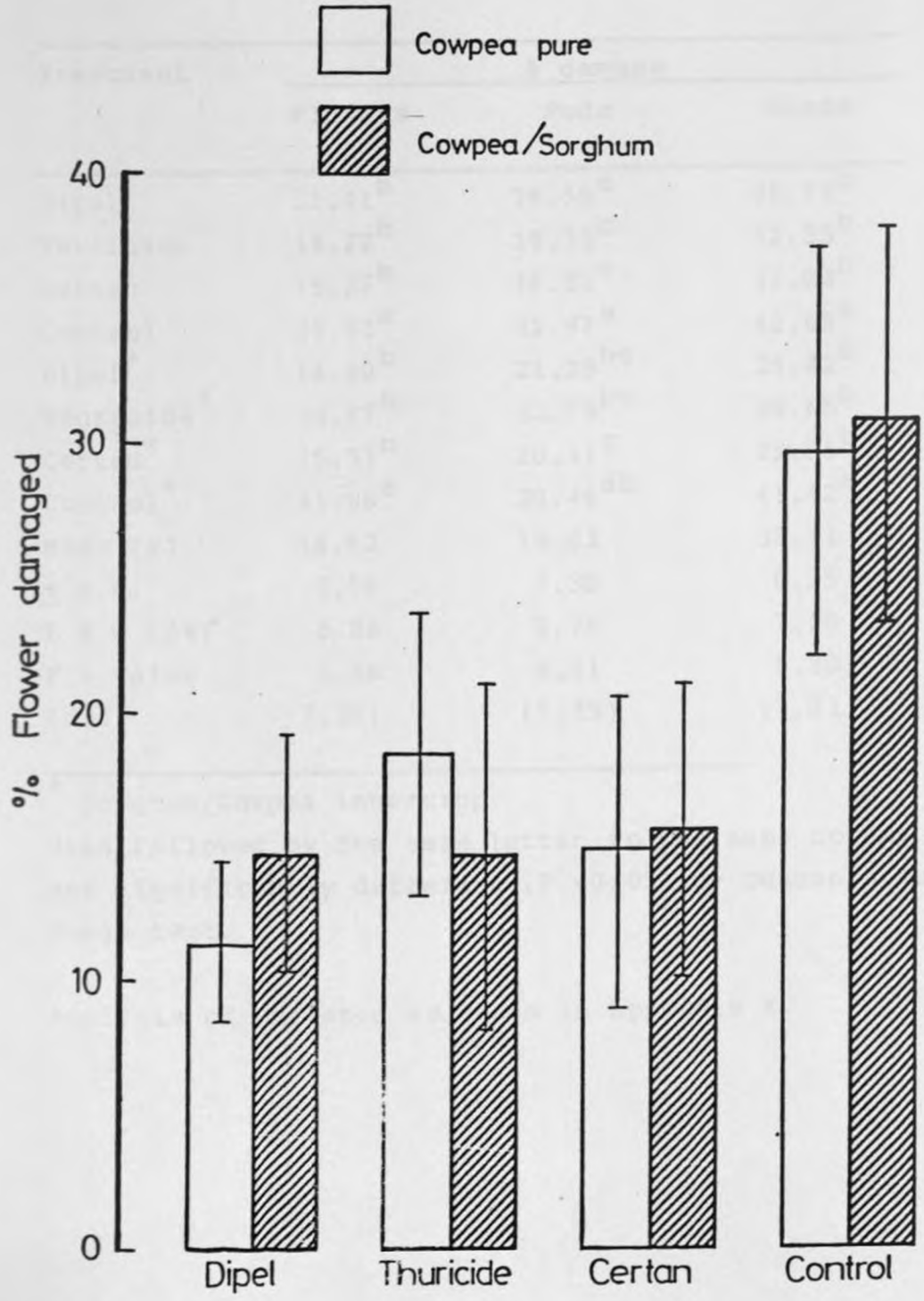

Cowpea pure
Cowpea/Sorghum
% Flower damaged
40
30
20
10
0
Dipel
Thuricide
Certan
Control

Table 9: The effect of various B.t. commercial formulations on cowpea flower, pod and seed damage by Maruca testulalis in different planting patterns after treatment with B.t. commercial formulation.

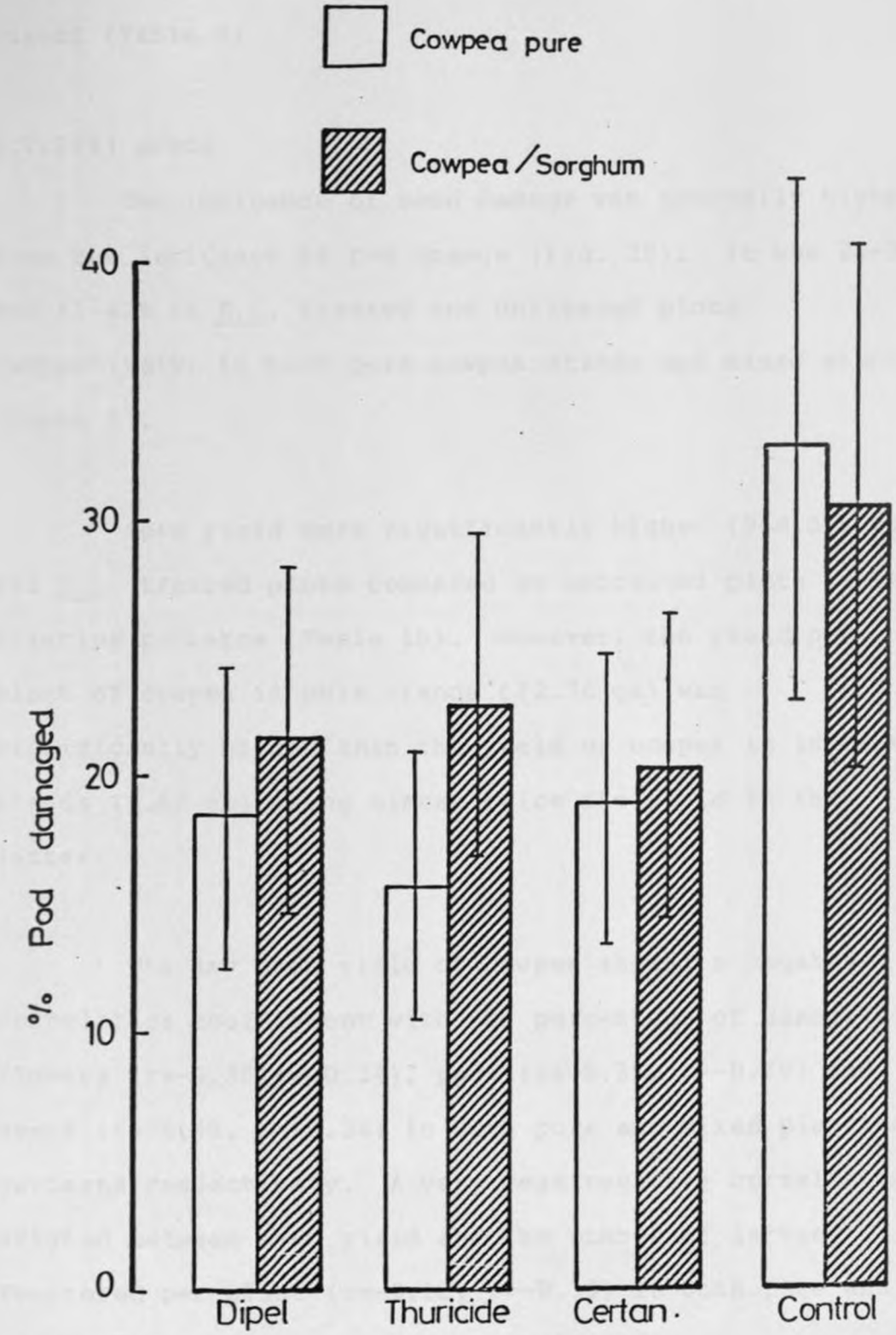
Treatment	% damage		
	Flowers	Pods	Seeds
Dipel	11.41[b]	18.55[c]	28.71[b]
Thuricide	18.22[b]	15.72[c]	32.33[b]
Certan	15.37[b]	16.52[c]	31.84[b]
Control	29.21[a]	32.97[a]	42.65[a]
Dipel*	14.90[b]	21.29[bc]	29.42[b]
Thuricide*	14.87[b]	22.73[bc]	28.65[b]
Certan*	15.51[b]	20.41[c]	29.85[b]
Control*	31.08[a]	30.46[ab]	41.42[a]
Mean (x)	18.82	19.83	33.11
± S.E.	2.56	3.30	0.35
L S D (5%)	8.88	8.79	7.25
F - value	5.48	4.21	5.30
(d.f)	7,35)	(7,35)	(7,21)

* Sorghum/Cowpea intercrop

Mean followed by the same letter in the same column are not significantly different (P =0.05) by Duncan's multiple range test.

Analysis of variance is shown in Appendix X.

Figure 22: The effect of various B.t. commercial formulations on pod damage by Maruca testulalis in different planting patterns


Cowpea pure
Cowpea / Sorghum
% Pod damaged
40
30
20
10
0
Dipel
Thuricide
Certan
Control

between 15 -22% in treated stands and 30 - 32% in control stands (Table 9)

3.7.2(4) Seeds

The incidence of seed damage was generally higher than the incidence of pod damage (Fig. 23). It was 28-32% and 41-42% in B.t. treated and untreated plots respectively, in both pure cowpea stands and mixed stands (Table 9).

Seed yield were significantly higher (P=0.05) for all B.t. treated plots compared to untreated plots in both planting patterns (Table 10). However, the yield per plant of cowpea in pure stands (12.76 gm) was significantly higher than the yield of cowpea in intercrop stands (6.51 gm) being almost twice the yield in the latter.

The dry seed yield of cowpea showed a negative correlation coefficient with the percentage of damage to flowers (r=-0.36,r=-0.34), pods (r=-0.35, r=-0.69) and seeds (r=-0.48, r=-0.38) in both pure and mixed planting patterns respectively. A very weak negative correlation existed between seed yield and the number of larvae recovered per plant (r=-0.22, r=-0.19) in both pure and mixed planting patterns respectively.

Figure 23: The effect of various B.t. commercial formulations on cowpea seed damage by Maruca testulalis in different planting patterns.

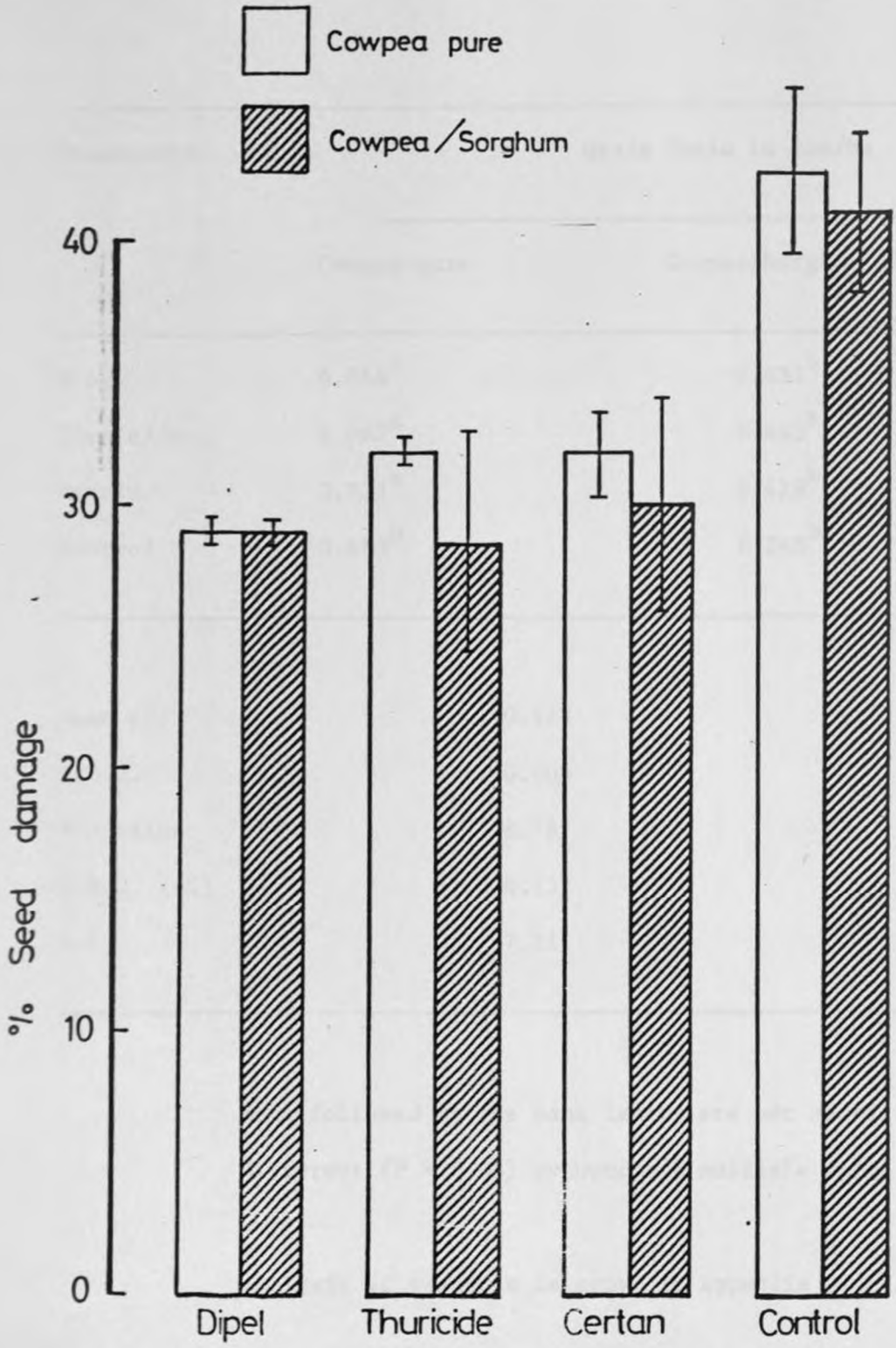

Cowpea pure
Cowpea / Sorghum
% Seed damage
40
30
20
10
0
Dipel
Thuricide
Certan
Control

Table 10: The effect of various B.t. commercial formulation on grain yield of cowpea in different planting patterns

Treatments	Grain Yield in ton/ha	
	Cowpea pure	Cowpea/Sorghum mixed
Dipel	0.864[a]	0.431[b]
Thuricide	0.867[a]	0.443[b]
Certan	0.821[a]	0.429[b]
Control	0.483[b]	0.248[b]

Mean ($\bar{x}$)	0.573
± S.E.	0.008
F - value	8.78
L.S.D. (5%)	0.237
D.f.	7.21

Mean followed by the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.

Analysis of variance is grown in Appendix xi.

C H A P T E R F O U R

D I S C U S S I O N

From the results obtained it was quite clear that the B.t. (HD-1) culture provided was viable and could grow well on Nutrient Agar medium. The observed microscopic and colonial characteristics of B.t. i.e. rod-shaped, spore former, whitish-cream colonies on Nutrient Agar with rough edges, were in agreement with those reported by other investigators. Also confirmed was the fact that B.t. produces a parasporal body.

The studies on the pathogenicity of B.t. demonstrated that the microbial preparation used was lethal to young and mature larvae of C. partellus, M. testulalis and B. fusca. The B.t. was successfully reisolated from the infected larvae of these pests, and it can therefore be concluded to be the agent responsible for death. The susceptibility of C. partellus in the field and M. testulalis in the laboratory had earlier been shown by Berger (1981) and Taylor (1968) respectively. Experiments by Taylor indicated that the microbial preparation of B. thuringiensis var. thuringiensis was

lethal to young and mature larvae of M. testulalis over the whole range of dilutions he used. The young larvae (third instar) were more susceptible than the mature (fourth or fifth instar) larvae. Both young and mature larvae first react by ceasing to feed then become relatively inactive within 6 - 24 hours. The reaction may be caused by combination of the phase of midgut paralysis following the ingestion of sporulated Bacillus and general body paralysis (Heimpel and Angus, 1959). The young larvae (1st and 2nd instars) were more susceptible and succumbed to infection earlier than the mature larvae (3rd, 4th and 5th instars).

By the 12th hour after providing food materials treated with B.t., the majority of 1st instar larvae had died and by the end of the 72 hours, 100% mortality had been achieved with all the instars while using food materials treated with a B.t. spore concentration of 1 x 10^9/ml. A direct relationship was shown to exist between the size of the infective dose of B.t. and the subsequent rate of mortality. The higher the concentration of B.t. the higher the percentage mortallity observed in a given time. This is an observation that is in agreement with observations made by other investigators (Ignoffo, Hostetter, Pinell and Garcia, 1977; Sne.. et al., 1981 and Morris 1973). They noted that the larvae were

less susceptible to B.t. as they became older. Similar observations were made by Taylor (1968) while working with M. testulalis. The application of B.t. will therefore be more efficient if directed to larvae at their relatively younger susceptible stages. In this regard, the lower dosage required for the younger instars will make control cheaper.

The results also indicated that starved larvae were more susceptible than nonstarved larvae. Similar observations were reported by Broesma et al.(1967) Busvine (1971) and Sneh et al., (1981). They noted that food has some influence on the resistance of insects to insecticides. The quantity and quality of food in the diet on which they were reared may affect their size and survival capacity. Prefeeding may affect susceptibility in that there may be differences in tolerance between recently fed individuals and those starved for various periods.

After death, the larvae turned brownish to black in colour usually initially at the anterior and posterior ends. This was particularly so with C. partellus and B. fusca which turned completely black. This has previously been noted as typical death symptoms associated with B.t. toxaemia (Norris, 1978; Poinar and Thomas, 1978; and Taylor, 1968).

Several factors influence the effectiveness of entomogenous bacteria employed for microbial insect control. From the time the bacterium leaves the application equipment until it is consumed by the target pests, it is exposed to all the physical and chemical elements of the environment such as temperature, solar radiation and plant phytocides (Falcon, 1971).

For the whole temperature range (5 - 85°C) to which B.t. was exposed, temperature had little effect on the subsequent pathogenicity of B.t. A mortality of more than 80% was achieved even after exposure to the low and high temperatures used. However, such extremely high and low temperatures are unlikely to be encountered under natural conditions. Franz (1971) observed that mostly, temperature conditions affect the pathogenicity of B.t. indirectly in that, it influences the development of the disease. Temperature normally influences the feeding behaviour of the insect and hence the rate of B.t. ingestion (Raun and Jackson, 1966; Burges, 1982). Pathogenicity of B.t. has been shown to be greatest at temperatures where growth of the bacterium and the metabolic rate of the insect are most rapid. With B.t. the lower the temperature, the later the toxic symptoms appear (Lacey and Federici, 1979), otherwise in

temperatures which permit host survival but little feeding, the bacterium will be ineffective. Korchagin (1983) and Falcon (1971) reported that field application of B.t. was most favourable and significantly effective against pests when the air temperature exceeded 18°C. Under the local environment, B.t. will actually not be affected by temperature since environmental temperatures are not extreme enough to affect the feeding behaviour of pests and hence development of disease.

The major disadvantage of B.t. has been shown to be lack of persistence in the environment for long enough to allow maximum ingestion by the pest. Solar radiation appears to be the major deleterious enviromental factor (Burges, 1982; Falcon, 1971; Franz 1971). The main influence by sunlight has been shown to be its Ultraviolet component (Cantwell et al., 1966; Burges et al., 1975 and 1976; Goldberg and Fond, 1980; Sneh et al., 1981; Sneh et al., 1983; Salama et al., 1983). However, different researchers have different views on whether the U.V. effect is on spore, delta-endotoxin or both. The results of this study have clearly indicated that the pathogenicity of B.t. to test larvae is greatly reduced by exposure to U.V. radiation and sunlight . Exposure of B.t. to U.V. light from a U.V. lamp source rapidly denatured it. Exposure of B.t. to sunlight, also resulted in a reduced pathogenicity

although to a lesser extent than when a U.V. lamp acted as the U.V. source. B.t. exposed on sorghum leaves in the screenhouse showed a slight decrease in pathogenicity compared to that exposed in the sunlight. Under these conditions (U.V, sunlight and sorghum leaves in the screen house), the time taken to have 50% loss of B.t. pathogencity was estimated to be 0.62 hr 9.3hr and 17.8 hr respectively using C. partellus third instars as the assay larvae. The decrease in pathogenicity of B.t. was highly correlated to the period of exposure to the U.V. lamp. The U.V. radiation being the major deleterious component in solar radiation, it then follows that sunlight, and not temperature, is a major contributor to the loss of B.t. pathogenicity in the field beside any other factor that may be involved, for example rain. Salama et al., (1983) observed a clear reduction in activity of B.t., 1 day after application on cotton leaves in Egypt and since a correlation existed between the decrease in spore viability with exposure time but none with temperature, U.V. appeared to be the dominant factor affecting spore viability.

By the time B.t.(HD-1) kept in sunlight lost 50%, of its insecticidal activity, B.t. kept in the shade and darkness could still cause more than 80% mortality after 72 hrs. exposure of 3rd instar larvae. From results obtained concerning the persistence of B.t. it indicates

that after about 3 days the effect of B.t. was very minimal in the field causing only 30% mortality to the test larvae. This actually was very high in comparison to B.t. exposed directly to solar radiation where only about, 10% activity was left. Probably, the high activity still left on leaves was due to. B.t. deposits on the underside of the leaf. The implications of these results is that B.t. can be used to control pests whose host plant grow under the canopy of other plants. Effective application of B.t. to cover even the underside of the leaves may prolong the activity of B.t. Further investigation comparing the persistence of B.t. on either side of the leaf is needed. The time of B.t. application, probably late in the evening, may help to reduce the destruction of B.t. by sunlight. Frye, Hard Carey and Dix (1983) conducted studies on field application of B.t. commercial formulation (Dipel) by day and night against forest pests. They noted that although the survival of spores applied by night was better than that applied by day, night - time spraying results were no better than day-time spraying results in terms of protection against defoliation. The only mechanism that perhaps could reduce destruction of B.t. by sunlight is the use of U.V. protectants and other adjuvants. Formulations incorporating U.V. protectants and stickers as well as B.t. preparations may prolong persistence of the activity on the leaves and thus

increase the efficiency of the spray and enable increased interval time between sprays (Sneh et al., 1981). Field trials by Sneh et al.(1983) revealed that addition of 1% of the adjuvant "Coax" to B.t. var. entomocidus gave protection to the insecticidal activity of B.t. spray deposits: for 4 days and partial protection for 8 days; but the addition of 0.5% did not give adequate protection. The use of Coax as a phagostimulatory adjuvant in microbial insecticides had also been suggested by Bell and Romne (1980) and its protective effect was considered to be the result of masking of U.V radiation (Sneh et al., 1981).

Results of the tests of the three B.t. commercial formulations used against C. partellus and M. testulalis in the laboratory showed them to be pathogenic to these larvae. A mortality of more than 70% was achieved with a concentration of 0.16mg/ml, a dosage lower by ten-fold than the one recommended for field use after three days. All the three formulations performed similarly in the laboratory and in the screen house except that Certan showed unaccountably slightly reduced pathogenicity in the screenhouse.

In the field, all three B.t. commercial formulations were found to be effective in the control of

target pests both in sorghum and cowpea in the different planting patterns. Crops in stands treated with B.t. formulation appeared healthy and gave high yields (Plate 12).

Several parameters were assessed during sampling in sorghum stands in both types of planting patterns to determine the efficacy of B.t. commercial formulations in controlling stem borers. By taking into account all these parameters, for example, number of stem borers recovered, plant height, foliar damage, stem tunnelling, "dead-hearts" and seed yield; it is clear from the results that the biocides sprayed to protect the crop from stem borers was quite effective. The grain yield in the protected stands, which is the most important parameter in the whole exercise of pest control, was double that in the nonprotected stands. The high negative correlation between "deadhearts" foliar damage, number of larvae recovered and, stem tunnelling (in that order) and grain yield shows the importance of each in contribution to yield loss. Berger (1981) reported a high yield of maize realized after control of C.partellus with Thuricide commercial preparation of B.t. The three B.t. commercial formulations used reduced the number of stem borers per plant in comparison with untreated stands thus ensuring less damage to leaves, a high proportion of plants

Plate 12: FIELD APPLICATION: In all the B.t. treated stands of sorghum and cowpea pure, and sorghum/cowpea intercrop, good control of target pest was achieved as compared to untreated plots. (a) Sorghum pure. (b) Cowpea pure (c) Sorghum/cowpea intercrop.

a)

b

c

attaining maturity and finally a healthy plant with strong stem supporting water and nutrient trasporting system, all accounting for the high yield.

In the unprotected stands, there was a large number of stem borers resulting in poor grain yield. Due to the damage on leaves the photosynthetic surface was reduced.

The stem tunnelling interfered with the water nutrient transporting system while the destruction of the entire plant ("deadheart") resulted in a reduced plant population per stand. All these factors directly contributed to the total yield loss in unprotected stands.

The percentage stem tunnelling started increasing from the 9th week after germination. This coincided with the increased number of borers. Stem borer increase at this time was probably due to the emerging second generation of C. partellus and appearance of the other stem borers (B. fusca, S. calamistis and E. saccharina). Also the fact that the application of B.t. was reduced to once every fortnight might have contributed to the increased number of borers. However, the number of stem borers were higher in unprotected stands than in protected stands; 5.5 and 2.2 respectively in both type of planting patterns. So knowledge of when the 2nd generation of C.

partellus emerges and when other stem borers appear is very important so that control measures could be enhanced by correct timing of application. The major stem borer damage was localized at the upper part of the sorghum stem supporting the head (peduncle) and also on the forming heads. This was very serious in the unprotected stands thus resulting in "chaffy heads" in the case of the plants which survived destruction during vegetative growth. The tunnelling of peduncle resulted in poor water and nutrient transport to the maturing head and hence poor seed filling (Plate 13).

This type of damage resulted in drying up of the head before proper seed formation and even breaking of the entire stem. In the case of protected stands, the boring of peduncle was quite minimal and slow, but before the transporting systems were destroyed the seeds had already matured and dried, ready for harvest (Plate 13) The fact that only the original plant was maintained during the whole period of the experiment by removing tillers, may explain why the stem borer number per plant was relatively high in all the treatments. The original plant was the only target for the incoming pests. So future consideration of whether to maintain the tillers during the growth of the plant is very important. More plants per hill may mean less damage, but consequently, more B.t.

has to be sprayed to cover all of them.

It was interesting to note that parasitization of stem borers by other agents such as Dentichasmias sp. and Appanteles sp. occured both in B.t. treated and control stands.

No particular advantage could be associated with either of the planting patterns as far as borer control was concerned.

As observed in sorghum, the three B.t. commercial formulations used were found to be effective in the control of podborer in cowpea under field conditions. However several factors prevailing in the environment interfered with the performance of the crop thus diluting the effectiveness of the parameters used in assessment of the damage by M. testulalis. Firstly, the growth of cowpea during the seedling stage was affected by too much water because the soil was water-logged in some blocks, a soil condition that is known to be unfavourable in cowpea production. This resulted in low flowering and pod formation. Secondly certain pests of cowpea which are not lepidopterans, damaged the cowpea thus interfering with damage and yield assessement. Such pests include thrips which fed largely on flower and flower buds and Bruchids spp. that bored seeds during the process of drying up.

However despite these effects, a significant difference was observed in percentage flower damage, pod damage (Plate 14) and seed damage. Also a significant difference was noted in terms of pod borer recovered and seed yield between protected and unprotected stands.

Yield of cowpea in cowpea/sorghum intercrop was significantly lower than the yield of cowpea in monoculture. The major reason of this difference may have been associated with the shading effect of sorghum on cowpea which may have resulted in poor seed filling in pods. This may have contributed to the low yield realized. So intercrop in this case was not an added advantage in attempt to control M. testulalis. Similar findings were reported by Ikechukwu and Taylor (1984) who found that there was no advantage of mixed cropping over monoculture in exercising M.testulalis control.

A weak negative correlation coefficient observed between the number of pod borers recovered and yield suggested that other factors besides pod borer damage might have interfered with the cowpea yield.

In conclusion, it is quite clear that B.t. (HD-1) is quite effective against lepidopteran pests found locally. The efficacy of B.t. in controlling these pests can quite well be extended to the field where good

protection of host crops have been shown to occur using commercial formulations. Other agents parasitizing lepidopteran pests were found to operate where B.t spraying was done.

(a)

(b)

Plate 13: SORGHUM YIELD: Sorghum heads from (a) B.t. treated stands. (b) untreated stands.

(a)

(b)

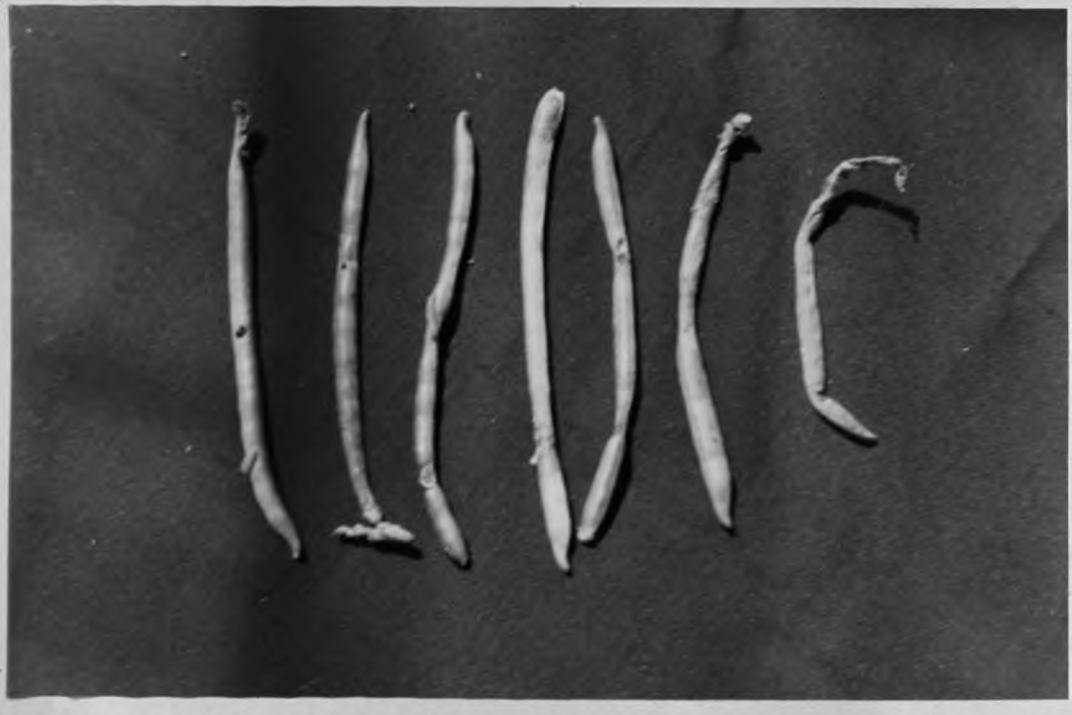


Plate 14: COWPEA YIELD: Cowpea pod damage .a) B.t. treated stands. (b) untreated stands.

SUMMARY OF B.t. AND FUTURE WORK

B.t. (HD-1) (var. kurstaki) and B.t. commercial formulations used in the study have clearly indicated that they are pathogenic to C. partellus, M. testulalis and B. fusca and therefore can be used to control them in the field. B.t. has a narrow spectrum range, killing only pests belonging to the Order Lepidoptera. It has been demonstrated in this study that other agents which parasitizes and kill lepidopteran pests continue to do so even after application of B.t. B.t can therefore be a good component in an IPM programme. It is compatible with other chemicals and can therefore be mixed to control a wider range of crop pests. It is not harmful to human and domestic animals as opposed to chemical toxicity associated with other pesticides. B.t. is highly pathogenic, even at low concentrations and the cost of making formulations may not be very high. B.t. is easier than chemical insecticides to handle and store because of the long shelf-life of spores and delta-endotoxin. Although B.t. does not have a knockdown effect, its overall effect shows a reduction in damage on the host plant.

The major disadvantage of B.t. as demonstrated in this study is its vulnerability to solar radiation. U.V. was found to be deleterious to B.t. although no effort was made to distinguish whether it is delta-endotoxin or spore that is affected. Due to the delayed action after ingestion, some damage may occur in the crops to be protected thus making it unpopular with farmers who would prefer a quick knockdown effect. Due to lack of knowledge of its existence in our local market, B.t. cost-effectiveness is difficult to assess and the use of B.t. is therefore limited to researchers at the moment.

This study gives a positive indication of the applicability and potential success of the use of such preparations as 'Thuricide', 'Dipel' and 'Certan' at the recommended dose for the control of the pest used in the study. The method is likely to constitute an important aspect of the integrated control of the pest complex on host crops.

However, improvement in the technical development of formulations (in view of improving U.V. protectants, stickers and other stabilizers), spray applications, and efficacy assessment should be continued. To the farmers, spraying to cover all parts of the plant (including both sides of the leaf) early in the morning or in the evening would be recommended. In this connection a quantitative

method of measuring deposits at the feeding sites is needed to relate deposit rate of the active ingredient and its effectiveness in plants growing under different planting patterns.

The search for more efficaceous formulations should continue. A field survey should be conducted with the objective of isolating or identifying a local strain of B.t. that is well adapted to local conditions and effective against the pests.

The correct time of spraying in relation to the prevailing weather conditions and the onset of infestation by the pest should be established. In the study conducted, it was noted that several generations of one pest may occur in one growing season of the crop thus requiring several applications of the control agent.

Field dosage and volume/response relationships should be established to provide the pest manager with cost/benefit options, especially with local strains that might be identified for commercial use.

Finally large scale field trials (pilot projects) should be designed to help in evaluating actual potential benefits of B.t. in controlling many species of lepidopteran pests in different ecological zones in a country.

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Appendix I: Analysis of variance for the mean time of death of different larval instars of *C. partellus* *M.testulalis* exposed to *B.t.*(HD-1)

(a) *C.partellus*:

Source of variation	df	SS	MS	F
Replications	3	24.93	8.31	0.88 NS
Treatments	4	166.27	41.57	4.42 *
Expt. Error	12	112.84	9.40	
Total	19	304.04		

* Significant at 5% level
NS Not significant.
Coefficient of variation (CV)= 14.14%
LSD for testing differences within treatments
$LSD_{0.05} = 4.72$.

b) *M. testulalis*:

Source of variation	df	SS	MS	F
Replications	3	27.11	9.04	1.46 NS
Treatments	4	528.57	132.14	21.39**
Expt. error	12	74.12	6.18	

* Significant at 1% level
NS Not significant
Coefficient of variation (CV)= 12.07%
$LSD_{0.05} = 3.83$

Appendix II: Analysis of variance for mean percentage mortality achieved after exposing B.t. (HD-1) to third larval instars of C. partellus and M. testulalis, kept in sunlight for different periods of time .

(a) C. partellus

Source of x variation	df	SS	MS	F
Replications	3	681.22	227.07	1.14NS
Treatments	4	9112.33	2278.08	11.47**
Expt. Error	11	2185.40	198.67	
Total	18	11978.95		

NS Not significant

** Significant at 1% level

CV = 63.60%

$LSD_{0.05}$ = 21.94

(b) M. testulalis

Source of Variation	df	SS	MS	F
Replications	3	95.00	31.67	0.37 NS
Treatments	4	15650.00	3912.50	45.58 **
Expt Error	12	1030.00	85.83	
Total	19	16775.00		

NS Not significant

* * Significant at 1% level

CV = 28.51 %

$LSD_{0.05}$ = 14.27.

Appendix III: Analysis of variance for mean number of stem borers recovered/sorghum plant in different stages of development for all the treatments the different planting patterns.

a) Seedling stage

Source of variation	df	SS	MS	F
Replications	3	3.00	1.00	6.17**
Treatments	7	34.29	4.90	30.22**
Expt.Error	21	3.40	0.16	
Total	31	40.70		

** Significant at 1% level

CV = 38.75%

$LSD_{0.05} = 0.59$

b) Vegetative stage

Source of Variation	df	SS	MS	F
Replications	3	33.83	11.28	18.75
Treatments	7	82.36	11.77	19.56
Expt. Errors	21	12.63	0.601	
Total	31	128.82		

** Significant at 1% level

CV = 41.80%

$LSD_{0.05} = 1.14$

c) Reproductive stage

Source of Variation	df	SS	MS	F
Replications	4	22.63	5.66	5.42**
Treatments	7	148.09	21.16	20.23**
Expt. Error	28	29.22	1.04	
Total	39	199.94		

** Significant at 1% level
CV = 20.15%
LSD 0.05= 1.32

Appendix IV : Analysis of variance for means of sorghum plant height in different stages of development for all the treatments in both planting patterns.

a) Seedling stage

Source of Variation	df	SS	MS	F
Replications	3	13180.55	4393.52	43.04**
Treatments	7	2237.55	319.6	3.14*
Expt. Error	21	21.43.52	102.0	
Total	31	1756.8		

** Significant at 1% level
* Significant at 5% level
CV = 15.16%
$LSD_{0.05}$ = 14.86

b) Vegetative stage

Source of Variation	df	SS	MS	F
Replications	3	1630.20	543.4	3.29*
Treatments	7	23202.96	3314.71	20.25**
Expt.Error	21	3437.83	163.71	
Total	31	28270.98		

** Significant at 1% level
* Significant at 5% level
CV = 10.54%
LSD 0.05 = 18.81

c) Reproductve stage

Source of Variation	df	SS	MS	F
Replications	4	1165.18	291.29	3.23*
Treatments	7	23847.49	3406.79	37.72**
Expt. Error	28	2528.88	90.32	
Total	39	27541.56		

** Significant at 1% level
* Significant at 5% level
CV = 7.78%
LSD 0.05 =13.77

Appendix V : Analysis of variance for mean percentage stem tunnelled at different stages of development of sorghum in different planting patterns.

a) Seedling stage

Source of VAriation	df	SS	MS	F
Replications	3	3.07	1.02	1.42 NS
Treatments	7	40.16	5.74	7.96**
Expt.Error	21	15.13	0.72	
Total	31	58.36		

** Significant at 1% level
NS Not significant
CV = 30.42%
$LSD_{0.05}$ =1.25

b) Vegetative stage

Source of Variation	df	SS	MS	F
Replications	3	351.73	117.24	30.19**
Treatments	7	467.33	66.76	17.19**
Expt. Error	21	81.55	3.88	
Total	31	900.62		

**Significantly at 1% level
CV = 27.18%
$LSD_{0.05}$ = 2.90

c) Reproductive stage

Source of Variation	df	SS	MS	F
Replications	4	1514.11	378.66	33.81**
Treatments	7	1775.05	253.41	22.62**
Expt. Error	28	313.61	11.2	
Total	39	3602.11		

** Significant at 1% level
CV =10.57%
LSD 0.05 = 4.34

Appendix VI : Analysis of variance for mean foliar damage for all the treatments in the different planting patterns.

Source of Variation	df	SS	MS	F
Replications	3	3.59	1.20	0.94 NS
Treatments	7	127.72	18.25	14.37**
Expt. Error	21	26.66	1.27	
Total	31	157.97		

NS Not significant
** Significant at 1% level
CV = 44.51%
$LSD_{0.05}$ = 1.66

Appendix VII : Analysis for variance for mean number of "dea hearts"/10 plants in all the treatments in the different planting patterns.

Source of Variation	df	SS	MS	F
Replications	3	1.44	0.48	1.59NS
Treatments	7	339.75	48.54	161.47**
Expt.Error	21	6.31	0.30	
Total	31	347.50		

NS Not significant
** Significant at 1% level
CV = 19.94%
$LSD_{0.05}$ = 0.81

Appendix VIII : Analysis of variance for mean grain yield in ton/ha of sorghum for all the treatments in the different planting patterns.

Source of Variation	df	SS	MS	F
Replications	3	2.66	0.89	4.39**
Treatments	7	18.24	0.60	12.92**
Expt.Error	21	4.23	0.20	
Total	31	25.13		

** Signigicant at 1% level
CV 23.30%
$LSD_{0.05}$ = 0.66

Appendix IX : Analysis of variance for average number of larva in the flowers and pods/plant in both planting patterns.

Source of Variation	df	SS	MS	F
Replications	5	72.99	14.6	24.44**
Treatments	7	18.99	2.57	4.31**
Expt Error	35	20.90	0.6	
Total	47	111.89		

** Significant at 1% level

CV = 82.25%

$LSD_{0.05}$ = 0.91

Appendix X : Analysis of mean variance for average percentage damage in cowpea for all the treatment in the different planting patterns.

a) Damage Flowers

Source of Variation	df	SS	MS	F
Replications	5	6978.69	1395.74	24.3**
Treatments	7	2204.51	314.93	5.48**
Expt. Error	35	2010.22	57.44	
Total	47	11193.43		

** Significant at 1% level

CV = 40.27%

$LSD_{0.05}$ = 8.88

b) Damaged Pods

Source of Variation	df	SS	MS	F
Replications	5	11303.92	2260.79	40.22**
Treatments	7	1656.67	236.67	4.21**
Expt. Error	35	1967.20	56.21	
Total	47	14927.79		

** Significant at 1% level

CV = 33.57%

$LSD_{0.05}$ = 8.79

c) Damaged Seeds

Source of Variation	df	SS	MS	F
Replications	3	60.46	20.16	0.83 NS
Treatments	7	903.16	129.02	5.30**
Expt. Error	21	511.09	24.34	
Total	31	1474.71		

**Significant at 1% level

NS Not significant

CV = 14.90%

$LSD_{0.05}$ = 7.25

Appendix XI : Analysis for average grain yields of cowpea for every treatments in both planting patterns.

Source of Variation	df	SS	MS	F
Replications	3	0.57	0.19	7.35**
Treatments	7	2.59	0.23	8.78**
Expt. Error	21	0.54	0.03	
Total	31	2.71		

** Significant at 1% level

CV = 28.14%

$LSD_{0.05} = 0.24$