EVALUATION OF *Bacillus thuringiensis* BERLINER FOR THE MANAGEMENT OF RICE LEAFFOLDERS

By

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THESIS

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Department of Agricultural Entomology

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DECLARATION

I hereby declare that this thesis entitled "Evaluation of *Bacillus thuringiensis* Berliner for the management of rice leaffolders" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis entitled "Evaluation of *Bacillus thuringiensis* Berliner for the management of rice leaffolders " is a record of research work done independently by Smt. Asha. R., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

INTRODUCTION

Rice is the most important and extensively grown food crop occupying nearly 40 per cent of the area under cereals in our country. Great strides acheived in the rice yields beginning from early 1960s to mid 1970s started receding in early 1980s and reached stagnation in recent years due to many constraints. With almost no hope for increasing area under rice cultivation, the only way out for stepping up rice production is to increase the productivity.

The productivity of rice crop is arrested by the panorama of different insect pests invading at the various phases of crop growth. Of this multitude, less than 20 species are considered as important pests (Dale, 1994). The introduction of high yielding varieties in the intensive multicropping system led to drastic changes in the ecosystem. This created a large number of minor pests to assume the status of major pests. Gall midge, leaffolders, brown planthopper (BPH), green leafhopper (GLH), white backed planthopper (WBPH) and case worm have now gained greater importance (Zhang, 1991). Among the lepidopterous pests, leaffolders are considered to be economically very important.

A complex of leaffolder species occur in rice, in Kerala of which three are of major importance. *Cnaphalocrocis medinalis* (Guenee) is the endemic and widely found species of leaffolders in all the rice ecosystems. Apart from *C.medinalis, Marasmia patnalis*(Bradley), *M. exigua*(Butler) are the other species of leaffolders found to attack rice crop in the state. Prior to feeding, the larvae fold the leaves longitudinally by fastening the leaf margins, with stitches of thread like rolls. Construction of the silk strands, results in a tubular structure open at both ends; the leaf roll or leaf folds (Kraker, 1996). Sometimes a feeding chamber is created by stitching two or three adjacent leaves together (Fraenkel and Fallil, 1989). The larvae feed inside the folds by scrapping off the green mesophyll tissues between the viens. This results in leaves with linear, pale white striped injury symptoms while heavily injured leaves may dry up completely (Velusamy and Subramaniam, 1974). Generally only one larva is found per leaf fold and after feeding within a fold for several days, the larva moves to another leaf. In this way, a larva makes three to four folds during its development (Barrion *et al.*, 1991).

Leaffolder larvae affect the growth of the rice crop in various ways. Consumption of mesophyll reduces the photosynthetically active area of the crop. Moreover the rate of photosynthesis in the remaining green tissues in leaf fold may be reduced (de Jong,1992). Pandya *et al.* (1994) noted that every unit per cent increase in the leaffolder incidence at tillering , early earing and milky stage led to 1.98, 2.22 and 1.22 per cent yield loss respectively during summer and 2.18, 2.5 and 1.27 per cent yield loss during wet season.

Farmers predominantly use synthetic pesticides to control rice leaffolders (Heong *et al.,* 1994). To prevent unwanted application of insecticides, researchers have attached great emphasis on the establishment of economic injury levels and threshold levels for arriving decisions on pesticide application. For leaffolder, thresholds of 1 to 2 injured leaves per hill have been recommended (Kraker, 1996).

Control tactics other than broad spectrum insecticides include microbial insecticides and release of egg parasitoids. These agents have the advantage that they are harmless to naturally occurring predators and parasitoids. Field trials elsewhere showed promising results with commercial and local formulations of *Bacillus thuringiensis* against rice leaffolder (Peng *et al.*, 1984 and Barrion *et al.*, 1991)

Rice leaffolders have a large and diverse complex of natural enemies, which include more than 200 different species of parasitoids, predators and pathogens as recorded from all over Asia and Pacific (Khan *et al.*, 1988).

The occurrence of nuclear polyhedrosis virus and granulosis virus(GV) on the leaffolder has already been reported in Kerala (Jacob *et al.*,1973a). The investigations on bacterial diseases elucidated the cause of death and details of the infection process, thus providing background data for the field application (Steinhaus, 1964). All sporeforming bacteria produce endospores. As spores, these are capable of remaining viable for long periods of time, either in dead host or exposed to environmental elements outside of their host. Once ingested by a host organism, endospores germinate in the gut to produce vegetative bacterial cells. These enter the haemocoel where they cause septicemia. Granuloses represent a distinct group of insect virus diseases which like the polyhedroses are characterised by a special type of virus inclusion bodies. Under the light microscope, they appear as minute "granules" with dimensions of approximately 300 to 500mμ. It was for this reason that diseases of this group were called granuloses by Steinhaus (1949). Though its occurrence has been recorded, no serious attempt has been made so far to collect the required basic information for judging the suitability of the pathogens for field application. Identification of efficient natural enemies including pathogens and use of them against lepidopterous pests will help to go a long way in the successful implementation of the much needed integrated pest management programme in rice. Hence the present investigation was taken up with the objective of gathering detailed information on the following aspects.

i) Survey and identification of the natural enemies of leaffolders and other lepidopterous pests of rice.

ii) Efficacy of different formulations of *B.t.* on rice leaffolders under field conditions.

iii) Compatibility studies of the promising *B.t.* formulation along with granulosis virus of *Cnaphalocrocis medinalis*(*C.m. gv*).

REVIEW OF LITERATURE

The literature available on different aspects of *Bacillus thuringiensis* pertaining to the present study are classified under the following heads:

2.1 Natural enemies of leaffolders and other lepidopterous pests of rice
2.2 Effect of *Bacillus thuringiensis* on some lepidopterous pests.
2.3 Effect of *Bacillus thuringiensis* on *Cnaphalocrocis medinalis* Guenee.
2.4 Effect of *Bacillus thuringiensis* on other rice pests
2.5 Effect of *Bacillus thuringiensis* in combination with other pathogens
2.6 Safety of *Bacillus thuringiensis* to the natural enemies
2.7 Cross infectivity of the granulosis virus

2.1 Natural enemies of leaffolders and other lepidopterous pests of rice

In 1972, larvae of *C.medinalis* collected from rice fields in Trivandrum, Kerala, India, showed symptoms of a granulosis virus. They at first turned pale and then

Review of literature

milky white. The body became distended and the cuticle raptured releasing a milky white fluid. A GV was isolated and its pathogenicity was proved (Jacob *et al.*, 1973).

During a survey of the microbial diseases of insect pests of rice at Cuttack in India, severe infection by the fungus *Beauveria* was observed *in S.incertulas, C.auriculius, Parnara sp. and Nephotettix virescens* (Rao, 1975). The pathogen was isolated from diseased insects and it was found that the pathogen could be transmitted to all the species mentioned and also to *S.inferens, C.medinalis, Hieroglyphus sp. and Oxya sp.*

Srivastava and Nayak (1978 b) noticed the bacterium *Serratia marcescens* on the larvae of *S.inferens* and *Melanitis leda ismene* on rice in Orissa, India. Later it was found that larvae of *S. incertulas, C.auricilius, C.medinalis* and *Pelopidas mathias* were also susceptible to the disease.

Barrion *et al.* (1979) identified seven species of larval and larval pupal parasites. These were *Cardiochiles philippinensis, Macrocentrus philippinensis, Temelucha stangh, Trichomma cnaphalocrocis, Xanthopimpla* *flavolineata, Goniozus indicus* and *Argyrophylax nigrotibialis* Predators attacking included *Desera geniculata,* a species of *Chlaenius,* a species of *Odontomachus* and spiders *Oxyopes javanus, Argyrope aemula* and *Nephila macula, Solenopsis geminata rufa* preying on the larvae.

Mun (1982) found *Apanteles opacus, A.cypris* and *Temeleucha philippinensis* to be the common parasites of *C.medinalis* in Malaysia. The beetles *Casmoidea interstialis, Paederus fuscipes* and *Micraspis discolor* preyed on the larvae and spiders of four genera *Tetragnatha, Trochosa, Araneus* and *Clubania* were common.

Philip *et al.* (1982) isolated rod shaped bacteria from diseased larvae of *C.medinalis* on rice fields in Kerala, India and these were found to be *Bacillus cereus.*

A survey conducted in thirteen areas of Taiwan revealed the presence of a total of 25 species of natural enemies of rice pest *C.meidnalis* including 21 parasites, two predaceous spiders and two pathogens. Of these the parasites *Trichogramma chilonis* and *Apanteles cypris* were the most abundant (Chen and Chiu, 1983).

Five adults of coccinellids, *Micraspis crocea* and *Synharmonia octomaculata* and nymphs and adults of the mirid *Cyrtorhinus lividipennis* were found to cause 21-32 per cent egg mortality in leaffolder in fields in Philippines (Bandong and Litsinger, 1986). Egg numbers were reduced by 85 and 73 per cent by five individuals of gryllids *Metioche vittaticollis* and *Anaxipha longipennis* respectively.

Manoharan and Chandramohan (1986) reported the natural infection of *C.medinalis* by the entomophagous nematode *Agamermis* sp.

Four parasites of rice leaffolder were recorded at Regional Research Station, Haryana during Kharif, 1987 (Bharati and Kushawaha, 1988). They were *Xanthopimpla flavolineata, Xanthopimpla* sp., *Brachymeria* sp. *laseus* and *Tetrastichus ayyari.*

Ahmed *et al.* (1989) identified *Trichogramma* (egg parasitoid), *Apanteles angustibasis* (larval parasitoid) and *Brachymeria* sp.(pupal parasitoid) to be the main parasitoids of *C.medinalis* in rice fields in Pakistan. The main predators were *Componotus sp., Solenopsis geminata* and *Ischnura forcipata.*

In field surveys conducted in Assam, India *Aulosaphes* sp. was recorded as the dominant parasite of *C.medinalis* followed by *Bracon* sp. Other parasitoids included *Goniozus* sp., *Cardiochiles philippinensis* and *Temelucha* sp. (Borah and Sahara, 1989).

In a survey conducted in Arunachal Pradesh, India, pupae of *Pelopidas mathias, Melanitis ismene and C.medinalis* were found to be infected and killed by *Beauveria velata* (Padmanabhan *et al.*, 1990). The percentage of infected pupae were 12.5 - 51.1, 20.0 - 43.6 and 27.7 - 60.0 for the three pests respectively.

Barrion *et al.* (1991) reported two entomopathogens *Beauveria bassiana* and *Nomuraea* to be effective against leaffolders in the laboratory and field.

Srinivas and Prasad (1991) reported two per cent of the leaffolder larvae collected from rice fields in Andhra Pradesh, India to be infected by *Neoaplectana carpocapsae.*

An entomogenous fungi was found from populations of *C.medinalis.* The fungus identified as *Zoophthora radicans* (*Erynia radicans)* was recorded from India for the first time . Mortality was 100 per cent both under field and green house conditions [Ambethgar, 1995]

2.2 Effect of *Bacillus thuringiensis* on some lepidopterous pests.

Afefy *et al.* (1971) reported that the exposure of the larvae of *Ephestia kuehniella* to sublethal doses of *B.t.* prolongs the larval stage.

Ignoffo and Gregory (1972) demonstrated prevention of mouth part development after ingesting the β-exotoxin of *B.t.* in the larvae of *Heliothes zea, Heliothes virescens, Trichoplusia ni, Spodoptera exigua, Estigmene excraea* and *Pectinophora gossypiella* in the laboratory in Illinois.

Field tests in Tamilnadu, showed that commercial preparations of *B.t.* (Thuricide HP, Biotrol, BTB 254, Bactospeine PM and Thuricide dust) were effective in controlling second and third instar larvae of *Spodoptera litura* on castor (Babu and Subramaniam, 1973)

The effectiveness of sprays of Dipel (a WP preparation of HD-1 of *B.t.*) in controlling pest species of Lepidoptera including *Sylepta derogata, Anomis leon,* *Heliothes armigera, Spodoptera littoralis , Earias insulana* and *E.biplaga* on okra was determined in the laboratory and field plots in Nigeria. The results indicated that application of 3.4 X 10^9 IU $acre^{-1}$ gave effective control (Taylor, 1974).

Govindarajan *et al.* (1976) reported Thuricide 90TS at concentration of 1ml in 400-600 ml of water to be effective against castor semilooper, *Achaea janata.* The time taken for all larvae to be killed averaged 1.19, 2.03, 6.11 and 6.16 days in the first, second, third and fourth instar.

Varma and Gill (1977) reported that two bacterial preparations of Thuricide HPSC and Dipel WP (both with a titre of 16 X 10^3 IU mg^{-1}) were found to be more promising to *Plutella xylostella* especially at 1g and $1.5gl^{-1}$ of water.

B.t. has been reported to be effective against *Orgyia antiqua* (Dronka *et al.*, 1978).

Three preparations of *B.t.* viz. Dendrobacillin, Homelin and Toxabacterin were tested against the forest pest *Dendrolimus superans* [Malokvasova, 1979]. It was found that Dendrobacillin and Homelin were highly effective against the pest where as Toxabacterin was relatively ineffective.

Dipel caused 50 per cent mortality in larvae of *Athalia lugens proxima* after three days of application. All three concentrations of 0.2, 0.1 and 0.05 per cent of spores recorded 100 per cent mortality after four days (Saxena, 1979).

Studies conducted by Dirimanov and Lecheva (1980) on the effectiveness of some microbial preparations against larvae of *Lycia hirtaria,* an important pest of cherry and apple showed that various *B.t.* preparations could cause up to 100 per cent mortality of the larvae depending on the preparation used and larval age.

The effectiveness of Bactospein, Dipel and Thuricide HP was tested against *Anticarsia gemmatalis.* Mortality reached a maximum of about 75-82.5 per cent among larvae confined to leaves taken immediately after treatment with Dipel or Thuricide (Mielitz and Guz, 1982).

Application of Thuricide HP (*B.t. kurstaki*) @ 0.5kg ha^{-1} from a helicopter in field test in Hungary was effective against only first and second instar larvae of *Hyphantrea cunea*. Larvae that had survived treatment for seven days had retarded physiological processes and feeding had been ceased in 80 per cent of the larvae [Jasinka, 1984].

Haider *et al.* [1986] reported that the purified crystal δ-endotoxin of *B.t.* var. *colmeri* to be highly toxic to lepidopteran, *Pieris brassicae* larvae.

Moris (1986) showed *B.t.* to be effective against *Mamestra configurata*.

The effectiveness of *B.t.* as Thuricide or Dipel and of *Trichogramma evanescens* against *Ostrinia furnacalis* and *H.armigera* on maize was evaluated in fields of Philippines. All treatments were found effective on the basis of yield. Dipel was the most effective treatment and also highest net return was obtained with Dipel (Tandan and Nillama, 1987)

Hernandez (1988) reported two strains of *B.t.* var. *kenyae and one of B.t. var.toleworthi* to be highly toxic to neonates of fall army worm *Spodoptera frugiperda*.

Studies conducted by Nahnoud *et al.* (1988) to determine the effect of Certan (*B.t. preparation),*on the survival and development of the first three larval instar of wax moth *Galleria melonella* indicated that the second instar larvae was more susceptible.

Bartninkaite (1989) reported efficacy of *B.t* against *Opheroptera brumata.*

In field trials in India, on the control of the pyralid *Plutella xylostella* on cauliflower, *B.t.* gave better control than all the chemical insecticides tested (Justin *et al.*, 1990).

Brownbridge and Onyango (1992) reported that *B.t.* formulation based on sub sp *kurstaki (B.t.k.)* was highly toxic to all larval instars of spotted stem borer *Chilo partellus* where as the formulation based on sub species *aizawai* was non toxic.

Field experiments were carried out in Geravata, Pernambuco, Brazil to evaluate eight insecticides O.P., pyrethroids and *B.t.* sub sp. *kurstaki*. *B.t.* product Dipel was by far the most effective treatment. Poor results were obtained with pyrethroid insecticides (Barros *et al.*, 1993).

Three formulations of *B.t.* Dipel containing 25 X 10^9 viable spores g^{-1} of the final product of *B.t.* var. *kurstaki*, Thuricide HP containing 30 X 10^6 viable spores of *B.t.* var. *kurstaki* g^{-1} final product and Bactospeine containing 1 X 10^8 viable spores of *B.t* sub sp. *thuringiensis* were assessed for their pathogenicity to *Pericallia ricini*. Dipel was found to be the most effective. A minimum time of 48 h was required to initiate the kill and a maximum of 120 h was required to induce 89.9 per cent mortality at the concentration of 0.5 per cent (Mathur *et al.*, 1994).

2.3 Effect of *Bacillus thuringiensis* on *Cnaphalocrocis medinalis* Guenee.

Srivastava and Nayak (1978b) reported the efficiency of *B.t.* products, Thuricide and Dipel in causing mortality of three day old larvae of *Cnaphalocrocis medinalis*.

Yang (1979) reviewed the work carried out in China on the use of *B.t.* for the control of *C. medinalis* under the general headings of separation and characterisation of different strains, production of the bacterium and practical application.

Investigations were carried out in China on the pathological changes occurring in the midgut tissues of the fifth instar larvae of *C.medinalis* infected with *B.t.* sub sp. *kurstaki* (HD-1) and apparent changes were observed from 6 to 22 h after infection in the midgut tissues (Zhu *et al.,* 1986).

B.t. sub sp. *kurstaki* (Dipel) was more effective against the second and third instar larvae of *C.medinalis* (Aguda *et al.,* 1988)

Barrion *et al.* [1991 b] reported *B.t.* to be promising as microbial insecticide against *C.medinalis.*

A truncated δ-endotoxin cry IA(b) of *B.t.,* which has the specific biological activity against lepidopteran insects was introduced into Nipponbare, a japonica rice variety. Bioassay using R_2 generation plants with two major pests, striped stem borer, *Chilo suppressalis* and leaffolder *C.medinalis* indicated that transgenic rice plants expressing the cry IA(b) protein are more resistant to these pests than the untransformed plants (Fujimoto *et al.,* 1993).

A laboratory bioassay that incorporated *B.t.* purified crystal proteins on dip assay for leaffolder has identified toxins to be effective against two species of leaffolders *C.medinalis* and *Marasmia patnalis* [Aguda *et al.*, 1994).

2.4 Effect on *Bacillus thuringiensis* on other rice pests

Investigations in India, showed that sprays of Thuricide HP at 1 per cent were ineffective against egg, pupae and adults of rice pests, *Scirpophaga incertulas, Sesamia inferens* and *Chilo auricilius.* But all larvae of *S.incertulas* that fed on cut rice stems that had been soaked in 0.25 to 1 per cent Thuricide HP at the time of hatching reduced the incidence of dead hearts by 76.36 per cent, white heads by 67.45 per cent and number of living larvae in the tillers by 76.87 per cent [Nayak *et al.*, 1978].

The toxicity of Thuricide HP at different concentrations on cut rice stems to larvae of *S. inferens* seven and 21 days old was determined. The larvae of both ages were found equally susceptible [Yadava, 1978].

The effectiveness of spray residues of 0.25 to 1 per cent suspensions of Thuricide HP applied on rice seedlings for the control of *Nilaparvata lugens* released on plants 1 h after treatment was determined in laboratory tests in India. Mortality counts made 24 and 48 h after release showed that all the Thuricide HP treatments gave 100 per cent mortality at both times [Rao and Rao, 1979].

In the Maritime province of USSR, best results were obtained with Bitoxibacillin (*B.t.* sub sp. *thuringiensis)* applied @ 1-4 kg in 200 litres liquids ha^{-1} for the control of *Oulema oryzae* infesting rice which killed atleast 94 per cent of the larvae in five days (Buryi *et. al.,* 1991).

Demayo *et al.* (1994) reported a significant difference in the populations of striped stem borer *C.suppressalis* repeatedly against the purified cry IA (C) crystal endotoxin of the bacterium *Bacillus thuringiensis*.

2.5 Effect of *Bacillus thuringiensis* in combination with other pathogens.

Combined application of *B.t.* and GV has been reported to give effective control of Indian meal moth *Plodia interpunctella* (Hunter ,1970, Hunter and Hoffmann, 1973 and Hunter *et al.*,1973).

Schonerr and Ketterer (1979) reported that the application of nuclear polyhedrosis virus (NPV) at a normal dosage (10^6 POBs ml^{-1}) mixed with sublethal doses of *B.t.* (3.75×10^6 spores ml^{-1}) resulted in a higher mortality of *Lymantria monacha* larvae than the single pathogen. Larvae treated with the pathogen combination fed enough to get an infection with virus, but their feeding activity decreased soon and growth diminished. A shortening of the incubation period of the virus disease could not be acheived by the addition of *B.t.* but defoliation of the trees was significantly reduced.

The pathogenicity of combinations of *B.t* and *T.ni* NPV to larvae of *T.ni* was determined in the laboratory tests in the USA The mortality from such combinations was additive when the NPV doses were atleast equivalent to the LD_{45}. However antagonism occurred when the NPV doses were low (Young *et al.,* 1980).

Khodyrev (1985) reported that the combined effect of application of Bitoxibacillin and Virin - EKS (NPV) against larvae of *Mamestra brassicae* was more than either preparation alone.

Su (1991) reported the efficacy of combined application of three formulations of *B. t.* (Biobit, Thuricide and Dipel) along with granulosis viruses against *Plutella xylostella* and *Artogeia rapae* and it lasted for fourteen days in the field.

Peters and Coaker (1993) found that the degree of susceptibility of *Pieris brassicae* larvae to *P. brassicae* granulosis virus (PbGV) was improved particularly when PbGV was applied in mixtures with low doses of either permethrin or *B.t.* sub sp. *kurstaki*.

2.6 Safety of *Bacillus thuringiensis* to the natural enemies

Laboratory studies were conducted to determine the effect of *B.t.* on *Cardiochiles* a parasite of *H. virescens*. Ingestion of the active material in sugarwater by this parasite resulted in significant decreases in life span. Topical treatment with *B.t.* did not decrease in post treatment life span, although there seemed to be an initial effect on survival. Parasites that were exposed to tobacco leaves treated with monocrotophos died within 24 h but parasites were not affected by exposure to tobacco leaves treated with Dipel. Post treatment length of life span of *Talysus spinosus* was not decreased by exposure to tobbacco leaves treated with *B.t.* (Dunbar and Jhonson, 1975).

In tests in Rumania, in forests of oak, Dipel and Turingin applied to control outbreaks of *Lymantria dispar, Tortrix* viridana, *Erannis marginaria* had no adverse effects on the activity of *Formica* sp. (Pascovici *et al.*, 1978).

Laboratory studies were carried out on the effects of *B.t.* var. *kurstaki* on seven species of parasites and one species of predator of *Yponomeuta evonymellus.* Two Tachinids *Bessa fugax* and *Zenillia dolose* as well as Inchneumonid, *Agrypon* sp. were not susceptible to this variety (at a dose of 10^8 - $5X10^8$ spores ml^{-1}). The hymenopteran, *Diadegma arinillata, Pimpla turionellac, Ageniaspis fuscicollis* and *Tetrastichus evonymillae* were found to be susceptible after taking spores of *B.t.* with food sap (Hamed, 1979).

Legotal (1980) studied the safety of *B.t.* preparation BT 202 to the larvae and adults of *Chrysoperla carnea* and *Coccinella septumpunctata,* the ant *Formica pratensis, Trichogramma evanescens* and the spider *Paedosa* *agrestis*. The results confirmed relative harmlessness of the products unless considerable amounts were ingested which led to mortality.

Tests in Bulgaria showed Dipel to be harmless to birds and in particular to those that prey on lepidopterous pests of apple and walnut(Nedkova *et al.*,1980).

Temerak (1980) reported that *B.t.* had deleterious effect on progeny, longevity and developmental duration of *Bracon brevicornis* when exposed to it directly or through the host larvae of *Sesamia actica*.

Temerak (1982) reported that two bacterial pathogens could be transmitted by ovipositor of *B.brevicornis* into the body of *Sesamia cretica*.

Hassan (1983) found Dipel and Thuricide HP (*B.t.* sub sp. *kurstaki)* to be harmless to the egg parasites of the genus *Trichogramma*.

Hassan (1984) reported Dipel to be atleast harmful to thirteen species of beneficial arthropods used for study.

During field trials conducted in Egypt to evaluate Dipel against *Spodoptera littoralis* on cotton it was observed that the population curve of most predators of *S.littoralis* in the region of the coccinellids, *Coccinella undecimpunctata, Scymnus interruptus* and *S. syriacus,* the staphylinid, *Paederus alfierii,* the anthocorid, *Orius* sp and chrysopid, *Chrysoperla carnea* was slightly affected as a result of spraying. This was related to the population reduction of *S.littoralis* and the predator population could rebuild together with that of the host. So, it was concluded that Dipel could be used for the control of *S.littoralis* with no great harmful effects on the predators (Salama and Zaki, 1984).

Field trials on the evaluation of *B.t.* product Thuricide on *Plutella xylostella* on cabbage indicated that it had no adverse effect on hymenopteran parasites of *P. xylostella* [Brunner and Stevens, 1985].

Cheng and Lon (1990) reported *B.t.* products applied to control *Manduca quinquemaculata* in tobbaco fields, to be safe to the parasitoid, *Cotesia congregata.*

Cortes *et al.* (1990) reported that application of *B.t.* to control *Phyllonorycter* sp. attacking beans did not affect the natural parasitism by *Apanteles* sp. Pathogens

2.7. Cross infectivity of the granulosis virus

Granulosis virus of *Pieris brassicae* was successfully transmitted to *P.rapae* and *Pieris napi* (Kelsey, 1958 and Smith, 1959 and 1960).

Studies by Hukuhara et al. (1969) showed that granulosis of *Hyphantrea cunea* was not infective for larvae of *Bombyx mori*.

Laboratory studies by Lipa and Ziemnicka (1972) showed that first, second and third instar larvae of *Agrotis exclamations* , *A. segetum, Heliothis armigera, H. zea and Discestra trifolii* were susceptible to infection with a granulosis virus isolated from larvae of *A. segetum.* Larvae of *Noctua fimbriata* and *N. pronuba* were resistant to infection but died as a result of cytoplasmic polyhedrosis that had apparently been activated by the presence of the granulosis virus.

Hunter and Hoffmann (1972) showed that *Plodia interpunctella* to be moderately susceptible to a similar virus isolated from *Ephestia cautella.* Capsules in cross infected larvae of *P. interpunctella* were generally abnormal in form, some containing up to eighteen virions.

Philip (1978) studied the cross infectivity of granulosis virus of *Pericallia ricini* to larvae of *Diacrisia obliqua, Utethesia pulchella, S. litura, Euproctis fraterna, Sylepta derogate, S. mauritia and Plusia peponis*. No detectable symptoms of granulosis was seen from any of the test insects and no death occurred due to virus infection.

Materials and methods

MATERIALS AND METHODS

The materials used and methods followed for the survey of the natural enemies and for evaluating the efficacy of various *B.t.* formulations against rice leaffolders are given below:

3.1. Survey and identification of natural enemies of leaffolders and other lepidopterous pests of rice

3.1.1. Survey

The survey was conducted in the rice fields in Agricultural Research Station, Mannuthy and Kerala State Agricultural Department Seed Farm, Mannuthy during July 1995 to October 1995 and from July 1996 to October 1996 adopting the following two methods.

i) Periodical collection of dead and dying insects from the field and making observations in the laboratory.

ii) Collection of healthy insects from the field and mass rearing in the lab.

3.1.2. Diagnosis

The parasitised larvae were identified by

i) Sluggishness of the larvae
ii) Lack of feeding
iii) Change of colour
iv) Presence of the grubs of the parasite on the host larvae or the presence of the pupal cocoons near the dead larval hosts.

The diseased larvae were identified by the close pathological symptoms.

3.2 Study of the effect of different commercial formulations of *B.t.* on the rice leaffolders.

The field experiment was conducted at the Agricultural Research Station, Mannuthy under the Kerala Agricultural University, Vellanikkara, Thrissur. The experiments were laid out in a randomised block design (RBD) with eight treatments and three replications and conducted both during Kharif 1995 and Kharif 1996 seasons. The plot size maintained was 6 x 6 m^2 . The rice variety culture 10-1-1 was used for the first field experiment during Kharif 1995 and variety Jaya was used during Kharif 1996.

The details of the treatment are given below:

SL. No.	Treatment	Dose	No. of sprays given based on ETL Experiment	
			I	II
1.	Dipel	1000 ml/ha	1	1
2.	Biolep	1 kg/ha	1	1
3.	Biotox	1 kg/ha	1	1
4.	Delfin	1 kg/ha	1	1
5.	Agree 50 WP	1 kg/ha	1	1
6.	BTT	1 kg/ha	1	1
7.	Monocrotophos	600 ml/ha	1	1
8.	Untreated check		---	---

For having an effective comparison with the six commercial formulation of *B.t.* there was a standard check of the recommended insecticide monocrotophos and an untreated control were included The *B.t.* products were obtained from AICRP on BCCP&W, College of Horticulture, Vellanikkara.

The treatments were applied based on ETL (10 per cent leaf damage at vegetative growth stage and 5 per cent leaf damage at flowering stage). Spraying was done with high volume knapsack sprayer and the quantity of spray fluid used was 500 l ha^{l-1}. The efficacy was assessed by estimating the percentage damage. For working out the estimate, the total leaves and damaged leaves from 10 hills selected at random in each plot were counted and recorded. The population of predators (spiders, coccinellid beetle, staphylinid beetle) was also recorded from 10 hills/plot. A sample number of ten larvae per plot was collected, reared in the laboratory and observed for the extent of parasitism by the larval parasitoids before and after each application of treatment. The above observations were recorded one day prior to treatment and 10 days after the application of the treatment. The number of larvae from ten hills were counted and the mean was recorded as the number per plot. Observations were also taken on the yield parameters viz. number of panicles per hill, percentage of filled grains per panicle and thousand grain weight. The grain yield was recorded for individual treatments and computed per hectare.

Statistical analysis

For comparing the percentage of damaged leaves per hill analysis of covariance was done taking the percentage of damaged leaves per hill before application as the covariate. Then the difference in the percentage of damaged leaves per hill after the application of treatments was compared by conducting Duncans multiple range test (DMRT). For comparing the decrease in percentage of damaged leaves per hill, analysis of covariance was done as in the previous case after taking the difference of the percentage of damaged leaves per hill before and after application of treatments.

The data on the other yield parameters were subjected to simple analysis of variance and the means were compared using DMRT.

The data on the percentage of parasitism were subjected to angular transformation and analysis of variance was done and means compared using DMRT.

3.3 Compatibility studies of promising *B.t.* formulation B.T.T. along with granulosis virus of *C.medinalis*.

The compatibility studies were conducted at the Central Nursery of the Kerala Agricultural University, Vellanikkara.

3.3.1 Mass rearing of leaffolder larvae

Mass culturing of leaffolder on potted rice plants was done as per the method suggested by Waldbauer and Marciana (1979), Fujiyoshi *et al.* (1980) and Goldase and Dumbre (1982).

Larvae were collected from the field and brought to the insectary for obtaining stock culture for the experiment. These larvae were reared on 60 day old potted rice plants (var. Jaya). The plants were grown in earthen ware pots (30 x 30cm^2). The pots were covered with a cylindrical frame (100 cm tall and 35cm diameter). Nylon net cloth was used as a wrap around over the metallic frame. The top of the cage was also covered with nylon net cloth. A sleeve of 50cm length and 20cm diameter was provided in the middle of the cage with the nylon net cloth. The sleeve was tied over with rubber band when not in use. The adults were allowed to emerge inside the cages. Every day morning, freshly emerging adults were collected using a glass vial.

Oviposition cages

Twenty/ Thirty sexed moths were released in oviposition cages. Sustained nutrition of adult moths confined in ovipositional cages was ensured by providing honey after extending with distilled water to get a terminal concentration of 25 per cent volume. Honey thus diluted was offered in cotton swabs placed on the tip of broom stick which was inserted into the mud in the pots.

The leaves of the tillers on which eggs were laid were held together by a thread around. This helped in retaining the first instar caterpillars in groups (when they emerged) on the leaves, which otherwise tend to run about on emergence and fall on the ground. When the tied up leaves were released on the third or fourth day of egg laying, first instar larvae could be collected easily with a camel brush. The caterpillars were reared on potted plants. When the larvae reached second or third instar, larvae were released into experimental pots.

3.3.2. Preparation of the primary inoculum of granulosis virus

The primary inoculum was obtained from field collected diseased larvae. Diseased larvae with typical symptoms as outlined by Jacob *et al.* (1972) were hand picked and stored in distilled water in large conical flasks. It was allowed to decay at room temperature for several weeks.

3.3.2.1 Determination of the concentration of capsules

In the absence of an electron microscope no attempt was made to count the number of granules in the suspension. But at the start of the work itself three litres of a suspension containing granules derived from 1150 dead larvae was prepared and stored under refrigeration at 4° C. This was used throughout.

3.3.3. Application of treatments

Required number of plants were raised in earthernware pots of 30 X 30 cm^2 size filled with paddy soil. 22 days old rice seedlings were planted in each pot. In each pot, two hills were maintained. When 35 days old ten numbers of second or third instar larvae were released in each pot. The plants were covered with net as described under `mass rearing'. When the percentage of leaf damage reached ten per cent in each pot, spraying of the treatment insecticides was done. In the case of BTT 2 ml per litre of the product was used. For spraying each pot 100 ml of the stock solution was used in the case of *C.m.* gv. For *C.m.*gv + BTT, 100 ml of BTT and 100 ml of gv was mixed and from this mixture 100 ml was sprayed per pot.

Observations recorded

i) Total number of leaves per hill
ii) Total number of damaged leaves per hill
iII) Number of larvae per hill

Statistical analysis

The percentage of leaf damage and the number of larvae were subjected to simple CRD analysis. The difference in the percentage of leaf damage before and after application of the treatments and the percentage of larvae dead was worked out and simple CRD analysis was done.

3.4 Cross infectivity of the granulosis virus of *C.medinalis (C.m.)*

Cross infectivity of the granulosis virus of *C.medinalis* to the following species of Lepidoptera were studied.

1) *Melanitis leda ismene*
2) *Psalis pennatula*
3) *Pelopidas mathias*
4) *Spodoptera mauritia*

Ten larvae of the above pests were field collected and fed with rice plants sprayed with virus suspension. After feeding on the treated leaves for one day the larvae were transferred to fresh untreated plants and reared till pupation or death.

Results

RESULTS

4.1 Survey and identification of natural enemies of lepidopteran pests of rice

Results of the survey conducted in the rice fields of the Agricultural Research Station, Mannuthy and the government farm, Mannuthy from June 1995 to October, 1995 and also during June 1996 to October 1996 are summarised in Table 1 and Table 2.

4.1.1 Microbial infections on lepidopteran pests of rice

A total of 133 dead and dying specimens were collected through eight accessions. Symptomatological and pathogenicity studies revealed the occurrence of five viroses, two bacterioses and a nematode.

No pathogenic micro organisms could be isolated from the other specimens. The microbial diseases detected are listed below in Table 1 in relation to their respective host insects.

Table : 1 **Accession and record of diagnosis of microbial diseases of lepidopteran pests of rice**

Sl. No.	Date of collection	Locality of collection	Name of host insect	Condition of larvae	Diagnosis
1.	3-7-95	Agricultural Research Station, Mannuthy	Rice skipper *Pelopidas mathias*	Larva pale yellow coloured, hanging with their hind prolegs attached to the leaf tip and with liquified body contents. All others are healthy.	Nuclear polyhedrosis infection.
2.	11-7-95	Agricultural Research Station, Mannuthy	Army worm *Spodoptera mauritia*	Pale yellow larvae, sluggish in nature, few were mummified. All others are healthy.	Virosis caused by nuclear polyhedrosis virus
3.	22-7-95	Agricultural Research Station, Mannuthy	Rice skipper *Pelopidas mathias*	Three cadavers dark brown coloured. All others are healthy.	Bacterial infection
4.	23-7-95	Kerala State Agricultural Department Seed Farm, Mannuthy	Case worm *Nymphula depunctalis*	Dead larvae hanging from the leaf tips as well as from the standing water, dead larvae in cases. All others are healthy.	Nuclear polyhedrosis virus infection
5.	7-8-96	Agricultural Research Station, Mannuthy	Rice horned caterpillar *Melanitis leda ismene*	Light brown coloured larva hanging with its hind prologs attached to the leaf lip. All others are healthy.	Nuclear polyhedrosis
6.	20-9.-96	Agricultural Research Station, Mannuthy	*Cnaphalocrocis medinalis*	Larvae sluggish, milky white in colour. All others are healthy.	Virosis caused by granulosis virus.
7.	20-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*Cnaphalacrocis medinalis*	Larvae sluggish, later becomes brown coloured and dead, oozing of brown coloured fluid. All others are healthy.	Bacterial infection
8.	20-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*Cnaphalocrocis medinalis*	Larvae sluggish, with pinkish red coloured marking in the centre. Next day larve died and 2 large nematodes coming out from the anal end could be seen. All others are healthy.	Mermethid infection identified as ***Hexamermis*** ***sp.***

4.1.2 Parasitoids of lepidopterous pests of rice

A total of 53 dead and dying specimens could be collected through sixteen accessions. Symptamatological studies revealed the occurrence of four Braconids,three Ichneumonids, two Tachinids, one Chalcidid, one Bethylid and two unidentifed parasitoids. No parasitoids could be reared from the other specimens. The parsitoids are listed below in relation to their respective host insects in Table 2.

4.2 Evaluation of the efficacy of *Bacillus thuringiensis* formulations against the leaffolders on rice.

Two experiments were conducted at the Agricultural Research Station, Mannuthy during Kharif 1995 and Kharif 1996 to determine the field efficacy of *B.t.* formulations against the leaffolders.

4.2.1 The results of the field experiment conducted during Kharif 1995 are summarised below.

4.2.1.1 Percentage of leaf damage

The results on the efficacy of *B.t.* formulations against leaffolders on rice based on the percentage of leaf damage are given in Table 3.

Table 2 Accessions and record of diagnosis of parasites of lepidopteran pest of rice.

Sl. No.	Date	Locality of collection	Name of host insect	Condition of larvae	Diagnosis
1.	27-7-95	Agricultural Research Station, Mannuthy	Rice skipper *Pelopidas mathias*	Few sluggish larvae collected. Later grub of the parasite emaerged from the larvae. Within few hours turned into a brown coloured pupa. All others are healthy.	Tachinid parasite, *Argyrophylax nigrotibialis* emerged.
2.	5-8-95	Agricultural Research Station, Mannuthy	Rice skipper *Pelopidas mathias*	A few Black coloured cadavers of the host larvae sorrounded by a white coloured cocoon. All others are healthy.	Adults of the Braconid parasite, *Apanteles sp.* emerged.
3.	5-8-95	Kerala State Agricultural Department Seed Farm	Yellow hairy caterpillar *Psalis pennatula*	Few larvae when collected sluggish, feeding slowly. Beside the larva a light brown coloured hairy cocoon of parasite could be seen. All others are healthy.	Adults of Braconidae emerged. (un identified)
4.	5-8-95	Kerala State Agricultural Department Seed Farm, Mannuthy-	Yellow hairy caterpillar *Psalis pennatula*	Near the dead larval host, light brown colured sculptured pupa hanging from the rice leaf could be seen. All others are healthy.	Ichneumonid parasite emerged. Identified as *Charops brachypterum*.
5.	9-8-95	Kerala State Agricultural Department Seed Farm, Mannuthy	Rice skipper *Pelopidas mathias*	Few sluggish larvae were collected. Later near dead larvae brownish black coloured oval pupa with whitish powdery coating could be seen. All others are healthy.	Tachinid parasite emerged, *Argyrophylax nigrotibialis*.

6.	30-9-95	Kerala State Agricultural Department Seed Farm, Mannuthy	*P. mathias*	A few light brown coloured skipper pupa collected. All others are healthy.	Orange coloured large Ichneumonid adult emerged. Identified as *Itoplectis sp.*
7.	30-9-95	Agricultural Research Station, Mannuthy	*Cnaphalocrocis medinalis*	A dead larva and by the side a number of small silken pupal cocoons could be collected. All others are healthy	Black coloured adults emerged. Identified as *Apanteles sp., Braconidae.*
8.	20-9-95	Agricultural Research Station, Mannuthy	*Cnaphalocrocis medinalis*	A few larvae with white cocoons were collected. All others are healthy.	A number of adults, metallic green coloured and clear winged emerged from a single cocoon. Identified as *Tetrastichus sp., Eulophidae.*
9.	20-9-96	Kerala State Agricultural Department Seed Farm,, Mannuthy	*Cnaphalocrocis medinalis*	A few black coloured cadavers sorrounded by white cocoon. All others are healthy.	Adult parasites emerged, *Apanteles sp., Braconidae.*
10.	15-8-96	Agricultural Research Station, Mannuthy	*C. medinalis*	Larva sluggish, on death inside white silken cocoons, parasite gubs could be seen	Adults emerged, *Goniozus triangulifer, Bethylidae.*
11.	14-9-96	Agricultural Research Station, Mannuthy	*C.medinalis*	Few larvae were collected. After few days all pupae dead and parasite emerged. All others are healthy.	Black coloured adult emerged. Identified as *Brachymeria sp.,* Chalcididae
12.	17-9-96	Agricultural Research Station, Mannuthy	*C. medinalis*	A few leaf folder larvae collected. After few days within silken cocoon, dead leaf folder larvae. 2 white pupae of parasite seen. All others are healthy.	Adults of *Chelonus sp.* emerged. Adults largeblack coloured with yellow markings. Black coloured marking on the forewing.

13.	17-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*C. medinalis*	A few discoloured larvae were collected. All others are healthy.	Adults Ichneumonid emerged. Identified as *Trichomma cnaphalacrocis*.
14.	30-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*C. medinalis*	Larvae sluggish, later parasite grubs emerged, pupated inside silken cocoons. All others are healthy.	Adults of *Goniozus triangulifer* (Bethylidae) emerged.
15.	30-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*S. mauritia*	Two light brown coloured larvae, sluggish with bulged body, later larvae dead and nearby the dead larvae, oval brown pupal of the parasite could be seen	Adults of Tachinidae emerged (unidentified).
16.	30-9-96	Kerala State Agricultural Department Seed Farm, Mannuthy	*C. medinalis*	Dead larvae sorrounded by small white cocoons collected. All others were healthy.	A number of black parasites emerged. Identified as, *Apanteles angustibasis, Braconidae.*

The leaf damage in the treated plots after treatment ranged from 5.5 to 7.3 per cent which were significantly lower than the untreated check that recorded 22.750 per cent damage by the leaffolder. The percentage of decrease in leaf damage was highest in the *B.t.* treated plot Biotox (-1.566), followed by BTT and Biolep which showed 1.399 and 1.268 percentage decrease respectively. Monocrotophos, Dipel and Delfin had values of 0.089, 1.560 and 1.755 per cent respectively which means a slight increase. Statistically all the *B.t.* and monocrotophos treatments were found to be on par and significantly different from the untreated check (16.93).

The results on the efficacy of *B.t.* formulations on rice leaffolders were also compared without taking the difference in leaf damage before and after treatment, based on the percentage of leaf damage after spraying. The adjusted values for the percentage of leaf damage after spraying also showed that Biotox and BTT to be the best treatments recording the lowest percentage of leaf damage of 4.810 and 4.977 respectively. However the difference recorded in treated plots was statistically on par between each other. In general the damage percentage in the treated plots ranged from 5.5 to 7.3 per cent which was much below the percentage of damage in the untreated check (23.3 per cent). All treatments were significantly different from the untreated check.

Table 3 Efficacy of *B.t.* formulations against leaf folders on rice (Kharif, 1995) based on the percentage of leaf damage

Treatments	Percentage of leaf damage		Percentage difference
	Before treatment	After treatment	
Dipel	7.00	7.300 (7.936) b	0.300 (1.560)b
Biolep	5.60	5.900 (5.108) b	0.300 (-1.268)b
Biotox	5.70	5.500 (4.810) b	- 0.200 (-1.566)b
Delfin	7.23	7.260 (8.131) b	0.030 (1.755)b
Agree 50 WP	6.40	6.100 (6.124) b	- 0.300 (-0.252)b
BTT	5.72	5.647 (4.977) b	- 0.073 (-1.399)b
Monocrotophos	6.44	6.400 (6.465) b	- 0.040 (0.089)b
Untreated check	6.92	22.750 (23.30) a	-15.830 (-16.93) a

Values in parantheses indicate adjusted means used for DMRT.

4.2.2 Yield parameters

The results on the yield parameters of rice as influenced by the application of *B.t.* formulations against leaffolders on rice is presented in Table 4.

4.2.2.1 Number of filled grains per panicle

The number of filled grains per panicle varied from 44.33 to 83.00. Monocrotophos recorded the highest number of filled grains per panicle (83.00) followed by BTT and Biotox that recorded 77.33 grains per panicle. The treatments monocrotophos, BTT, Biotox and Agree 50 WP were on par with each other. All the *B.t.* treatments except Dipel were significantly different from control.

4.2.2.2 Thousand grain weight

The thousand grain weight on the different plots ranged from 13.42g to 20.01g. The *B.t.* treated plot Agree 50WP recorded the highest value of 20.01g followed by Dipel and Biotox which recorded 19.6g and 19.58g respectively. But statistically all the *B.t.* treatments were on par with monocrotophos and all these significantly different from the untreated check.

4.2.2.3 **Percentage of filled grains per panicle**

Monocrotophos was found to be the best in terms of percentage of filled grains per panicle. BTT, Agree 50 WP and Dipel was also better treatments recording 86.3, 86.3 and 84.53 per cent filled grains per panicle respectively and standing on par with monocrotophos. *B.t.* treated plots Biolep, Biotox, Delfin were found to be on par with each other. All the treatments were significantly different from the untreated check.

4.2.2.4 **Grain yield**

The grain yield ranged from 1.45 to 3.5 t ha^{-1}. BTT recorded the highest yield of 3.55 t ha^{-1} which was statistically on par with the yield recorded in the Monocrotophos treated plot (3.42 t ha^{-1}) and both producing significantly higher yields than all other treatments. Dipel, Biolep, Biotox, Agree 50WP were on par and found significantly superior to untreated check. The percentage increase in yield over untreated check in different treatments in the order BTT (144.8), monocrotophos (135.86) Dipel(43.24) and Biotox (42.76), Biolep (39.3), Agree 50WP (33.103) and Delfin (4.83).

Table 4 Yield parameters as influenced by the application of *B.t.* formulations against leaffolders on rice (Kharif, 1995)

Treatment	Number of filled grains per panicle	1000 grain weight (g)	Percentage of filled grains per panicle	Grain Yield (t ha^{-1})	Percentage increase over control
Dipel	56.67 cd	19.6 a	84.53 abc	2.077 b	43.240
Biolep	60.00 c	19.43 a	80.30 bc	2.020 b	39.300
Biotox	77.33 ab	19.58 a	80.30 bc	2.070 b	42.760
Delfin	63.33 bc	18.41 a	77.33 bc	1.520 cd	4.830
Agree 50 WP	69.00 abc	20.01 a	86.33 ab	1.930 bc	33.103
BTT	77.33 ab	18.2 a	86.30 ab	3.550 a	144.800
Monocrotophos	83.00 a	18.83 a	89.00 a	3.420 a	135.860
Untreated check	44.33 d	13.42 b	67.30 d	1.450 d	

4.3 Effect of *B.t.* formulations on the predators and parasitoids of rice pests

4.3.1 Effect of *B.t.* on the predatory spiders

The results on the effect of *B.t.* on predatory spiders of rice pests are presented in Table 5. The species located in the rice ecosystem were wolf spider, *Lycosa pseudoannulata* and the long jawed spider, *Tetragnatha javana*. Before the application of *B.t.* formulations the mean population varied from 2.5 to 3.67 per hill for *L.pseudoannulata* and 1.33 to 2.33 for *T.javana*. After the application of the highest mean density, in case of both the predators, was recorded in the untreated check plot where as it was the lowest in the insecticide check plot. The density of the predatory spiders does not fluctuate much in all other *B.t.* treated plots.

4.3.2 Effect of *B.t.* on the density of other predators

The data gathered on the density of three other predators viz. *Micraspis* sp., *Ophionea* sp. and the rove beetle, *Paederus fuscipes* of rice pests are given in Table 8. In the case of *Micraspis* sp., the population ranged from 3.1 to 4.9 per hill before the application of *B.t.* For *Ophionea* sp. the density varied from 3.7 to 4.6

Table. 5 Effect of *B.t* on the predatory spiders of rice pests. (Kharif 1995)

Treatments	Density per hill			
	Lycosa pseudoannulata		*Tetragnatha javana*	
	BT	AT	BT	AT
Dipel	2.33	3.33	1.33	1.67
Biolep	3.30	3.60	2.00	2.00
Biotox	2.90	3.00	2.33	2.30
Delfin	2.50	3.00	2.00	2.67
Agree 50 WP	3.67	4.00	2.00	3.00
BTT	3.00	3.90	3.00	3.10
Monocrotophos	2.90	0.40	1.33	0.10
Untreated check	3.33	4.20	2.33	3.30

BT - Before Treatment
AT - After Treatment

Table 6 Effect of *B.t* on the other predators of rice pests (Kharif, 1995)

Treatments	Density per hill					
	Micraspis sp.		*Ophionea* sp.		*Paederus* sp.	
	BT	AT	BT	AT	BT	AT
Dipel	3.90	4.50	4.60	4.50	2.30	2.60
Biolep	3.10	3.40	4.00	4.70	3.30	3.90
Biotox	3.80	4.10	4.30	4.90	2.67	2.90
Delfin	4.40	4.40	4.10	4.30	2.90	4.00
Agree 50 WP	4.30	4.40	3.90	4.50	3.00	3.90
BTT	4.00	4.10	3.70	4.20	4.00	4.00
Monocrotophos	4.40	0.20	4.00	0.50	4.50	0.30
Untreated check	4.90	4.70	4.10	4.80	3.30	3.90

BT - Before Treatment
AT - After Treatment

and *for Paederus* sp. it varied from 2.3 to 4.5. After the application of *B.t.* the density varied from 0.2 to 4.7 for *Micraspis* sp., 0.5 to 4.9 for *Ophionea* sp. and 0.32 to 3.5 for *Paederus* sp.

In all the three cases, after the application the density of the predators was lowest in the monocrotophos treated plot. Numerically highest density of *Micraspis* sp. was recorded in the untreated check (4.7), for *Ophionea* sp., it was in Biotox treated plot (4.1) and for *P. fuscipes* it was in Delfin treated plot (4.5).

4.3.3 Effect of *B.t.* on the parasitoids of leaffolder

The data gathered on the percentage of parasitism of the leaffolder after the application of the treatments are presented in Table 7. The parasitism ranged from 13.30 to 20 per cent in the *B.t.* treated plots which were on par with the parasitism in the untreated check which recorded 23.3 per cent. In the monocrotophos treated plot the percentage of parasitism was the lowest (3.33 per cent), and significantly lower than untreated check but on par with the *B.t.* treated plots.

Table 7 Effect of *B.t.* formulations on the parasitism of leaffolder (Kharif, 1995)

Treatments	Percentage of parasitism	
	BT	AT
Dipel	15.5	16.67 (0.1770) ab
Biolep	15.0	13.30 (0.1339) ab
Biotox	18.5	20.00 (0.2014) ab
Delfin	15.0	13.33 (0.1433) ab
Agree 50 WP	15.5	16.67 (0.1676) ab
BTT	17.5	16.67 (0.1676) ab
Monocrotophos	18.0	3.33 (0.0500) b
Untreated check	18.5	23.30 (0.2358) a

BT - Before Treatment
AT - After Treatment

Values in parantheses are angular transformed values

4.4 The results of the field experiment conducted during Kharif 1996 are presented below

4.4.1 Percentage of leaf damage

The results on the efficacy of *B.t.* formulations based on the percentage of leaf damage (Kharif, 1996) are presented in Table 8. The leaf damage ranged from 5.9 to 8.8 per cent before the application of treatments. Ten days after the application of the treatments the damage ranged from 5.8 to 15.99 per cent. The percentage of decrease of leaffolder's damage (adjusted) was higher in Dipel treated plot (-0.350 per cent) followed by Biotox and monocrotophos (-0.321 and -0.109 respectively, Table 10). However all the *B.t.* treatments were on par with each other and monocrotophos but significantly different from untreated check with a percentage decrease of -7.768.

The adjusted means of leaf damage in different plots ranged from 7.235 per cent in Dipel to 15.535 per cent in the untreated check. Though statistically all the *B.t.* treatments were found to be on par with each other and monocrotophos, *B.t.* formulations Dipel recorded the lowest percentage of leaf damage (7.235) followed by Biotox (7.264) and monocrotophos (7.477). All the *B.t.* treatments were significantly superior over the untreated check but they were on par with each other.

Table 8 Efficacy of *B.t.* formulations against leaf folders on rice based on percentage difference in leaf damage (Kharif, 1996)

Treatments	Percentage of leaf damage		Percentage difference in leaf damage
	BT	AT	
Dipel	5.900	5.800 (7.235) b	-0.100 (-0.350) b
Biolep	7.300	7.367 (7.610) b	0.067 (0.024) b
Biotox	7.980	7.600 (7.264) b	-0.387 (-0.321) b
Delfin	6.670	6.790 (7.570) b	0.120 (-0.016) b
Agree 50 WP	8.800	8.950 (7.916) b	0.150 (0.330) b
BTT	7.500	7.700 (7.773) b	0.200 (0.187) b
Monocrotophos	8.200	8.000 (7.477) b	-0.200 (-0.109) b
Untreated check	8.333	15.990 (15.535) a	7.657 (7.768) a

BT - Before Treatment
AT - After Treatment

Values in parantheses indicate the adjusted means used for DMRT.

4.4.2 **Larval mortality**

The results obtained on the efficacy of the *B.t.* formulations based on the larval mortality are given in Table 9.

Before the application of the *B.t.* formulations the number of leaffolder larvae per hill varied from 4.4 to 5.72. After the application of treatments the number per hill of the larvae varied from 0.3 to 6.44. The adjusted treatment means for the decrease in the number of leaffolder larvae was highest in the monocrotophos plot (4.788) followed by *B.t.* treatments Dipel (4.662) and Delfin (4.132). All the *B.t.* treatments were found to be significantly superior over untreated check .

The adjusted treatment means for the number of lear folder larvae per hill, ten days after the spraying of the commercial formulations ranged from 0.193 to 6.45 (Table 9). The *B.t.* treatments Dipel, Biolep, Delfin, Agree 50wp, were found to be on par with monocrotophos treated plots. Statistically all the *B.t.* treatments were found to be significantly superior over untreated check.

Table 9 Efficacy of *B.t* formulations against leaf folders on rice based on the larval mortality (Kharif, 1996)

Treatments	Number of leaf folder larva per hill		Number of leaf folder larvae dead
	BT	AT	
Dipel	4.52	0.40 (0.320) cd	4.120 (4.662) ab
Biolep	5.20	1.20 (1.238) bcd	4.003 (3.743) abc
Biotox	5.25	1.88 (1.927) bc	3.370 (3.055) bc
Delfin	4.52	0.93 (0.850) cd	3.590 (4.132) ab
Agree 50 WP	4.40	1.03 (0.929) cd	3.370 (4.053) ab
BTT	5.40	2.60 (2.673) b	2.800 (2.309) c
Monocrotophos	5.72	0.30 (0.193) d	5.420 (4.788) a
Untreated check	5.04	6.44 (6.450) a	-1.400 (-1.468) d

BT - Before Treatment
AT - After Treatment

Values in parantheses indicate the adjusted means used for DMRT.

4.5 Yield parameters

The results on the yield parameters as influenced by the application of *B.t.* formulations against leaffolders on rice is presented on Table 10.

4.5.1 Number of filled grains per panicle

The number of filled grains per panicle ranged from 77.5 to 118.30. Monocrotophos recorded the highest number of filled grains per panicle (118.30) followed by Dipel (103.9), BTT (99.29), Biotox (98.9) and Agree 50 WP (96.58). All the treatments were significantly different from untreated check.

4.5.2 Thousand grain weight.

Among the *B.t.* formulations Dipel was found to be the best and found to be on par with monocrotophos. Thousand grain weight varied from 25g to 29.3g. The other *B.t.* formulations such as BTT and Agree 50 WP were on par with each other and Dipel but inferior to monocrotophos. Statistically Biolep, Biotox, Delfin and Agree 50 WP were found to be on par with the untreated check.

Table 10 Yield parameters as influenced by the application of *B.t.* formulations against leaf folders on rice (Kharif, 1996)

Treatments	Number of filled grains per panicle	1000 grain weight (g)	Percentage of filled grains per panicle	Grain Yield (t ha^{-1})	Percentage increase over untreated check
Dipel	103.90 b	28.00 ab	83.20 ab	3.20 ab	25.00
Biolep	88.38 bcd	25.33 cd	78.10 abc	3.30 ab	39.06
Biotox	98.90 bc	25.00 cd	71.67 c	3.25 ab	26.95
Delfin	86.26 cd	25.00 cd	74.00 bc	3.10 ab	21.09
Agree 50 WP	96.58 bc	25.60 bcd	82.50 ab	2.99 bc	16.79
BTT	99.29 bc	26.60 bc	84.00 a	3.43 ab	34.00
Monocrotophos	118.30 a	29.30 a	85.00 a	3.58 a	39.84
Untreated check	77.50 d	23.80 d	69.00 c	2.56 c	

4.5.3 Percentage of filled grains per panicle

Percentage of filled grains per panicle varied from 69.00 to 85.00 per cent. BTT was found to be the best among the *B.t* formulations with the highest percentage of filled grains (84 per cent). However other *B.t.* treatments Dipel, Biolep, Agree 50 WP were also on par with BTT and with monocrotophos. Biolep, Biotox, Delfin were found to be on par with each other and with untreated check.

4.5.4 Yield of grain

All the treatments except Agree 50 WP were on par with each other and significantly superior to the untreated check. The grain yield ranged from 2.56 to 3.58 t ha^{-1}. Among the *B.t.* formulations BTT recorded the highest yield of 3.433 t ha^{-1} which was on par with the yield obtained in monocrotophos treated plot (3.580 t ha^{-1}).

The percentage increase over untreated check varied from 16.792 to 39.84 per cent. Among the *B.t.* fromulations BTT recorded the highest percentage of increase in yield over the untreated check.

4.6 Effect of *B.t.* on the predators and parasitoids of rice pests.

4.6.1. Effect of *B.t.* on the density of predatory spiders.

The results on the effect of *B.t.* on density of predatory spiders (Kharif 1996) are presented in Table 11 Before the application of *B.t.* formulations mean density varied from 1.67 to 3.3 for *L. pseudoannulata* and from 1.0 to 2.3 for *T. javana.* After the application of *B.t.* formulations the mean density ranged from 0.5 to 5.7 for *L. pseudoannulata* and 0.2 to 3.3 for *T. javana.* In the insecticide treated plot the mean density decreased in both the cases after the application of *B.t.* formulations where as in the untreated check, the mean density increased. But in the *B.t.* treated plots there was not much difference in the mean density before and after application.

4.6.2 Effect of *B.t.* on other predators of rice pests.

The result on the effect of *B.t.* on the other predators of rice pests (Kharif,1996) is presented in Table 12. Before the application, the mean density varied from 2.0 to 3.7 for *Micraspis* sp., 2.0 to 3.67 for *Ophionea* sp. and 2.3 to 3.67 for *Paederus sp.* After the application of *B.t.* the mean density ranged from 0.2 to 4.33 for *Micraspis* sp., from 0.7 to 4.0 for *Ophionea* sp. and from

Table 11 Effect of *B.t.* formulations on the predatory spiders of rice pests (Kharif, 1996)

Treatments	Density of spiders per hill			
	Lycosa pseudoannulata		*Tetragnatha javana*	
	BT	AT	BT	AT
Dipel	2.67	2.90	1.00	1.33
Biolep	3.30	3.30	2.00	1.67
Biotox	1.67	2.60	1.33	1.33
Delfin	3.33	3.67	2.33	2.00
Agree 50 WP	2.67	2.90	2.00	2.00
BTT	3.67	3.70	2.33	2.00
Monocrotophos	3.33	5.00	2.00	0.20
Untreated check	3.30	5.70	2.33	3.33

Table 12 Effect of *B.t* formulations on the other predators of rice pests (Kharif,1996)

Treatments	Density of pedators per hill					
	Micraspis sp.		*Ophionea* sp.		*Paederus* sp.	
	BT	AT	BT	AT	BT	AT
Dipel	3.67	4.33	2.00	3.33	2.30	3.00
Biolep	2.00	3.90	2.67	3.00	3.00	3.20
Biotox	2.33	4.30	3.00	3.33	2.67	3.00
Delfin	3.00	4.30	3.00	3.00	3.67	3.67
Agree 50 WP	3.33	3.30	3.66	2.67	3.30	3.67
BTT	3.33	3.66	2.78	3.30	2.67	3.00
Monocrotophos	2.67	0.20	3.30	0.70	2.97	0.20
Untreated check	3.60	4.30	3.67	4.00	3.67	3.67

BT - Before Treatment
AT - After Treatment

0.2 to 3.67 for *Paederus* sp. In all other cases the lowest mean density was recorded in insecticide treated plot and there was no much difference between the *B.t.* treated plots and untreated check.

4.6.3 Effect of *B.t.* on the parasitism of rice leaffolder

The result on the effect of *B.t.* on the parasites of rice leaffolder (Kharif, 1996) are presented in Table 13. After treatment the percentage of parasitism of rice leaffolder ranged from 6.67 per cent to 20 per cent. Though statistically all the treatments were on par with each other, monocrotophos recorded the lowest parasitism value of 6.67 per cent.

4.7 Compatibility of BTT with granulosis virus of *Cnaphalocrocis medinalis (C.m. GV)*

Compatibility studies of *B.t.* formulation BTT with granulosis virus of *Cnaphalocrocis medinalis* was conducted in the pot culture in the Central Nursery, Kerala Agricultural University.

Results of the experiment conducted to evaluate compatibility of *B.t* formulation BTT with *Cnaphalocrocis medinalis* granulosis virus (*C.m.* GV) are given below

Table 13 Effect of *B.t.* formulations on the parasitism of leaf folder (Kharif, 1996)

Treatments	Percentage of parasitism	
	BT	AT
Dipel	17.5	20 (0.201) a
Biolep	17.6	20 (0.201) a
Biotox	18.1	20 (0.204) a
Delfin	18.5	16.67 (0.177) a
Agree 50 WP	19.1	23.3 (0.247) a
BTT	16.0	16.67 (0.168) a
Monocrotophos	17.5	6.67 (0.075) a
Untreated check	18.5	20 (0.201) a

BT - Before Treatment
AT - After Treatment

4.7.1 Percentage of damaged leaves

The results on the compatibility of BTT with the granulosis virus of *C.medinalis* (*C.m.* GV) based on percentage of damaged leaves is presented on Table 14

The percentage of damaged leaves ranged from 11.635 to 14.66 in the test plots before the application of treatments, twenty day after transplantation. In the case of the untreated check, the percentage of damaged leaves increased from 14.224 to 21.709 ten days after the first spraying and it went upto 37.8 after second spraying . In all the other treatments, the percentage of damaged leaves went on decreasing. For BTT it was 9 per cent after first spraying, a 5.66 percent decrease and 3.598 per cent after second spraying, a 5.4 per cent decrease. The percentage of damaged leaves was 7.91 and 3.617 for C.m. GV, 8.47 and 3.91 for the combination of BTT and *C.m.* GV and 6.68 and 2.60 for insecticide check plot ten days after first and second spraying respectively.

After first spraying treatments BTT, *C.m.* GV and combination of BTT and *C.m.* GV were on par with each other. The treatments *C.m.* GV and BTT + *C.m.* GV were on par with monocrotophos which showed lowest damage. All the

Table 14 Compatibility of BTT with the granulosis virus of *C.medinalis* (*C.m. GV*) based on the percentage of leaf damage

Treatments	Percentage of damaged leaves per hill			Decrease in percentage of damaged leaves per hill	
	20 DAT	30 DAT	40 DAT	30 DAT	40 DAT
BTT	14.660	9.00 b	3.598 bc	- 5.660 c	- 5.402 b
C.m. GV	11.635	7.91 bc	3.617 bc	- 3.725 b	- 4.293 b
BTT + *C.m.* GV	12.560	8.47 bc	3.910 b	- 4.087 b	- 4.563 b
Monocrotophos	12.350	6.68 c	2.600 c	- 5.640 a	- 4.080 b
Untreated check	14.224	21.71 a	37.800 a	7.485 a	16.09 a

DAT - Days after transplanting

treatments were significantly different from the untreated check. The treatments BTT, *C.m.* GV were on par with monocrotophos treated plot.

Based on the difference in the percentage of leaf damage BTT was found to be the best (5.66 per cent decrease) and on par with the monocrotophos (5.64 per cent decrease) plot after first spraying. After second spraying all the treatments were on par with monocrotophos treated plot.

4.7.2 Larval mortality

The results on the compatibility of BTT with *C.m.* GV based on the larva mortality is presented in Table 15. The number of leaffolder larvae per hill varied from 6.0 to 7.5 in the test pots before the application of the treatments. In the case of the untreated check, the number of larvae per hill increased from 7.0 to 10.0, after first spraying and from 10.0 to 12.1 after second spraying. But there was substantial reduction in the larval population in the case of the treatments BTT, *C.m.* GV, combination of BTT and *C.m.* GV and monocrotophos which were on par with each other.

Table 15 Compatibility of BTT with the granulosis virus of *C.medinalis* (*C.m.* GV) based on the larval mortality

Treatments	Number of leaf folder larvae per hill			Percentage of larvae dead per hill	
	20 DAT	30 DAT	40 DAT	30 DAT	40 DAT
BTT	7.5	2.5 b	0.5 b	67.13 a	84.17 ab
C.m. GV	6.0	2.0 b	0.3 b	66.37 a	86.00 ab
BTT + *C.m.* GV	6..0	2.5 b	1.0 b	58.89 a	66.41 b
Monocrotophos	6.5	1.0 b	0.0 b	83.97 a	100.00 a
Untreated check	7.0	10.0 a	12.1 a	- 45.15 b	- 23.09 c

DAT - Days after transplanting

The percentage of larvae dead after spraying was 67.13 in BTT, 66.37 in *C.m.* GV, 58.89 in the combination of BTT and *C.m.* GV and 83.97 per cent for monocrotophos.However the larval population increased in the untreated check by 45.15. All the microbial treatments were on par with monocrotophos treated plot. After second spraying there was 100 per cent mortality in monocrotophos. In the BTT treated pot it was 84.17, for *C.m.* GV it was 86.00 per cent and for combination of BTT + *C.m.* GV it was 66.41. The treatments BTT, *C.m.* GV and BTT + *C.m.* GV were on par and BTT and *C.m.* GV were on par with monocrotophos. All the treatments were significantly different from the untreated check.

4.8 Cross infectivity of the granulosis virus of *C.medinalis* to other lepidopterous pests of rice.

The results given in Table 16 showed that the granulosis viruses of *C. medinalis* was highly host specific. All the larvae of *Melanitis leda ismene, Psalis pennatula, Pelopidas mathias* and *Spodoptera mauritia* were not susceptible to GV of *C. medinalis*.

Table 16. Infectivity of Granulosis virus of *Cnaphalocrocis medinalis* to other species of rice pests.

Sl. no.	Test Insect	Total number of larvae inoulated	Instar of the larvae inoculated with *C.m.* GV	Number of larvae dead due to		Infectivity	Percentage of adult emergence
				GV	Others		
1.	*Melanitis leda ismene*	10	3	0	2	- ve	80
2.	*Psalis pennatula*	10	3	0	0	- ve	100
3.	*Pelopidas mathias*	10	3	0	5	- ve	50
4.	*Spodoptera mauritia*	10	3	0	3	- ve	70

* There was no mortality in control

Discussion

DISCUSSION

Biological suppression of noxious insects on rice has been successfully demonstrated in many locations as reported by Srivastava and Nayak (1978), Nayak *et al.* (1978), Chen and Chiu (1983) and Demayo *et al.* (1994). First hand information on the native biological agents including microbial pathogens is a prerequisite in attempting such a programme. Hence the studies in the present investigations have been made with the main objectives of survey and identification of the natural enemies of the leaffolders and other lepidopterous pests and to asses the possibility of using the various commercial formulations of *B. thuringiensis* in the control of rice leaffolders under field conditions.

Survey was conducted in the rice fields of the Agricultural Research Station, Mannuthy and the Kerala State Agricultural Department Seed Farm, Mannuthy from June 1995 to October 1995 and also during June 1996 to October 1996 on the microbial infections and parasitoids of the lepidopterous pests of rice. The study revealed the occurrence of five viroses, two bacterioses and a nematode infection. The studies also brought to limelight the occurrence of one Chalcid, three Ichnenmonids, four Braconids, one Bethylid and two Tachinid parasitoids on various lepidopterous pests of rice.

5.1. **Microbial infections on lepidopterous pests of rice**

Incidence of nuclear polyhedrosis of *Spodoptera mauritia* has been reported earlier by Bianchi (1957) in Hawaii. Jacob *et al.* (1973b) reported widespread incidence of a nuclear ployhedrosis in populations of *S. mauritia*, on paddy in Kerala. The external and internal symptoms of the dead larvae collected in the present studies were similar to those observed by Jacob *et al.* (1973b). Thus the occurrence of the nuclear polyhedrosis virus infection in *S. mauritia* was confirmed.

Symptoms of the nuclear polyhedrosis of *Nymphula depunctalis* generally resembled those described by Devanesan (1979).

Larvae of *Pelopidas mathias* infected by the NPV exhibited loss of appetite and sluggishness and appeared paler in colouration compared to the healthy larvae. Similar observations were made by Nayak and Srivastava (1978) by allowing healthy larvae of *P. mathias* to feed on rice plants sprayed with crude suspension of NPV. Nayak and Godse (1986) during a survey on microbial diseases of insect pests of rice in India, observed a NPV in *P. mathias* in rice fields in Orissa. Microscopic examination of haemolymph and tissue smears revealed a large number of refractile polyhedral bodies which were negative to staining by geimsa.

The occurrence of NPV as a pathogen on the rice horned caterpillar, *Melanitis leda ismene* was detected in the present studies. No previous record on this is available in literature.

The symptoms of the nuclear polyhedrosis of the lepidopterous pest of rice detected in the present study resembled those described for nuclear polyhedrosis of other lepidopterous larvae as reviewed by Aizawa (1963) and (Smith) 1967.

Steinhaus and Marsh (1962) identified *C. medinalis* larvae in Fiji to be infected by a GV. Jacob *et al.* (1973) reported the occurrence of GV in larvae of *C.medinalis* collected from rice fields in Trivandrum, Kerala. The infected larvae especially the fourth and fifth instars turned white in colour, body became distended and cuticle ruptured releasing a milky white fluid Philip (1978) also noted similar colour changes in the larvae of *Pericallia ricinii*. Many of the larvae collected from rice fields in Mannuthy in the present studies exhibited similar symptoms.

Bacterioses of *P.mathias* caused by *Bacillus* sp. is reported for the first time. Symptoms closely resembled those described by Steinhaus (1951) for most species of Lepidoptera. The infected larvae especially later instars assumed slightly greyish and finally dark brown to black colour.

In squash bugs, diseased insects became slightly darker as death approached when infected by *Bacterium entomotoxicon* (Dugger, 1895). White (1923) also noted similar colour change in hornworm infected by *Bacterium spingidis.*

In the present study, it was found that the dead or dying late instar larvae of *C. medinalis* showed typical symptoms of bacteriosis as described by Steinhaus (1951). Philip *et al.* (1982) isolated rod shaped bacteria from diseased larvae of *C.medinalis* on rice fields in Kerala, India which was found to be *Bacillus cereus.*

This was the first time that the nematode, *Hexamermis* sp. is recorded as a pathogen on rice leaffolder, *C.medinalis* in India. In the present studies it was found that the dying late instar larvae exhibited the typical symptoms of nematode infection. The infected larvae showed loss of appetite and became sluggish in movement. As the infection advances the larvae assumed a pinkish red colour in the centre of the body. Microscopic examination of the female juveniles of the nematode indicated the presence of a caudal appendage which is a characteristic feature of *Hexamermis* sp.Similar observations were made by Subbiah (1990) in *Cydia leucostoma* infected with *Hexamermis sp.* It was generally believed that the caudal appendage of *Hexamermis* juveniles might be helpful in piercing the body wall of the host insect.

Manoharan and Chandramohan (1986) also observed identical symptoms in larve of *C. medinalis* when infected by the nematode, *Agamermis* sp. Similar observations were made by Srinivas and Prasad (1991) in *C. medinalis* infected by the nematode DD-136, *Neoaplectana carpocapsae.*

5.2 Parasitoids of lepidopteran pests of rice.

The density of the parasitoids of lepidopteran pests of rice were estimated and it was found that the incidence of braconids were high in the rice fields surveyed. Among the braconids *Apanteles* sp. was the important species of parasitoid attacking the larvae of lepidopterous pests. Many authors have reported the occurrence of *Apanteles* sp. as the important parasitoid of rice pests. Reissig *et al.* (1986) recorded the occurrence of *Apanteles* sp. in rice skipper, *P. mathias.* Mun (1982) conducted detailed investigations on *Apanteles opacus, A. cypris,* the common parasites of *C. medinalis* in Malaysia. Chen and Chiu (1983) made a detailed survey in Taiwan and identified *Trichogramma chilonis* and *Apanteles cypris* to be the most abundant species of parasitoids among 21 parasitoids attacking *C. medinalis.* It was generally noticed that lepidopterous larvae when infected by *Apanteles* were surrounded by a white coloured or silken cocoon and black coloured small adults emerged in groups from inside the cocoon. Similar observation was made by Ahmed *et al.* (1989) in *C. medinalis* infected by *A. angustibasis.*

The occurrence of the *Chelonus* as a parasitoid on *C. medinalis* was detected in the present studies. No previous record of this is available in the literature. The infected larvae showed, sluggishness and loss of appetite. After few days within sliken cocoon, pupa of parasite could be seen on the cadaver. Only one adult emerged from the cocoon. Adults were larger in size, black coloured with yellow markings. Black coloured markings could be seen on the forewing.

The next abundant parasitoid found to occur in Mannuthy was *Goniozus triangulifer*. In the case of the *G. triangulifer* infected *C. medinalis* larvae, the larvae showed sluggishness and died after few days . On the cadaver silken cocoons of the parasite grubs could be seen. Narayanan *et al.* (1964) also observed similar symptoms in *C. medinalis* larvae parasitised by *G. triangularis* and *Goniozus* sp. Barrion *et al.* (1979) identified *G. indicus* to be one of the seven species of parasites attacking *C. medinalis*.Arida and Shepard (1990) has also reported *G. triangulifer* to be one of the most common parasitoid of leaffolder larvae in Laguna Province, Philippines.

The Tachinid parasite, *Argyrophylax nigrotibialis* was the major parasite attacking the rice skipper, *Pelopidas mathias*. The skipper larvae when infected by *A. nigrotibialis* were sluggish, after few days near the dead larvae, single brownish black coloured pupa with powdery coating could be seen. Puparia with white coating is characteristic of *A. nigrotiabialis*. Barrion *et al.* (1979) has reported the parasite *A. nigrotibialis* to be infecting rice leaffolder, *C. medinalis*. Rajapakse (1990) observed similar symptoms in the *C. medinalis* larvae while conducting detailed studies on the *Argyrophylax* sp. on the *C. medinalis* larvae in rice fields in Srilanka.

In the present study, the Ichneumonid, *Charops brachypterum* was found to be a parasite of *Psalis pennatula*. The signs and symptoms in the infected larvae, resembled those described by Reissig *et al.* (1986) for *P. mathias* infected by *C.brachypterum*. The larvae of *P.pennatula* infected by *C. brachypterum* were sluggish with loss of appetite. As the larvae died within few days, a light brown and white coloured sculptured pupa could be seen hanging from the rice leaf on a brown silken thread.

The dead and dying late instar larvae of *C.medinalis* exhibited signs of Ichneumonid parasitisation. The infected larvae were discoloured. After few days, a single large Ichneumonid emerged from the dead host identifed as *Trichomma cnaphalocrocisi* (Uchida). The signs and symptoms observed in the present study agrees with the finding of Barrion *et al.* (1979). Ooi and Shepard (1994) has also observed *T.cnaphalocrocis* to be the major parasite of *C.medinalis*.

Tetrastichus sp. has been recorded as a pupal parasite of *C. medinalis*. *Tetrastichus* sp. has been recognised as parasites of lepidopteran rice pests by many authors. Narayanan *et al.* (1964) recorded *Tetrastichus* sp to be attacking *C. medinalis*. Bharathi and Kushawaha

(1988) in their detailed studies on the parasites of leaffolder in Haryana, India have reported *Tetrastichus ayyari* to be one of the important parasite of leaffolder. The symptoms observed in *C. medinalis* larvae in the present study agrees with the findings of the above authors. Ooi and Shepard (1994) has also indicated *T. ayyari* to be one of the important pupal parasite of *C. medinalis*.

The pupal parasitoid *Brachymeria* sp. has been recorded in the leaffolder in the present survey. Few leaffolder larvae collected after pupation died and single parasite emerged from the pupa. Similar symptoms were observed by Bharati and Kushawaha (1988) in *C. medinalis* infected by *Brachymeria* sp. nr. *laseus*. Ahmed *et al.* (1989) also identified *Brachymeria* sp. to be important pupal parasitoid of *C. medinalis*.

Apart from the above, an unidentified Braconid was recorded on the rice leaffolder and an unidentified Tachinid was found to attack *Spodoptera mauritia*.

5.3 Evaluation of efficacy of commercial formulations of *B.t.* against leaffolders

In the field experiments with six commercial formulations of *B.t.* during Kharif 1995, the reduction in the percentage of leaf damage was in the order of Biotox (1.566), BTT (1.399), Biolep (1.268), eventhough all the treatments were on par with each other statistically [Table. 3.]. In the insecticide treated plot the reduction in leaf damage was -0.089 per cent and in the untreated check it was -16.93 per cent. During Kharif 1996, the percentage of reduction in leaf damage was in the order of Dipel (0.350), Biotox (0.321), and Delfin (0.016). Here also all the *B.t.* treatments and monocrotophos were on par with each other statistically (Table 8). The reduction in the percentage leaf damage in monocrotophos treated plot was 0.109 and for the untreated check plot it was -7.768. Fig 1 and Fig 2 show that the percentage of leaf damage remains more or less constant in the treated plots where as in the untreated check there is an increase in the precentage of leaf damage.

Similar observations were recorded by Taylor (1974) during his studies on the use of Dipel in controlling pest species of Lepidoptera including *Sylepta derogata, Anomis leon, Heliothes* armigera, *Spodoptera littoralis, Earias insulana* and *E. biplaga* on okra in Nigeria.

In the present studies, the decrease in the number of leaffolder larvae after treatment showed that the larvae were highly susceptible to the treatment. Though the treatments Dipel, Biolep, Delfin, Agree 50 WP were on par

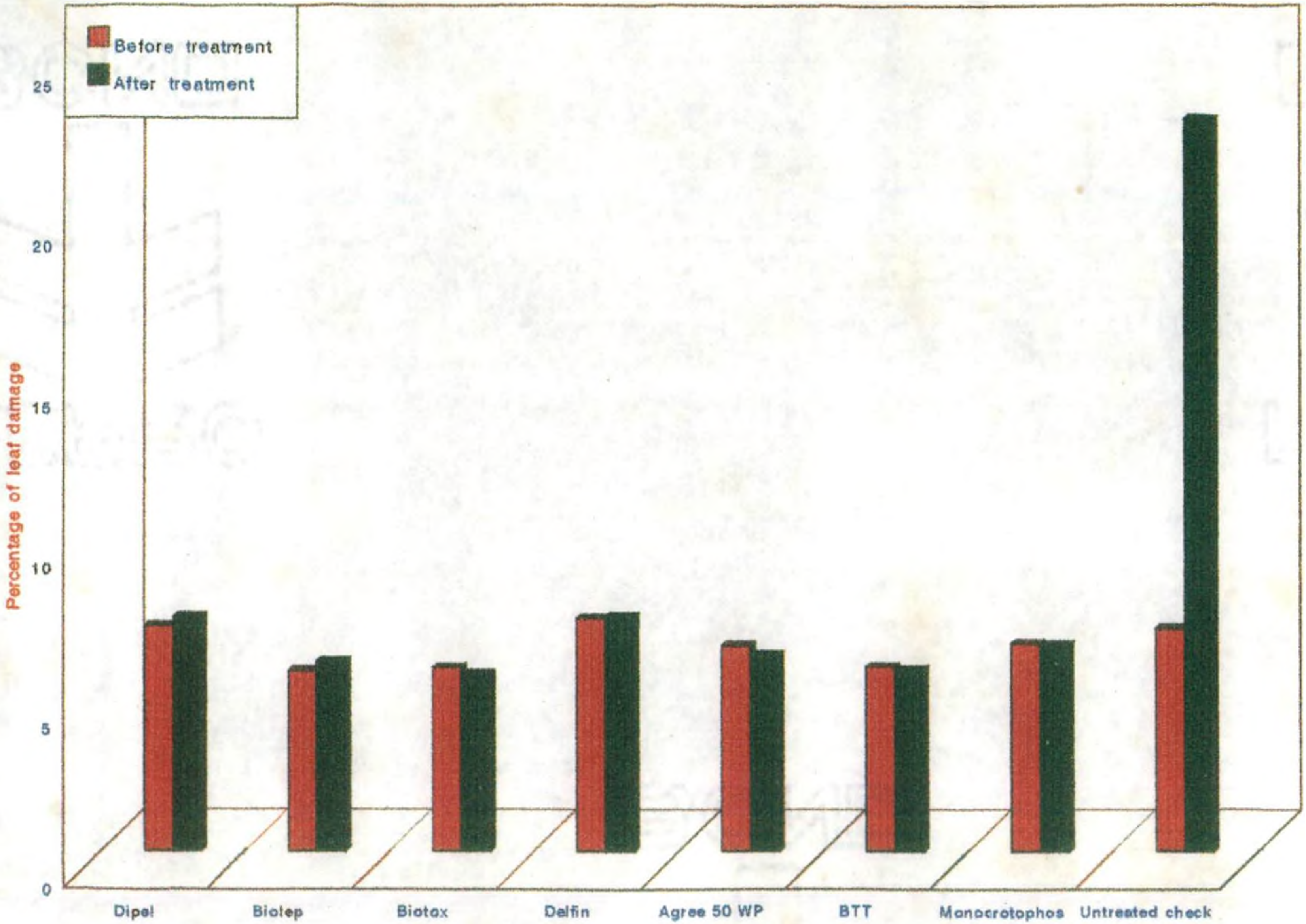

Fig 1 Efficacy of B.t. formulations against leaffolders on rice (Kharif, 1995) based on the percentage of leaf damage

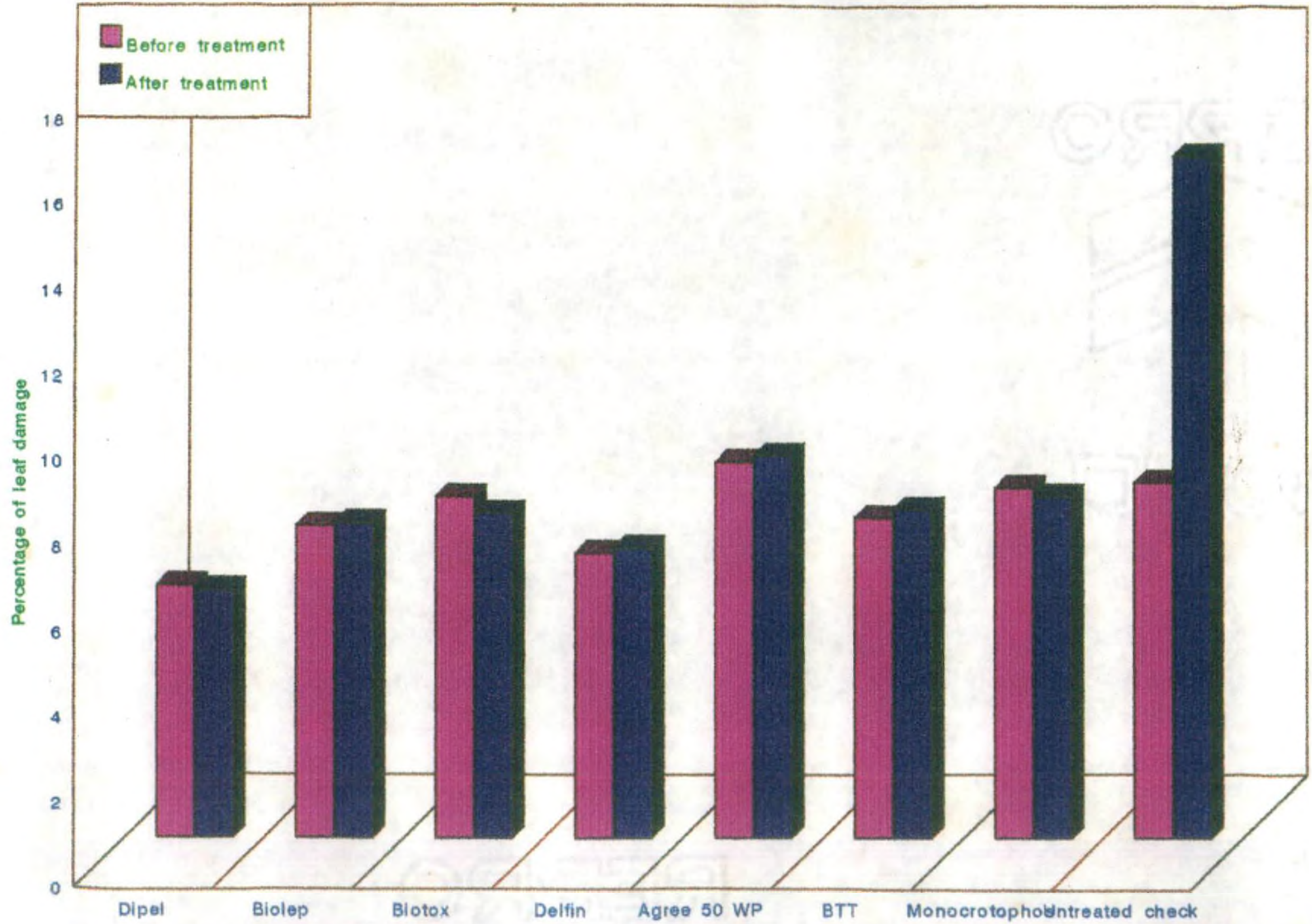

Fig 2 Efficacy of B.t. formulations against leaffolders on rice (Kharif, 1996) based on the percentage of leaf damage

with each other, the decrease in the number of larvae was highest in Dipel (4.662) followed by Delfin (4.132) and Agree 50 WP (4.053) [Table 9]. For the monocrotophos treated plot the decrease in number was 5.420 and in the untreatd check it was -1.468, showing an increase of 1.468 larvae. Srivastava and Nayak (1978) have also observed the susceptibility of *C. medinalis* larvae to the *B.t.* products Thuricide and Dipel. The present findings also agree with that of Barrion *et al.* (1991) who also obtained higher degree of efficacy against *C. medinalis* larvae with Dipel. Aguda *et al.* (1988) has reported the efficacy of *B.t.* sub sp. *kurstaki* (Dipel) against second and third instar larvae of *C. medinalis.*

Yield parameters

During Kharif 1995, monocrotophos recorded highest number of filled grains per panicle (83.00) followed by the *B.t.* treatments Biotox and BTT (77.33). However all the treatments execept Dipel were significantly different from the untreated check (Table 4). During Kharif 1996, monocrotophos recorded the highest number of filled grains per panicle (118.3). Dipel was the next best treatment recording 103.9 filled grains per panicle. All the treatments except Biolep and Delfin were significantly different from untreated check (Table 10).

The data indicate that test weight of culture 10-1-1 during 1995 was not altered by the treatment of different *B.t.* formulations and it remained on par with monocrotophos application. Application of both *B.t.* preparations and monocrotophos enhanced the test weight significantly over the untreated control (Table 4). The thousand grain weight was highest in *B.t.* product Agree 50 WP treated plot (20.01g) followed by Dipel (19.6g). For monocrotophos it was 18.83g and in the untreated check it was 13.42 g. Similarly during Kharif 1996, the *B.t.* prepartion Dipel and monocrotophos treated plots produced significantly high test weight in `Jaya' over the untreated control. Statistically bacterial preparations showed varied effect and only Dipel was comparable to monocrotophos, which showed maximum test weight of 26.3 g. The treatments Dipel, Agree 50 WP and BTT were found to be on par with each other and the values were 28.00g, 25.60 and 26.60g respectively [Table 10].

During Kharif 1995, the *B.t.* preparations Dipel, Agree 50 WP , BTT and monocrotophos were statistically similar in the percentage of filled grains per panicle, ie. 84.53, 86.33 , 86.3 and 89.00 respectively, and were superior to the untreated check (67.3 per cent) [Table. 4]. During Kharif, 1996, the percentage of filled grains per panicle in the case of BTT (84 per cent), Dipel (83.2 per cent), Agree 50 WP (82.5 per cent) and Dipel (78.1 per cent) was on par with the monocrotophos treated plot (85 per cent) and was superior to the untreated check where it was 69 per cent.

During Kharif 1995, BTT recorded the highest yield of 3.55 tha^{-1} followed by monocrotophos (3.42 tha), Dipel (2.077 tha^{-1}) and Biotox (2.07 tha^{-1}) which were statistically similar and superior to all other treatments. During Kharif 1996, all the *B.t.* treatments except Agree 50WP were on par with monocrotophos. Highest yield was in the monocrotophos treated plot (3.433t ha^{-1}) followed by Biolep (3.3 tha^{-1}) and Biotox(3.25t ha^{-1}). In the untreated check the yield was 2.56t ha which was statistically inferior (Table 10). Figure 3 and 4 graphically depict the yield variation between the treatments.

Yield is ultimately the product of number of panicles per hill, number of filled grains per panicle, test weight and not an outcome of single yield attribute.Number of panicles per hill remained the same and the other attributes that determine the yield are the number of filled grains per panicle and the 1000 grain weight .In the present studies it has been found that the application of *B.t.* formulations could significantly increase the number of filled grains per panicle and the 1000 grain weight over the untreated check.Hence there was an increase in yield.

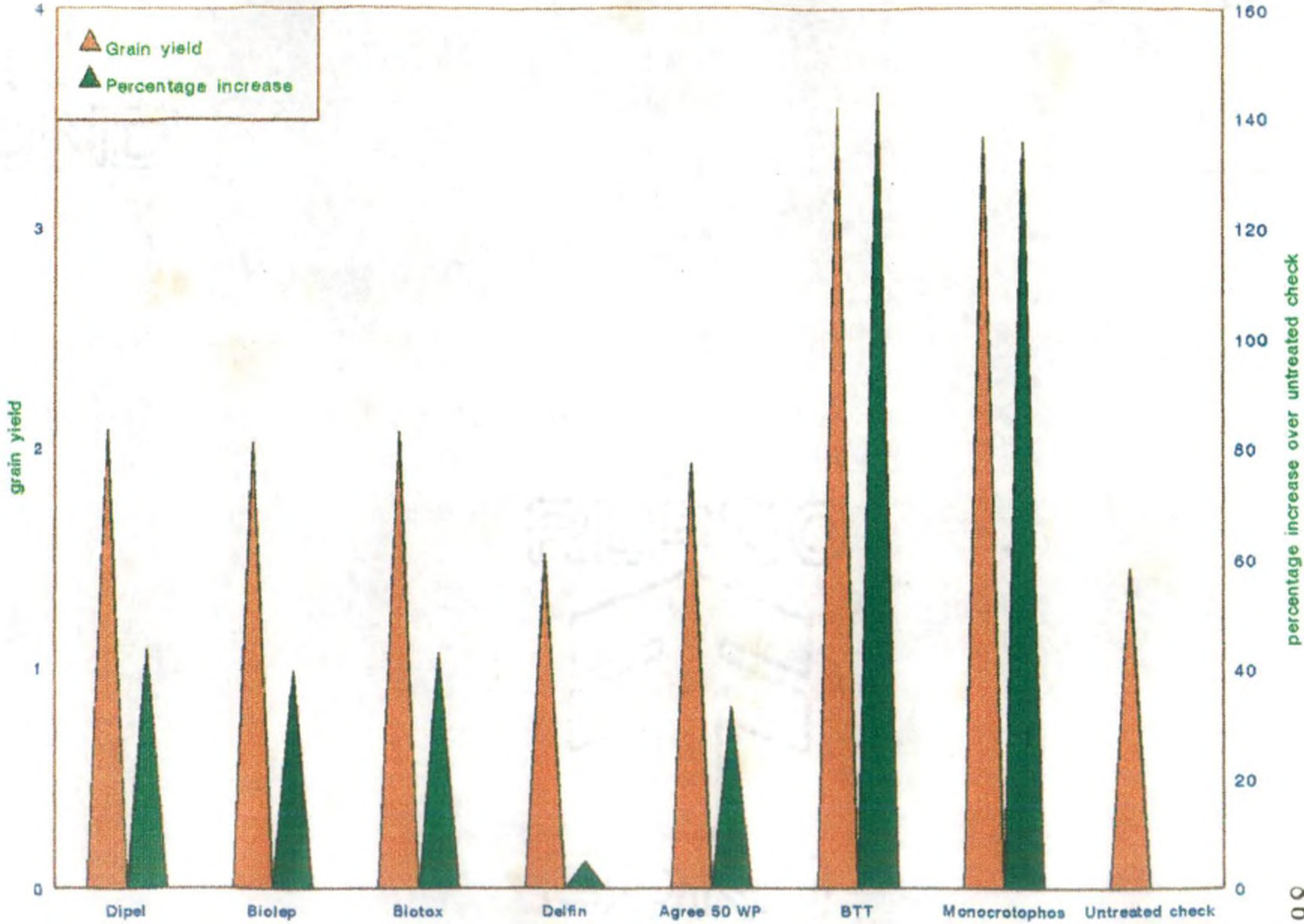

Fig 3 Grain yield as influenced by the application of B.t formulations against leaffolders on rice (Kharif, 1995)

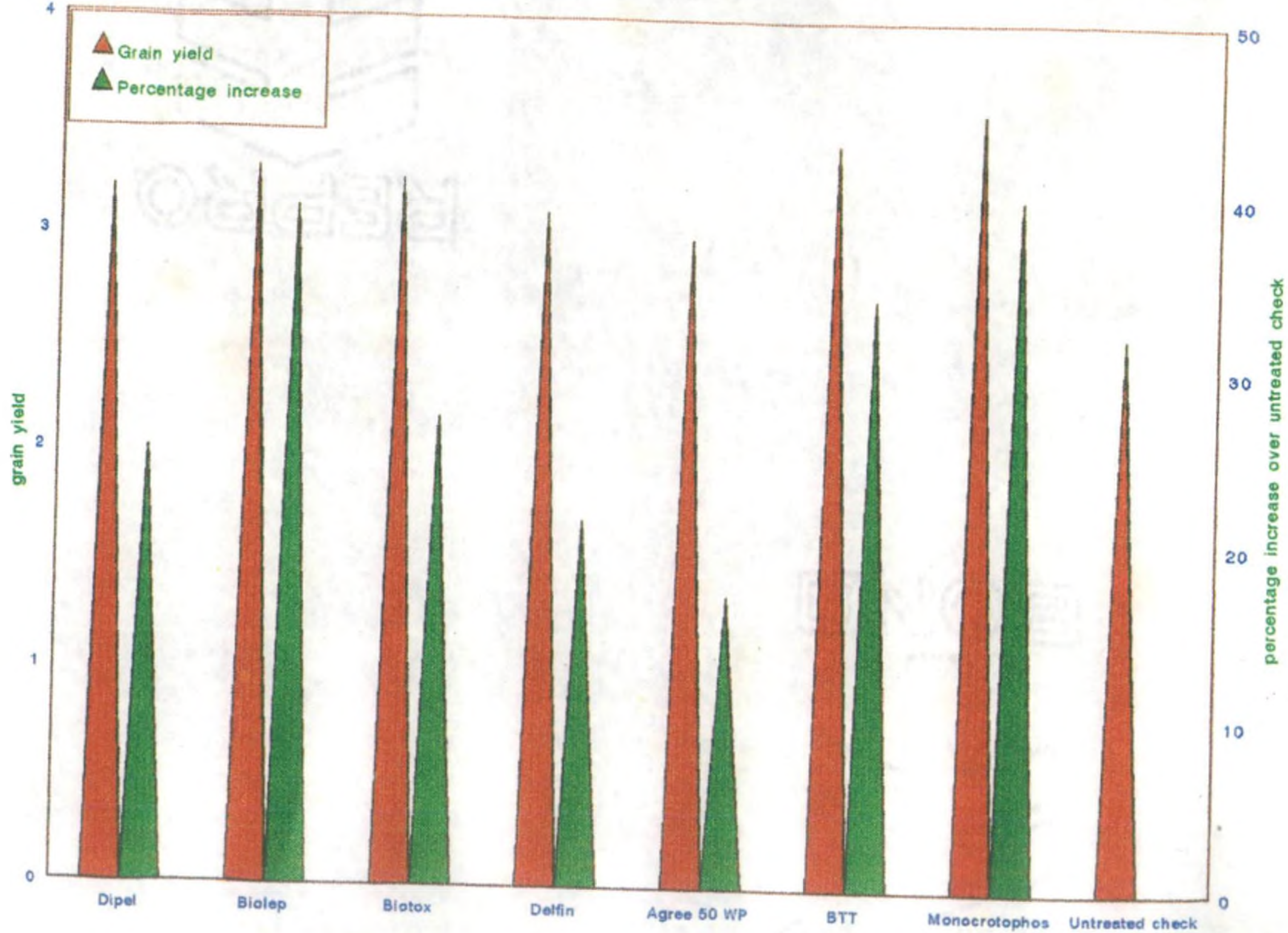

Fig 4 Grain yield as influenced by the application of B.t formulations against leaffolders on rice (Kharif, 1996)

Similar observations of increase in yield with the application of *B.t.* as Thuricide *or* Dipel was recorded by Tandan and Nillama (1987). They found Dipel to be the most effective treatment against *Ostrinia furnacalis* and *H. armigera* on maize. Justin *et al.*(1990) also obtained similar results in field trials with *B.t.* against *Plutella xylostella* on cauliflower. *B.t.* was found to be the best than all the chemical insectides tested. Barrion *et al.*(1991) reported that application of Dipel @ 2kg ha^{-1} at weekly intervals gave higher degree of efficacy against leaffolder larvae and recorded an yield of 5.5 t ha^{-1}.

5.4 Effect of commercial formulations of *B.t.* on the population dynamics of natural enemies of lepidopterous pests of rice.

5.4.1 Effect of *B.t.* formulations to the predators of rice pests

Based on the estimation of the density of predatory spiders and predators in the rice ecosystem after the application of *B.t.* formulations, it was found that the *B.t.* formulations were able to conserve the predators when compared to the insecticide check plot where there was a decrease in the number of predators after the application of treatments. During Kharif 1995, the density of *L.pseudoannulata* in the monocrotophos treated plot was 0.4

and in the untreated check it was 4.2, for *T.javana* it was 1 and 3.3 per hill respectively(Table 5).For *Micraspis* sp. it was 0.2 and 4.7, for *Ophionea* sp. it was 0.5 and 4.8 and *Paederus* sp. it was 0.3 and 3.9 per hill respectively in the monocrotophos and untreated check respectively (Table 6). During Kharif,1996, for *L. pseudoannulata* the density was 0.4 in the monocrotophos treated plot 4.2 in the untreated check.*T.javana* recorded 0.4 and 3.3(Table 11) *Micraspis* sp. 0.2 and 4.7,*Ophionea* sp. 0.5 and 4.8 and for *Paederus* sp. 0.3 and 3.9 per hill in the monocrotophos and untreated check respectively(Table 12).

Similar observations on the effect of *B.t.* on the predators have been observed by Pascovici *et al.* (1978).In Rumanian forests of oak, when Dipel and Turingin were applied for the control of *Lymantria dispar,Tortrix viridana, Eranis marginaria,*they could find no adverse effects on the activity of *Formica* sp. Legotal (1980) observed relative harmlessness of the *B.t.* preparation BT 202 to the larvae and adults of *Chrysoperla carnea and Coccinella septumpunctata, the ant Formica pratensis, Trichogramma evanescens* and the spider, *Paedosa agrestis* unless considerable amounts were ingested.

5.4.2 Effect of the *B.t.* formulations to the parasitoids

The studies conducted to estimate the percentage of parasitism of leaffolder larvae after the application of the treatments revealed that the *B.t.* products are harmless to the parasitoids in the rice ecosystem(Fig 5). The percentage of parasitism recorded during Kharif 1995 showed that the percentage of parasitism was highest in the *B.t.* treated plot Biotox (20.0) followed by Agree 50 WP, BTT, Dipel all recording 16.67 per cent parasitism. All the *B.t.* treatments were on par with untreated check which recorded 23.3 per cent parasitism (Table 7). During Kharif, 1996 though all the treatments were statistically on par with each other, *B.t.* formulation, Agree 50 WP treated plot recorded the highest percentage of parasitism of 23.3. The treatments Dipel, Biolep, Biotox recorded similar values as in the untreated check (20.0 per cent). Monocrotophos recorded the lowest value of 6.67 per cent (Table 13).

Similar field trials by Brunner and Stevens (1985) of the *B.t.* product Thuricide on *Plutella xylostella* on cabbage reavealed no adverse effect of the *B.t.* product on the parasite. Cheng and Lon (1990) in their field trials with *B.t.* products in tobbacco observed relative harmlessness of the *B.t.* products through the parasitoids of *Manduca quinquemaculata, Cotesia congregata.* Hassan and Smith (1995) has also observed that the *B.t.* to be safe to the parasitoids *Trichogramma bactrae and Microplitis demolitor.*

5.5 Compatibility studies of the promising *B.t.* formulation BTT with the granulosis virus of *C.medinalis* (*C.m.* GV)

The percentage of damaged leaves per hill ten days after first spraying was lowest in the monocrotophos treated pot (6.680) followed by *C.m.* GV (7.710), BTT + *C.m.* GV (8.473) and BTT (9.00). After the second spraying the percentage of damaged leaves was 2.6 per cent in the monocrotophos treated pot, 3.598 for BTT, 3.617 for *C.m.* GV and 3.910 for BTT + *C.m.* GV. For the untreated check it was 21.71 per cent and 37.8 per cent after the first and second application of treatments respectively (Table 14). After the first spraying the treatments BTT, *C.m.* GV, combination of BTT and GV were on par with each other. The treatments *C.m.* GV and BTT + *C.m.* GV were on par with monocrotophos. All the treatments were significantly different from the untreated check. After the second spraying all the microbial treatments were on par with each other. The treatments BTT, *C.m.* GV were on par with monocrotophos treated plot.

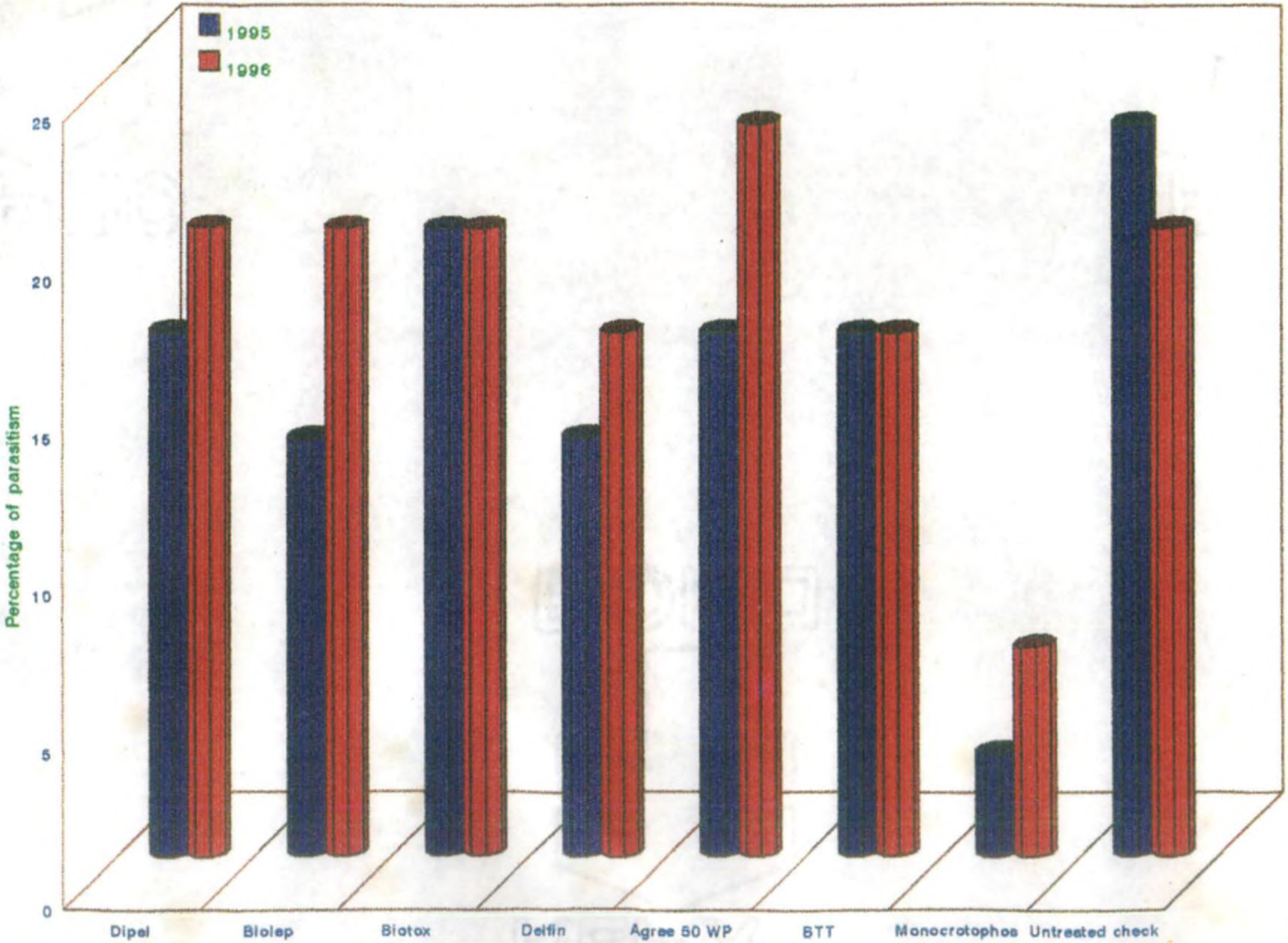

Fig 5 Effect of B.t. formulations on the parasitism of leaffolder

The percentage of larvae dead after the first spraying was highest in BTT (67.13) followed by *C.m.* GV (66.37) and BTT + *C.m.* GV (58.89). All the treatments were on par with each other and significantly different from untreated check. There was 83.97 per cent mortality in monocrotophos where as in the untreated check the percentage mortality was -45.15 per cent mortality which means 45.15 per cent increase in the leaffolder density (Table 15). After the second application of treatment monocrotophos recorded 100 per cent mortality, it was 84.17 for BTT 86.0 per cent for *C.m.* GV and 66.41 per cent for BTT + *C.m.* GV. In the untreated check it was -23.09 per cent.

Hunter(1970), Hunter and Hoffmann(1973) and Hunter *et al.* (1973) have also observed similar observations of the combined applications of *B.t* and GV to give effective control of Indian meal moth, *Plodia interpunctella*.

Su (1991) reported similar results with the combined application of three formulations of *B.t.* (Biobit, Thuricide and Dipel along with granulosis viruses against *P. xylostella* and *Artogeia rapae*.

Observations made by Peters and Coaker (1993) showed that the degree of susceptibility of *P.brassicae* larvae to *P. brassicae* granulosis virus (*Pb* GV) was improved particularly when *Pb* GV was applied in mixtures with low doses of either *B.t.* sub sp. *kurstaki* or permethrin.

5.6 **Cross infectivity of the granulosis virus of *C.medinalis* to other lepidopterous pests of rice.**

The results given in Table 16 showed that the granulosis virus of *C. medinalis* was highly host specific. All the larvae of *Melanitis leda ismene, Psalis pennatula, Pelopidas mathias* and *Spodoptera mauritia* were not susceptible to GV of *C. medinalis.*

One of the serious drawbacks of microbial pathogens is their high host specificity and consequent ineffectiveness in mixed populations of insect pests in rice ecosystem. In the present studies on the cross infectivity of GV of *C. medinalis* high host specifity was observed. Similar observations were also made with insect viruses by Smith (1967). Philip (1978) also reported the high host specificity of GV of *P. ricinii.*

It is clear from the above study that the density of predators and the pathogens are an important source of mortality of leaffolder.Broad spectrum insecticides applied to control leaffolders kill its natural enemies and there by disturb the natural equilibrium. Efforts to conserve these natural enemies include development of workable thresholds , a practical monotoring programme and selective chemical or microbial insecticides applied only when leaffolder population density reaches thresholds. In the case of leaffolders usually the economic thresholds levels were reached only at the fag end of the crop. In this context *B.t.* formulations applied at this stage were able to prevent the further spread of leaf damage. Because of their unique properties of safety to the biological control agents the *B.t.* formulations offer a potential scope for their inclusion in the integrated pest management programmes.

Summary

SUMMARY

Rice is the important and extremely grown food crop in India. The production of rice tend to reach stagnation in recent years due to many constraints. The introduction of high yielding varieties in the intensive multicropping system has allowed a large number of minor pests to assume the status of major pests. Among the other pests, leaffolders are considered economically very important. Farmers predominantly use synthetic pesticides to control rice leaffolders. Control tactics other than broad spectrum insecticides include microbial insecticides and release of egg parasitoids. No serious attempt has so far been made to understand the suitability of the pathogens in field application. Identification and use of efficient natural enemies including pathogens against lepidopterous pests will help to go a long way in the successful implementation of much needed integrated pest management programme in rice. Hence the present studies were taken up with the objective of gathering detailed information on the following aspects.

i) Survey and identification of natural enemies of leaffolders and other lepidopterous pests of rice

ii) Evaluation of efficacy of different formulations of *B.t.* under field conditions.

iii) Compatibility studies of the promising *B.t.* formulation along with the granulosis virus of *C. medinalis* (*C.m.* GV)

The survey was conducted in the rice fields of the Agricultural Research Station, Mannuthy and the Kerala State Agricultural Department Seed Farm, Mannuthy during Kharif 1995 and Kharif 1996. The pathogens and parasitiods were assessed by periodical collection of dead and dying insects from the field and making observations in the laboratory. Healthy insects were field collected and reared in the lab.

For the evaluation of *B.t.* formulations two field experiments were conducted during Kharif 1995 and Kharif 1996. The experiments were conducted at the Agricultural Research Station, Mannuthy. The experiments were conducted in a randomised block design with eight treatments and three replications. The treatments were applied based on ETL (10 per cent leaf damage at vegetative stage and 5 per cent leaf damage at flowering stage). Observations were taken on the number of damaged leaves per hill and the total number of leaves per hill before and after the application of the treatments. Observations on the density of predatory spiders and other predators in the ecosystem were taken. The extent of parasitism of leaf folder larvae was also recorded before and after the application of the treatments.

The compatibility studies of the *B.t.* formulation BTT with the granulosis virus was conducted in pot culture. The treatments included, BTT, *C.m.* GV, BTT + *C.m.* GV, monocrotophos and the untreated check. The results of the study are summarised below.

The survey revealed the occurrence of nuclear polyhedrosis infection in *Pelopidas mathias, Spodoptera mauritia, Nymphula depunctalis* and in *Melanitis leda ismene,* bacteriosis in *Pelopidas mathias* and *Cnaphalocrocis medinalis* and granulosis virus in *C. medinalis*.The nematode *Hexamermis* sp. is recorded for the first time in India in *C.medinalis* .

The Tachinid parasite *Argyrophylax nigrotibialis* was reared from *Pelopidas mathias* larvae. *Apanteles* sp. and *Apanteles angustibasis* were reared from larvae of *C.*

medinalis and *P. mathias*. Ichneumonid, *Charops brachypterum* was recorded on *Psalis pennatula* larvae. An unidentified *Braconid* was also reared from larvae of *Psalis pennatula*. Ichneumonid, *Itoplectis* sp. was found to attack *P. mathias* in Mannuthy. *Goniozus triangulifer*, *Tetrastichus* sp. and *Chelonas* sp. were reared from *C. medinalis* larvae. *Brachymeria* sp. was obtained from *C. medinalis* pupa. An unidentified Tachinid were reared from *C. medinalis*.

During Kharif 1995, the *B.t.* formulation Biotox, BTT, Biolep were found to be the best based on the percentage of leaf damage. With regard to the grain yield, the *B.t.* formulation BTT followed by Dipel, Biolep and Biotox were the next best treatments.

During Kharif 1996, Dipel followed by Biotox and monocrotophos were found to be the best based on the percentage of leaf damage. With respect to the grain yield during 1996, BTT was found to be the superior one followed by monocrotophos.

B.t. formulations were found to be safe to predators and parasites. The population density of the predators and extent of parasitism was lowest in the monocrotophos treated plot.

In the compatibility studies the results on the percentage of leaf damage and percentage of larval mortality showed the *B.t.* formulation BTT to be compatible with *C.m.* GV

The granulosis virus of *C. medinalis* was found to be highly host specific.

The cry for increased attention to ecological control infact indicates the necessity for the intensification of research on the feasibility of biological control, as natural enemies form the basis of biotic factor. In this context, the present studies have provided adequate information on the availability of pathogens and parasitoids in the rice ecosystem. Broad spectrum insecticides applied to control leaffolders kill its natural enemies, there by disturb the natural equilibrium. *B.t.* formulations were found to conserve the natural enemies and were able to prevent the further spread of rice leaffolders. In this context *B.t.* formulations offer a potential scope as a tool in integrated pest management programme.

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EVALUATION OF *Bacillus thuringiensis* BERLINER FOR THE MANAGEMENT OF RICE LEAFFOLDERS

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ABSTRACT OF THE THESIS

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ABSTRACT

The study was conducted with the objective of survey and identification of the natural enemies of leaffolder and other lepidopterous pests of rice and to evaluate the efficacy of *B.t.* formulations against the leaffolders of rice. The survey was conducted at the Agricultural Research Station, Mannuthy and the Kerala State Agricultural Department Seed Farm, Mannuthy. The field trials to evaluate the efficacy of rice leaffolders were conducted at the Agricultural Research Station, Mannuthy during Kharif 1995 and Kharif 1996.

The present study revealed the occurrence of five viroses, two bacterioses and a nematode infection in the various lepidopterous pests of rice. Four Braconids, three Ichneumonids one Eulophid, one Chalcid and a Bethylid parasite were also recorded from various lepidopterous pests of rice.

The *B.t.* formulations tested were found to be effective against the leaf folders on rice. The percentage of leaf damage during Kharif 1995 showed the *B.t.* formulations Biotox, BTT, Biolep to be the best treatments. With respect to the grain yield, BTT was the best treatment followed by

Dipel, Biolep and Biotox. During Kharif 1996, Dipel followed by Biotox and monocrotophos were found to be the best in terms of percentage of leaf damage. Based on the yield during 1996, BTT was found to be the superior one followed by monocrotophos.

B.t formulations were found to be safe to the predatory spiders, other predators and parasitoids of leaf folders.

The combination of BTT and *C.m.* GV was able to reduce the leaf damage and data on larval mortality showed the leaf folder larvae to be susceptible to the combination of BTT and *C.m.* GV.

The GV of *C. medinalis* was found to be highly host specific.