SYNERGISTIC INTERACTION OF BIOCIDES AND INSECTICIDES ON TOMATO FRUIT BORER *Helicoverpa armigera* (Hubner)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**Synergistic interaction of biocides and insecticides on tomato fruit borer *Helicoverpa armigera* (Hubner)**" is a bonafide record of research work done by me during the course of research and that this 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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LILY LEVIN

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INTRODUCTION

1. INTRODUCTION

Tomato, *Lycopersicon esculentum* Mill (Family: Solanaceae) originated in Peruvian and Mexican region. The Portugese introduced tomato to India. Tomato fruit borer, *Helicoverpa armigera* (Hubner) is a serious pest in the flowering and fruiting stages causing severe damage up to 50 – 55 per cent in tomato (Srinivasan, 1959; Narayanan and Gopalakrishnan, 1990 and Pokharkar ; Chaudhary, 1997).

Non-judicious application of synthetic pesticides led to main snags like resistance to chemicals, resurgence of sucking pests, residues in soil and environmental pollution. The persistence of toxic residues in/on edible part(s) limits the choice of pesticides and makes the management of pests of vegetables more sensitive and challenging.

The concern about the wide spread usage of broad-spectrum pesticides has led to the surge of research into alternative pest control technologies. The pesticidal formulations based on chemicals from living organisms have attracted particular attention because of their specificity to insect pests, their bio-degradable nature and a potential for commercial application.

Botanical pesticides and bio-pesticides are the best alternatives to keep the pests below the Economic Threshold Level (ETL) and provide security to mankind from the residues of pesticides. The plant world comprises a rich store house of bio - chemicals that could be tapped for use as insecticides. Among pesticides of plant origin, neem (*Azadirachta indica* Juss.) has been used since time immemorial in India. Besides neem, *Vitex negundo* Linn. ("Karinotchi"), *Andrographis paniculata* Wall. ("Kiriyathu") and *Acorus calamus* Linn. ("Vayambu") also possess highly

odoriferous chemical compounds and are gaining importance in modern pest management programmes.

Most of the microbial control agents are safe to other forms of life while they are capable of causing epizootics in populations of their specific hosts. But their high host specificity and long incubation period restrict their use in situations involving pest complexes and pest out breaks. They do not act fast and under such conditions chemical insecticides are preferred. But the unrestrained use of insecticides often leads to deleterious consequences. These problems have stimulated interest in evolving a pest control strategy involving the use of pesticides in harmony with other methods of control.

Bio - pesticides like the virus, Nucleo Polyhedrosis Virus (NPV), the bacteria *Bacillus thuringiensis* var. *kurstaki* (*Bt*), and entomopathogenic fungi (*Nomuraea rileyi* (Farlow) Samson, *Metarhizium anisopliae* (Metsch.) Sorokin, and *Beauveria bassiana* (Bals-Criv.) Yuill) are excellent tools for management of insect pests. The insecticidal toxicity of *Ha*NPV (Goud *et al.*, 1997 ; Chaudhari *et al.*, 2003) *B.thuringiensis* (Gujar *et al.*, 2000 ; Mandal *et al.*, 2003) and *N. rileyi* (Lu and Yin, 1998; Lingappa *et al.*, 2003 ; Ramanujam *et al.*, 2003) against insect pests had been well documented. The efficacy of Spinosad 48 SC @ 50 and 75 g a.i./ha against *H. armigera* was reported by several workers (Patil *et al.*, 1999; Vadodaria *et al.*, 2001).

Before chalking out a management programme the compatibility of the candidate pathogens with insecticides and botanicals has to be assessed. Insects undergoing a stress condition like over crowding, malnutrition and other environmental factors will be more susceptible to a disease (Steinhaus, 1958). There are reports indicating that insecticides act as stressors to viral infection (Benz, 1971).

Similarly there are also reports showing viral infections weakening the tolerance of insects to insecticides (Girardeau and Mitchell, 1968). The advance made in this area of research up to 1968 was reviewed by Benz (1971). Since then a number of workers have reported the successful potentiation of microbial agents when mixed with low doses of insecticides and botanicals. Such combinations would help in reducing the chemical and biological agents used for pest control while accomplishing adequate crop protection.

Keeping these in view, the following studies on the efficacy and interaction of different plant extracts, microbial pesticides and synthetic chemicals against *H. armigera* were taken up to avoid the after effects of commonly used insecticides and to consider its fitness in different management options.

1. Survey of *H. armigera* and its natural enemies in vegetable ecosystem during 2002-2004.
2. Screening of plant extracts and essential oils at different concentrations against third instar larvae of *H. armigera.*
3. To study the compatibility of botanicals, insecticides and biocides.
4. To assess the bio efficacy of botanicals, insecticides and biocides alone and in combination.
5. To know the activity of digestive enzymes in the host insect consequent to application of botanicals and biocides.
6. Evaluation of field efficacy of successful combinations in managing *H. armigera.*

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

A comprehensive review on "Synergistic interaction of biocides and insecticides on tomato fruit borer *Helicoverpa armigera* (Hubner)" is presented below.

2.1 NATURAL ENEMIES OF *H.armigera*

Our ecosystems are naturally endowed with a rich natural enemy fauna. A large number of them *viz.*, parasitoids, predators and pathogens were recorded on *H.armigera.* The following account gives details of the first record of major natural enemies of *H. armigera*

2.1.1 Parasitoids

The eggs of *H. armigera* on marigold, tube rose and corn were found to be heavily parasitised by *Trichogramma chilonis* (Ishii) (Manjunath *et al.,* 1970). On tomato and niger, 80 and 20 per cent parasitism by *Trichogramma* sp. was found (Manjunath, 1972). The eggs from tomato, lucerne and potato were parasitised heavily by *T.chilonis* (82.4 to 98.20 per cent) (Yadav and Patel, 1981).

Among the different larval parasitoids on *H.armigera, Campoletis chlorideae* (Uchida) and *Carcelia illota* (Curran) were most active on seasonal crops and also during most seasons. *Apanteles* sp. was the predominant endoparasitoid on chickpea, parasitising upto 85 per cent larvae and *C.chlorideae* attacked four per cent of them (Singh *et al.*, 1982). In Karnataka, parasitism of eggs of *H. armigera* on cotton and intercrop okra was 51.60 and 4.81 per cent respectively (Naganagoud and Thontadarya, 1984).

Eggs and early stage larvae were parasitised by hymenopterans whereas later instars were parasitised by dipterans. The tachinids *C.illota* and *Exorista xanthopsis* (Wiedemann) provided about 24.54 per cent parasitism on sunflower (Patel and Talati, 1987). The tachinid *C. illota,* the ichneumonids *Reiborus* sp. and *Charops* sp. and the trichogrammatid *Trichogrammatoidea armigera* (Nagaraja) were recorded as natural enemies of the noctuid *H. armigera* on cotton in Indonesia for the first time (Nurindah and Indrayani, 1989).

Koshiya and Patel (1987) reported the pupal mortality of *H.armigera* by *C.illota.* In Karnataka, *Trichogramma* sp., *C.chlorideae, C.illota* and *Hexameris* sp. were the important natural enemies on Tomato and *C.illota* parasitised about 20 per cent of the grown up larvae (Krishnamoorthy and Mani, 1990). Studies were carried out to determine parasitoid wasps associated with cotton bollworm, *H. armigera* in cotton fields. Three ichneumonid wasps observed were *Barylypa amabilis* (Tosquinet), *B. pallida* (Gravenhorst) and *Ichneumon sarcitorius* (Linnaeus). These wasps attack the last larval instars of the pest and the adult wasps emerged from pupae (Mojeni and Sedivy, 2001).

Out of the 77 parasitoids recorded in India so far, fifty belong to parasitic hymenoptera. *Banchopsis ruficornis, C. chloridae, Enicospilus* sp., *Eriborus* sp., *Bracon brevicornis* (Wesmael), *T. chilonis, T. armigera, Palexorista laxa* (Curran), *C. illota* and *Goniophthalmus halli* (Mesnil) were the common parasitoids (Singh *et al.*, 2002).

Neelam and Brar (2003) conducted field studies to record the incidence of gram pod borer, *H. armigera* and its natural enemies on black gram. The percentage parasitism due to *C. chlorideae* on black gram was two. *H. armigera* larvae on chickpea were parasitized by *Apanteles* sp., *Diadegma fenestrale* and *C. chlorideae.*

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2.1.2. Predators

The potter wasps *Delta* sp. (Vespidae) and *Chrysoperla* sp. (Chrysopidae) were observed to be important predators of *H.armigera* (Manjunath *et al.,* 1990). Ants were found to be potential predators in sunflower and pigeon pea ecosystem in Karnataka (Ballal, 1998).

Predators such as *Delta* (Vespidae), *Orius* (Anthocoridae), *Chrysoperla* (Chrysopidae), *Cheilomenes* (Coccinellidae), *Rhynocoris* (Reduviidae), *Geocoris* (Lygaeidae), *Nabis* (Nabidae), carabids (carabidae), ants, mantids, spiders and birds were observed on *H. armigera* eggs, larvae, prepupae and pupae (Singh *et al.*, 2002). Devi *et al.* (2002) reported that *Nesidiocoris tenuis* (Miridae) is an important predator known to prey on eggs and first instar larvae of *H.armigera* in tomato ecosystem.

A survey of predatory spiders of *H. armigera* in pigeon pea ecosystem was conducted and four predatory spiders were identified, i.e. *Oxyopus ratnae, Oxyopus shweta, Neoscona* sp. and *Plexippus paykullii*. Among these, *O. ratnae, O. shewta* and *Neoscona* sp. were present throughout the crop growing period (Borah and Dutta, 2003)

2.1.3 Pathogens

2.1.3.1 Virus

A cytoplasmic polyhedrosis virus (Rabindra and Subramaniam, 1973) and a granulosis virus (Narayanan, 1987) were recorded from *H.armigera* in India.The populations of third to sixth instar larvae of *H.armigera* declined by 22.75 to 23.08 per cent owing to infection by *Ha*NPV (Koshiya and Patel, 1987). Neelam and Brar (2003) observed natural mortality due to *Ha*NPV on black gram was 7.6 per cent.

2.1.3.2 Bacteria

Bacillus thuringiensis (Berliner) was isolated from *H. armigera* for the first time in India (Majumdar *et al.*, 1955). Singh (1972) reported the occurrence of *B. thrungiensis* subsp *kurstaki, B. thuringiensis* var. *galleriae* and *Serratia marcescens* in Russia. *B. cereus* and *B. thuringiensis,* var. namely, *aizawai, dendrolimus, entomocidus, galleriae, kurstaki, shandongenesis* and *thuringiensis* have been recorded on *H.armigera* in different areas of its distribution (Singh *et al.*, 2002).

2.1.3.3 Fungus

The important fungal diseases isolated from *H.armigera* include *Metarhizium anisopliae* Sorokin (Urs and Govindu, 1971), *Beauveria bassiana* Balsamo and *B.brongniarti* (Jayaramaiah, 1981) and *Nomuraea rileyi* Samson (Gopalakrishnan and Narayanan, 1988) in India.

Epizootiology studies of *N.rileyi* in field populations of *H.armigera* in India have revealed higher rates of fungal infection in *H.armigera* on *Cajanus cajan* (37 per cent) compared to *Phaseolus vulgaris* (28.20 per cent) and tomato (20.5 per cent) (Gopalakrishnan and Narayanan, 1989).

N. rileyi was found infecting third and fourth instar larvae of *H. armigera* on sorghum in Karnataka. Infection ranged from 3 to 47 per cent (Hugar and Hegde, 1996). In Andhra Pradesh, when outbreak of *H. armigera* occured on cotton and chickpea, more than 35 per cent of the infected larvae yielded mycelium and spores of *N.rileyi* and only about six per cent yielded the white muscardine fungus, *B. bassiana* (Singh, 1999).

Kulkarni and Lingappa (2002) observed the incidence of the fungus *N.rileyi* on *H. armigera* in cotton. *Spicaria farinose* var.*verticiloides, S. heliothis* and *Entomophthora* sp. have also been recorded on *H. armigera* (Singh *et al.*, 2002).

Manjula *et al.* (2003) reported the occurrence of *N. rileyi* on *H. armigera* on cotton, chilli, tomato, red gram, black gram and groundnuts. Cotton and groundnut recorded 100 per cent mycosed larvae of *H. armigera.*

2.1.3.4 Protozoa

The various protozoan pathogens reported on *H. armigera* were *Nosema* sp. *Vairimorpha* sp. and *Nattesia* sp. (Singh *et al.*, 2002).

2.1.3.5 Nematode

Parasitism by entomopathogenic nematode *Ovomermis albicans* was found to be higher on groundnut, tomato and weeds (Bhatnagar *et al.*, 1985). At Bangalore, during the rainy season, the nematode *Hexameris* sp. was very active on tomato crop and *O. albicans* was recovered from *H.armigera* infesting various crops and weeds (Singh *et al.*, 2002).

2.2 INSECTICIDAL ACTION OF BIOCIDES AND SYNTHETIC CHEMICALS

2.2.1 Plant extracts

Tropical plants provide a rich and intriguing source of natural products with potent biological activities. Insects undergo developmental abnormalities and mortality in various stages by the plant products (Gunathilagaraj and Sundarababu, 1987).

2.2.1.1 Sweet flag (*Acorus calamus* Linn.)

Insecticidal effect of powdered rhizomes of sweet flag, *A.calamus* was recognized against mosquitoes, houseflies, pulse beetles, bird lice, bed bugs. (Subramanian, 1942). Dixit *et al.* (1956) found that solvent extracts and essential oils of the rhizomes were toxic to housefly (*Musca nebulo* Wied), mosquito (*Culex fatigens* Wied) and carpet beetle (*Anthrenus vorax* Waterhouse).

Insecticidal properties of essential oil of *A.calamus* were reported against various storage pests (Koul, 1967; Abraham *et al.*, 1972; Agarwal *et al.*, 1973 and Yadav, 1974). Ether extract of rhizomes of *A.calamus* was toxic to *A. proxima* (Pandey *et al.*, 1976 and Sudhakar *et al.*, 1978). Pandey *et al.* (1982) reported that two per cent extracts of the rhizomes killed fifth instar larvae of potato tuber moth *Phthorimoea operculella*. Thirty four compounds have been identified in the oil and extracts of calamus among which, the glucoside bitter principle, acorin and β- asarone showed good biological activity (Duke, 1985).

The rhizome extract is having insect growth regulatory activity as well as juvenile hormonal effects (Deshmukh and Renapurkar, 1986). Saleela *et al.* (1988) reported the toxicity of *A.calamus* rhizomes against the land leach *Haemedispa sylvestris*.

Behera and Satapathy (1996) observed that *A.calamus* extract induced highest percentage of abnormalities due to morphogenetic effect both by leaf dip (38.60 per cent) and topical (37.30 per cent) applications in the treated *S. litura*. Rhizome extract of *A.calamus* caused 57.77 and 72.22 per cent larval mortality of *S. litura* and *H. armigera* respectively (Venkadasubramanian and David, 1999). On *S. litura* and *Lipaphis erysimi*, the extract caused more than 30 per cent mortality (Desai and Desai, 2000).

Some plant products, including calamus oil from *A. calamus*, clove oil from *Eugenia* sp. and karanj oil from *Pongamia glabra* [*P. pinnata*], were evaluated for their toxicity against *Aedes aegypti*, *Culex quinquefasciatus*, *Sitophilus oryzae*, *Callosobruchus chinensis*, *S. litura* and *Odontotermes obesus*. Calamus oil exhibited the highest toxicity against the filaria vector *Culex quinquefasciatus* and against adults of the stored grain pest *C. chinensis* (Bhonde *et al.*, 2001).

Laboratory experiments were conducted to assess oviposition deterrence effect of *A. calamus* extracts to the melon fly, *Bactrocera cucurbitae*. Both aqueous and solvent extracts showed the deterrent effect, the latter being more effective (Nair and Thomas, 2001). Suryadevara and Khanam (2002) investigated the insecticidal activity of some selected Indian medicinal plants (*A.calamus*, *Allium sativum* and *Gardenia gummifera)* against larvae of mosquito *C. quinquefasciatus*. The ethanolic extract of *A. calamus* was the most active among the extracts with an LC_{50} of 0.072 and LC_{90} of 0.243 ppm.

Five different plant materials were evaluated as protectants for milled rice against the lesser grain borer (*R. dominica*) at 0.25, 0.5 and 1.0 per cent. The rhizome of sweet flag (grits or powder) was highly toxic to the adult insects at all concentrations. Adult mortality was less than 10 per cent in the remaining plant materials. The grits or powder of sweet flag suppressed the progeny of *R. dominica* completely even at the lowest rate (Harish *et al.*, 2003).

The efficacy of *Vitex negundo* (25 kg/ha), *Prosopis juliflora* (25 kg/ha), *Nicotiana tabacum* (25 kg/ha), *A. calamus* (at 25 and 10 kg/ha), and *Ocimum basilicum* (25 kg/ha) dust formulations in controlling rice bug (*Lepticorisa acuta*) was investigated and compared with that of fenthion at 500 ml/ha. Among the plant products, *A. calamus* recorded the lowest pest population (Nelson *et al.*, 2003).

The effect of *Stemona tuberosa* and *A. calamus* ethanol extracts on the diamond back moth, *Plutella xylostella*, was investigated. It was found that a 0.4 per cent *A. calamus* extract and 0.5 per cent *S. tuberosa* extract had the best activity against third-instar larvae of *P. xylostella* by leaf dipping method. At 0.4 per cent, *A. calamus* extract gave 63.3 per cent accumulated mortality within 48 hours (Jiyavorranant *et al.*, 2003).

2.2.1.2 Kiriyathu (*Andrographis paniculata* Wall.)

The main constituent of *A. paniculata* has been isolated and established as andrographolide (Moktadar and Guha-sincar, 1939 ; Chakravarthi and Chakravarthi, 1952). *A. paniculata* extracts had higher antifeedant activities on third instar larvae of *S.litura* (Gunasekaran and Chelliah, 1985). In laboratory studies, extracts of *A. paniculata* acted as an antifeedant to *P. xylostella*, and as an oviposition deterrent to *C. chinensis* (Hermawan *et al.*, 1993).

A. paniculata extracts had higher antifeedant activities on fourth stadium larvae of *P. xylostella* (Hermawan *et al.*, 1994). The antifeedant activities of crude extracts and a major compound (andrographolide) of *A. paniculata* were tested against female adults of the rice leaf hopper *Nephotettix cincticeps* using artificial diet. The crude extract and andrographolide suppressed feeding of female adult at concentrations as low as one ppm (Widiarta *et al.*, 1997). The compound identified from *A.paniculata* was 14-de-oxy andrographolide and at 1000 ppm reduced the number of eggs laid by *P.xylostella* by about 50 per cent (Hermawan *et al.*, 1998).

Ethyl acetate fraction was found to be possessing highest ovipositional deterrency against *Spilarctia obliqua* Walker and the methanol fraction of *A.*

paniculata extracts had the highest growth inhibitory activity on larval and adult stages of *S.obliqua* according to Tripathi *et al.* (1999). Bioefficacy of different solvent fractions of *A. paniculata* was tested against the cowpea beetle, *C. chinensis* in terms of its effect on adult mortality, total egg output and emergence (eclosion) of F_1 adults. All the extracts were effective against the beetle. The efficacy was, however, more significant with respect to methanol and ethyl acetate extracts at the highest concentrations (1000 ppm) which led to 72.01 and 67.69 per cent adult mortality, respectively (Bright *et al.*, 2001). Suresh (2002) tested different plant extracts against third instar larvae of *S. litura* and found that the acetone extract of *A. paniculata* afforded higher leaf protection (78.3 per cent) and larval starvation (71.8 per cent) at five per cent levels.

Tripathi *et al.* (2003) evaluated ten medicinal plants at a dose of 10 mg/ml for insecticidal, ovicidal, feeding-deterrence, growth inhibition and morphogenetic effects against various life stages of a noxious lepidopteran insect-pest, *S. oblique*. The acetone extract of *V. negundo* was found to exhibit all the activities tested against *S. obliqua*. Other plants tested, *Ajuga remota*, *A. paniculata* and *Clerodendrum inerme*, were found to have low to moderate effects towards *S. obliqua*.

2.2.1.3 Karinotchi (Vitex negundo)

The essential oil extracted from dried leaves of *V.negundo* was found to contain terpenes, enole, 1- sabinene and sesquiterpene (Itikawa and Yamasita, 1940 and Manalo, 1982). Kalyanasundaram and Babu (1982) reported that petroleum ether leaf extract of the plant was effective against mosquito. Bai and Kandasamy (1985) reported that acetone extracts of *V.negundo* when treated on *S.litura* and *H.vigintioctopunctata* completely suppressed egg laying.

Acetone extracts caused cent per cent mortality of *S.litura* and *H.vigintioctopunctata* (Saradamma, 1989 ; Moore *et al.,* 1989). Kalavathy *et al.* (1991) reported the insecticidal activity of acetone extract of *V.negundo* against *E.vitella.* Saradamma *et al.* (1993) reported the juvenomimetic activity of *V.negundo* on the penultimate instars of *Dysdercus cingulatus.* Volatile oils from *V.negundo* caused upto 83 per cent mortality of *P.xylostella* eggs and topical application caused 91 per cent mortality in third instar larvae (Dayrit *et al.*, 1995).

Leaf extract caused 83 per cent mortality in *S.litura* (Sahayaraj and Sekar, 1996). Leaf extract at ten per cent concentration was not effective against *Sitotroga cerealella* under field condition (Ramamurthy and Venugopalan, 1997). Sahayaraj (1998) reported the morphogenetic effects of *V.negundo* extracts on *S.litura* larvae. The aqueous extract had no ovipositional deterrency and antifeedancy. But it had better ovicidal action (66.50 per cent) at ten per cent level.

Senguttuvan and Dhanakodi (1999) evaluated the efficacy of different plant extracts in comparison with monocrotophos against the groundnut pest *Aproaerema modicella* and reported that *V. negundo* leaf extract at five per cent and *Croton sparsiflorus* plant extract at five per cent were found to be effective, resulting in a 24.7 per cent lower larval population and 19.3 fewer damaged leaflets over the control.

Virendra *et al.* (1999) analysed volatile constituents of *V. negundo* leaves growing in Dehra Dun (India) and detected 66 compounds. The main compounds were viridiflorol (19.55 per cent), beta-caryophyllene (16.59 per cent), sabinene (12.07 per cent), 4-terpineol (9.65 per cent), gamma-terpinene (2.21 per cent), caryophyllene oxide (1.75 per cent), 1-oceten-3-ol (1.59 per cent), and globulol (1.05 per cent). viridiflorol was reported for the first time in the oil of a *Vitex* species.

Methanolic extracts of neem (*Azadirachta indica* A. Juss) seed kernel extracts (NSKE), *Pongamia pinnata* L. seed extracts (PPSE) and *V. negundo* L. leaf extracts (VNLE) were applied to cotton leaves and fed to the cotton bollworm, *H.armigera*, to assess their effects on feeding, survival and fecundity. Individually, NSKE was highly effective, whereas PPSE and VNLE were less so. Mixtures of the extracts increased feeding deterrency and mortality and decreased fecundity of *H. armigera*, and also initiated dose-dependent changes resulting in delayed metamorphosis with larval-pupal intermediates, abnormal adults and finally death (Babu *et al.*, 2000).

Four solutions of *V. negundo* leaf alkaloid extracts of different concentrations, at 1.0, 0.75, 0.50 and 0.25 per cent, and a control were tested against larvae of *Culex fatigans*. One hundred per cent larval mortality was observed on trays treated with 1.0 and 0.75 per cent concentrations. At 0.50 per cent concentration, 38 per cent of the larvae emerged as pupae but failed to reach the adult stage. At 0.25 per cent concentration, 52 per cent of the larvae emerged as pupae but also failed to reach the adult stage (Shah and Maheshwari, 2002).

Solvent as well as aqueous extracts of *V. negundo* had lower feeding inhibition at five per cent level. However, it caused higher rate of pupal and adult malformations of *S.litura* (Suresh, 2002). Petroleum ether, methanol and acetone extracts (at one per cent), essential oil (at 1, 0.5, 0.25, 0.125, 0.062 and 0.031 per cent) and two pure compounds (viridiflorol at 434 mg and agnuside at 2.3 g) isolated from *V. negundo* leaves were screened for insecticidal activity against *S. cerealella* infesting wheat seeds. Only the essential oil was effective against the pest and caused 100 per cent mortality. Emergence of new adults was completely prevented by 0.125 per cent of the essential oil (Singh *et al.*, 2002).

Virendra *et al.* (2003) evaluated *V. negundo* leaves for its chemical constituents and isolated twelve pure compounds. Viridiflorol (six mg/kg) from leaves exhibited a

dose dependent antifeedant activity on *S. oryzae* and ovipositional activity on *C. chinensis.*

2.2.2 Essential oils

2.2.2.1 Citronella oil (Citronella winterianus Jowitt*)*

The use of the repellent citronella oil against fruit-piercing moths and fruit-sucking moths was investigated in an apple orchard. Wads of cotton wool soaked in the oil were suspended from branches of apple trees. No moths were found on treated trees, as compared with up to eight adults of *Calpe emarginata* (F.), *Othreis materna* (L.), *O. fullonia* (Cl.), *Cyligramma latona* (Cram.), *Sphingomorpha chlorea* (Cram.) and other species on untreated trees (Bosch, 1971).

Dale and Saradamma (1981) tested eight essential oils of plant origin (citronella, palmarosa, geranium, eucalyptus, wintergreen, patchouli, citrodora and camphor oils) against third-instar larvae of *Pericallia ricini* at concentrations of 2.5, 5 and 10 per cent on castor leaves. All the oils had some antifeedant properties. The repellent effect of citronella oil on the stored products insects *Tribolium castaneum, Bruchus chinensis* [*Callosobruchus chinensis*] and *Periplaneta americana,* was studied under laboratory conditions. Repellency was noted up to 52 h after treatment (Saraswathi and Rao, 1987).

Essential oils of citronella (*Cymbopogon* sp.), clove (*Syzygium aromaticum*) and lemon were tested for toxicity to adults of *C. maculatus.* With reference to their LC_{50}s, the oils showed a ranked order of toxicity with significant differences in the order clove oil - citronella oil - lemon oil (Lale, 1991).

Exposure of freshly laid eggs of *Earias vittella* to volatiles from the essential oils of japanese mint (*Mentha arvensis*), peppermint (*M. piperita*), palmarosa (*Cymbopogon martinii*) and citronella (*C. winterianus*) for more than 24 h inhibited hatchability to varying degrees. This was, however, significant only in respect of the citronella and palmarosa oil vapour treatments when compared with the control. Male and female pupal weights of larvae that survived embryonic exposure to the volatiles of the four oils were significantly lower than those of the untreated ones (Marimuthu *et al.*, 1997).

The commercial product citrus clean (a mixture of citronella oil, pine oil, and oils extracted from lemon grass and marigold) applied to eggs of *Corcyra cephalonica* resulted in 100 per cent mortality when applied at concentrations of 50, 75 or 100 per cent. The lowest concentration (25 per cent) gave 91 per cent mortality (Dwivedi and Gray, 2000). Karanj oil and piperonyl butoxide exhibited synergism with deltamethrin against adults of a resistant strain of *T. castaneum*. Karanj oil, sesame oil, citronella oil and piperonyl butoxide showed an additive effect against adults of a susceptible strain (Sridevi and Dhingra, 2000).

Rao *et al.* (2000) evaluated *C. winterianus* oil at 0.5 per cent against second instar larvae of *H.armigera*. Significant antifeedant activity was recorded compared to untreated control. Nor-Azah *et al.* (2002) evaluated the toxicity of citronella oil against the 3rd to 4th instar larvae of a vector mosquito (*Aedes aegypti*). The results showed a stronger repellent activity. *C. winterianus* oil at 0.5 per cent strength exhibited moderate ovipositional deterrency and ovicidal action. At three and five per cent concentration it had higher leaf protection (65.37 and 85.05 per cent) and an LC_{50} value of 370 ppm on the third instar larvae of *S.litura* (Suresh, 2002).

*2.2.2.2 Kacholam oil (Kaempferia galanga*Linn. *)*

Rhizomes of *Curcuma xanthorrhiza, C. zeodoaria, K. galanga* and *K. pandurata* (obtained from Indonesia) were analysed for insecticidal constituents. Seventeen major compounds including flavonoids, sesquiterpenoids and cinnamic acid derivatives were isolated. All compounds were studied for contact toxicity against larvae of the polyphagous pest insect *S. littoralis*. Nine compounds, including the most active sesquiterpenoids, xanthorrhizol and furanodienone, showed pronounced toxicity against neonate larvae of *S. littoralis* (Pandji *et al.*, 1993)

Suresh (2002) reported the ovicidal action of kacholam oil at 0.1 and 0.5 per cent strengths (43.32 and 79.60 per cent respectively). Kacholam oil induced very high larval starvation of *S.litura* (62 to 82 per cent) at three and five per cent concentrations. It greatly affected the assimilation of ingested and digested food and induced pupal and adult malformations when applied on the third instar larvae of *S.litura*.

2.2.2.3 Lemongrass oil (Cymbopogon flexuosus Steud.*)*

Rajapakse and Jayasena (1991) observed that treatment with lemongrass oil reduced the damage caused by *S.litura* on peanut. Rao *et al.* (2000) reported that *C. flexuosus* oil at 0.5 per cent caused significant reduction in larval weight and adult emergence of *H.armigera.* Suresh (2002) reported the ovipositional deterrency and ovicidal action of *C. flexuosus* at 0.5 per cent. He also observed higher adult malformation of *S.litura* at one per cent concentration.

The efficacy of broken seeds of *A. indica, Pongamia glabra, Calophyllum inophyllum* and *C. flexuosus,* in controlling *Corcyra cephalonica* infesting rice was determined at 0.5 and 1.0 per cent concentration. The fecundity, egg viability and longevity of both males and female *C. cephalonica* decreased with increasing

concentrations of the extracts and the oils. Male and female longevity was lowest with lemongrass oil treatment (Meena and Bhargava, 2003).

2.2.2.4 Palmarosa oil (Cymbopogon martinii Roxb.*)*

Plant products (neem seed kernel powder, neem leaf powder and *Lantana camara* leaf powder) at 25 g/kg groundnut pods and two aromatic oils (citronella and palmarosa) at 15 ml/kg pods were evaluated against the groundnut bruchid, *Caryedon serratus* (Olivier), a serious pest of groundnut pods and kernels. Citronella oil and palmarosa oil gave total protection to groundnut pods by inhibiting oviposition by the bruchid for six months with an efficacy equal to that of malathion dust (Kumari *et al.*, 1998).

Venkadasubramanian and David (1999) reported that the palmarosa oil at one per cent caused 91 and 100 per cent mortality of third instar larvae of *H.armigera* and *S.litura* respectively. *C.martini* at 0.5 per cent resulted in significant reduction in larval weight of *H.armigera* and adult emergence (Rao *et al.*, 2000).

The effect of dust formulations of plant oils was evaluated on the development of *Sitotroga cerealella* (Olivier) and *C. chinensis* on IR 20 paddy grain and cowpea, respectively. Low adult emergence was recorded in palmarosa oil (PO)+neem oil (NO)+Iluppai oil (IO). Cowpea grains treated with PO+NO revealed only 3.0 eggs/50 g seeds while plant oils, palmarosa oil and neem oil at 1 per cent recorded 6.5 and 4.5 eggs/50 g seeds, respectively (Baskaran and Janarthanan, 2000).

Crude extracts of neem (*Azadirachta indica*, NK 26, NAVNEM, NEMKVR and NEKET) and palmarosa oil were evaluated for their larvicidal activity against fourth-instar *Culex quinquefasciatus*. The LC_{50} value of NK 26, palmarosa oil, NAVNEM, NEMKVR and NEKET were 2.31, 3.31, 6.08, 21.11 and 30.55 ppm,

respectively. Palmarosa oil and NK 26 exhibited the highest larvicidal activity (Alice, 2001). The palmarosa oil at five per cent concentration exhibited highest insecticidal toxicity with LC_{50} value of 178 ppm against *S.litura* larvae (Suresh, 2002).

2.2.3 Efficacy of new synthetic insecticides

2.2.3.1 Carbosulfan

The effect of three per cent carbosulfan granules on black vine weevil *Otiorhynchus sulcatus* (Curculionidae) larvae and adults was investigated. The mortality of middle instar larvae was 79.5 per cent at one or two g carbosulfan/pot. Young larvae were highly sensitive, while middle and final instar larvae displayed low sensitivity to carbosulfan (Masaki *et al.*, 1999). Mohamed *et al.* (1999) evaluated 13 insecticides against *H.armigera* on pigeon pea. The pod damage was significantly lower in carbosulfan (6.10 per cent) followed by triazophos (six per cent). The highest yield was recorded with sulprofos followed by quinalphos and carbosulfan.

The efficacy of carbosulfan (1000 g a.i./ha) against *S. incertulas* on rice cv. Red Triveni was studied in Pattambi, Kerala, India, during 1996 (second cropping), 1997 (first and second cropping), and 1999 (first and second cropping). In all years, carbosulfan effectively controlled *S. incertulas*. Carbosulfan gave a higher yield (3492 kg/ha) than the control insecticide 1000 g a.i. carbofuran/ha (3444 kg/ha) (Karthikeyan and Purushothaman, 2000)

In field conditions in Poland, preliminary tests were carried out with ten insecticides protecting *Pinus sylvestris* plantations against damage caused by *Hylobius abietis* (Curculionidae). It was found that the least damage occurred in the case of the application of Marshal 25 EC [carbosulfan] and a pyrethroid preparation (Korczynski, 2001). Wei and Liu (2001) investigated the toxicity of carbosulfan and engine oil and

their mixtures to *M. persicae* in the laboratory. The results showed that the mixture of carbosulfan and engine oil at 1:5 ratio had the most obvious synergism among the treatments, and co-toxicity coefficient was 278.

Field experiments were conducted in Dharwad, Karnataka, India, to test the efficacy of new compounds (lambda cyhalothrin, carbosulfan, profenofos and polytrin C) and test insecticides (MPO 62, RH 2485, spinosad and fipronil) against insecticide susceptible and resistant *S. litura* infesting groundnut. The new compounds were highly effective against the insecticide susceptible strain. However, against the resistant strain, only carbosulfan, profenofos and polytrin-C were highly effective (Ramegowda and Basavanagoud, 2001).

The toxic effects of several insecticides were evaluated on adults of *Phyrdenus muriceus* (Curculionidae). Soil surface applications of tefluthrine, carbosulfan and chlorpyrifos showed a highly toxic initial effect, which significantly declined by day 15. The same doses were much less toxic when mixed with the soil (Novo *et al.*, 2002). The results of 11 different insecticide treatments in a field experiment conducted in Junagadh, Gujarat, India, during 1998-99 rabi season on Indian mustard cv. GM-2 showed that the treatment with methyl-o-demeton 0.025 per cent, carbosulfan 0.04 per cent, methyl parathion two per cent dust at 25 kg/ha and monocrotophos 0.04 per cent were highly effective against mustard aphid, *Lipaphis erysimi* (Gami *et al.*, 2002).

Sahoo and Pal (2003) conducted a trial in West Bengal, India during the summer season of 2001 to investigate the alternate use of carbosulfan 25 EC at 0.05 per cent, chlorpyrifos 20 EC at 0.05 per cent and azadirachtin 0.15 per cent in controlling shoot and fruit borers (*Earias vittella*) infesting okra. Spraying carbosulfan at 15 days intervals showed the lowest fruit damage (2.75 to 12.91 per cent) and the highest fruit yield (100.92 q/ha).

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Kwon *et al.* (2003) determined the efficacy of methomyl (24.1 and 45 per cent), imidacloprid (10 per cent) and carbosulfan (20 per cent) in controlling *Rhopalosiphum nymphaeae* infesting *Alisma plantago* in a field experiment conducted in South Korea. Carbosulfan recorded the highest control of the pest after five (97.3 per cent) and ten days of spraying (92.1 per cent).

Field studies were carried out in Andhra Pradesh, India, during the rabi and kharif seasons of 2000 to evaluate the efficacy of insecticide granules and spray formulations in controlling insect pests of rice cv. Krishna hamsa. Granules of carbosulfan (1000 g a.i./ha) were at par with carbofuran (1000 g a.i./ha), with regard to efficacy against insect pest complex and in enhancing grain yield (Krishnaiah *et al.*, 2003).

2.2.3.2 Profenofos

Khaliq *et al.* (1995) tested different doses of cypermethrin, monocrotophos, dimethoate and profenofos against lepidopterous defoliators *H.armigera* and *Mythimna separata* attacking potatoes. They reported that cypermethrin and profenofos were the most effective insecticides giving 100 per cent mortality of larvae.

A field trial was conducted to assess the efficacy of profenofos 0.5-1 kg/ha, Profenofos + cypermethrin at 0.33 – 0.44 kg/ha, lufenuron at 0.03 kg/ha, dichlorvos at 0.76 kg/ha and cypermethrin 0.05 kg against *H.armigera* in tomato. Lowest infestation and highest yield of marketable fruits were recorded with profenofos + cypermethrin followed by profenofos (Walunj *et al.*, 1999).

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2.3.3 Spinosad

Spinosad is a potent compound for controlling lepidopterous pests and thrips and also has activity on various insect orders (Thomson and Hutchins, 1999). Spinosad 48SC (75 g and 50 g ai /ha) was found to be effective against *H. armigera* (Patil *et al* ., 1999; Dandale *et al* ., 2001; Vadodaria *et al*., 2001 ; Mansoor *et al*., 2001). Tracer (0.4ml /l) showed good potential for *H.armigera* control when compared to indoxacarb and methoxy fenozide (Knight *et al*., 2000).

Spinosad (0.4 ml/l) was found better than indoxacarb (Rao *et al* ., 2001a) when compared to conventional insecticides namely profenofos, thiodicarb and chlorpyriphos (Hansah *et al* ., 2001) and it was equally effective as emamectin 1.92 EC (1.0 ml /l) in controlling the larval population of *H. armigera* (Rao *et al* ., 2001b).

The effectiveness of spinosad (7.5, 10.0, 12.5, and 15.0 ml of commercial product/100 litre of water.) to control the citrus leaf miner, *Phyllocnistis citrella*, was compared with abamectin (Vertimec 18EC) and lufenuron (Match). Spinosad, abamectin and lufenuron were efficient for the control of citrus leafminer up to ten days after spraying (Gravena *et al*., 2002).

Spinosad (Success ® 2.5 SC) @ one ml/l was effective in reducing the larval population and fruit damage caused by *H. armigera* in bhendi and chillies (Shobanadevi, 2003) and in tomato (Suganyakanna, 2003). Spinosad 75 g ai/ha was found to be highly toxic to all instars of *H.armigera* (Sathish, 2003).

Toews *et al.* (2003) reported that spinosad has excellent (99 to 100 per cent) contact activity against adults of all stored product insects except *Tribolium* spp. Spinosad 2.5 SC at 20 g a.i./ha was found to be significantly superior to the remaining treatments in reducing the infestation of the diamondback moth larvae at two and six

days after application and increased the yield of marketable cabbage head (Tambe and Mote, 2003). Spinosad, the least used insecticide, was the most toxic insecticde against *P. xylostella* when compared to *Bacillus thuringiensis* var. *kurstaki*, cartap hydrochloride, cypermethrin, dichlorvos, malathion, carbaryl, endosulfan and monocrotophos. (Arora *et al.*, 2003).

In lettuce, at 25 ml/ha, spinosad controlled *S. littoralis* more effectively than the other two insecticides (Lannate (methomyl) at 200 ml/ha and (chlorpyrifos-methyl) at 300 ml/ha) and with no sign of phytotoxicity (Sannino and Piro, 2003). Prithwiraj and Chatterjee (2003) reported that spinosad at 0.005 per cent was the most effective in reducing the diamondback moth, *P. xylostella* population per plant when compared to novaluron, acetamiprid and cartap hydrochloride and in increasing the yield of the crop over the untreated control.

Avermectin was most effective when applied on cabbage in the first and second sprays against diamond back moth, where as *B. thuringiensis* and spinosad remained effective in both sprays after 96 h (Abro *et al.*, 2004).

2.2.3.4 Triazophos

The insecticide triazophos 0.05 per cent effectively controlled cotton boll worm *H.armigera* and increased the yield by 53 per cent (Sudhakar and Paul, 1991; Gupta *et al.*, 1998). Khurana (1997) found that triazophos, monocrotophos and chlorpyriphos were the most effective insecticides against early instars of *H.armigera* on chickpea. Toxicity of insecticides *viz.*, methomyl (0.025 per cent), thiodicarb (0.15 per cent), cypermethrin (0.0055 per cent) and triazophos (0.08 per cent) were evaluated against *H.armigera*. Cypermethrin resulted in the highest mortality followed by methomyl and triazophos (Pampapathy and Goud, 2000).

Kathuria *et al.* (2000) evaluated the efficacy of nine insecticides, endosulfan (0.07 per cent), malathion (0.05 per cent), quinalphos (0.05 per cent), monocrotophos (0.04 per cent), triazophos (0.04 per cent), methomyl (0.025 per cent), carbaryl (0.05 per cent), fenvalerate (0.006 per cent) and cypermethrin (0.006 per cent) as ovicides against eggs of *H. armigera* in the laboratory during 1999. Irrespective of the age of eggs, overall mean egg mortality in different insecticidal treatments ranged from 56 to 86 per cent. The synthetic pyrethroids, fenvalerate and cypermethrin, exhibited the highest egg mortality (80 per cent), followed by methomyl and triazophos (77 per cent each).

Studies carried out in Madhya Pradesh during 1996-97 with endosulfan and Spark [triazophos 35 EC and deltamethrin one per cent] showed that staggered applications of Spark at 0.09 per cent at the pod formation stage were the most effective treatment against *H.armigera* (Brave and Patil, 2000). The bio-efficacy of triazophos (350 or 700 g/ha), acephate (1000 or 1500 g/ha), cypermethrin (150 and 300 g/ha) and imidacloprid (50 or 70 g/ha) against the major pest complex of chilli was evaluated in a field experiment conducted in Rajendranagar, Hyderabad, Andhra Pradesh, India during the kharif season of 1997-98. Cypermethrin and triazophos (300 g/ha) were generally the most effective insecticides against borers (Kumar *et al.*, 2001).

Mann *et al.* (2001) evaluated the effectiveness of high potency neem-based insecticides, Neemazal (one per cent), Rakshakgold (one per cent), neem powder (0.5 per cent), and triazophos (0.1 per cent) against *Bemisia tabaci* during flowering and its impact on management of other insect pest complexes (*Amrasca biguttula, Aphis gossypii* and the bollworm, *H. armigera*) of cotton in a field experiment. Minimum boll damage and higher seed cotton yield was recorded in triazophos-treated plots.

2.2.4 Bacteria *(Bacillus thuringiensis* Berliner)

Creighton *et al.* (1961) reported that a commercial formulation of *B. thuringiensis* spore material containing $96x10^9$ spores/gm gave good control of the tobacco budworm *Heliothis virescens* on tobacco. The percentage of tomato fruits damaged by *H.armigera* in Dipel (0.5 kg/ ha) treated plots was significantly lower (7.41 per cent) than in untreated check (21.30 per cent) (Krishnaiah, *et al.*, 1981).

Karel and Schoonhoven (1986) observed that two applications (450g/ha) of *B.thuringiensis* controlled the larvae of *H.armigera* during the post flowering stage. Spraying of tomato crop with 1000 ppm of Delfin, a commercial formulation of *B.thuringiensis* resulted in 90 per cent mortality of third instar larvae of *H.armigera* (Reddy *et al.*, 1997).

Satpathy and Panda (1997) reported that the asporogenous and sporogenous formulation of *B.thuringiensis* subsp. *kurstaki* showed effective control of *H.armigera* when applied @ two kg ai/ha at 55 days after sowing. Kulat *et al.* (1999) observed cent per cent mortality of tomato fruit borer *H.armigera* with Delfin. *B.t.k* (Dipel 8 L) 750 ml/ha and was found to be more effective in minimizing *H.armigera* larvae population. Bioasp and Biobit @ 1.5 kg/ha were able to minimize the *H.armigera* population below ETL (Mahapatra and Gupta, 1999).

The effectiveness of *B. thuringiensis* was evaluated against larvae of *H.armigera* on safflower leaves in the laboratory. Maximum mortality (96.67 per cent) was observed with 17600 IU/mg after 72 h, when larvae were treated topically (Dhembare, 1999). The effect of *B. thuringiensis* subsp. *kurstaki* formulations/strains on the growth and development of *H. armigera* was studied *in vitro*. Of the formulations, Biobit was most toxic to the neonates (LC_{50} 0.02 a.i. ppm), followed by Biolep, HD-1, HD-73 and Dipel. The toxicity of these formulations was decreased

considerably against five-day old larvae. HD-1 showed the highest toxicity to the five-day old larvae (LC_{50} 1.71 a.i. ppm), followed by Biobit and Biolep (Gujar *et al.*, 2000).

Mandal *et al.* (2003) investigated the efficacy of three *B. thuringiensis* formulations (Biolep, Bioasp and Dipel) and nuclear polyhedrosis virus formulation (Virin) in controlling *H. armigera* infesting chickpeas. Among the biopesticides tested, Biolep gave the lowest number of larvae per 10 plants and pod damage, and highest mean yield and returns, whereas Dipel gave the highest benefit : cost ratio.

2.2.5 Viruses (Nuclear polyhedrosis virus)

The Nuclear Polyhedrosis Virus (NPV) concentration of 1.5×10^{12} Polyhedral Occlusion Bodies (POB) /ha was found to control the population of *H.armigera* and increase the yield of sunflower (Rabindra *et al.*, 1986), pigeon pea (Muthiah and Rabindra, 1991), groundnut (Muthuswami *et al.*, 1993), tomato (Satpathy *et al.*, 1999), chickpea (Cherry *et al.*, 2000) and egyptian clover (Batter *et al.*, 2001).

Sathiah and Rabindra (2001) found that a higher dose of 3×10^{12} POB/ha was required for the control of *H. armigera* on cotton ecosystem. Mohan *et al.* (1996) evaluated the virus for controlling *H. armigera* on tomatoes at three concentrations of NPV (300, 200, and 100 LE/ha). Application of NPV at 300 LE/ha gave the lowest per centage of damaged fruits and the highest yield of marketable tomatoes. Larvae of *H.armigera* resistant and susceptible to fenvalerate and endosulfan were tested for their susceptibility to a NPV. The LC_{50} values were less in resistant strains than in susceptible strains. The resistance ratio to *Ha*NPV was greatest in the fenvalerate resistant strain (0.88) followed by the endosulfan resistant (0.64) strain (Goud *et al.*, 1997).

Rawat and Shukla (2001) studied the efficacy of NPV with and without adjuvants and UV protectants, against *H. armigera* in chickpea crop in a field trial. Significantly higher seed yields of 1612.5 and 1550.0 kg/ha were observed in the treatments of NPV 250 LE + milk powder (1.0 per cent) and NPV 250 LE + Ranipal (0.5 per cent), respectively and both were on par.

Laboratory experiments were conducted to measure the efficacy *Ha*NPV. Third-instar *H. armigera* larvae were fed on artificial diet treated with NPV at 4×10^3, 4×10^4, 4×10^5, and 4×10^6 POB/ml. The LD_{50} and LT_{50} values for *Ha*NPV were 2.33 $\times 10^4$ PIB/ml and 175.39 h, respectively (Satpathy and Rai, 2002). A study was conducted in 1999-2000 and 2000-01 with different treatments for the management of leaf eating caterpillar *H. armigera* on potato. NPV (250 LE/ha) applied twice at 15 days interval after the appearance of the pest was effective in controlling the pest (Chaudhari *et al.*, 2003).

Thakre *et al.* (2003) conducted field experiment to assess the efficacy of different biocides (neem seed extract at five per cent; neem leaf extract at five per cent; *Ipomoea* leaf extract at five per cent; *Ha*NPV at 250 LE; *B. thuringiensis* at 1000 g/ha and endosulfan (0.035per cent)) for the management of pod borer complex of pigeon pea. *Ha*NPV was the most effective, followed by an equally effective treatment of *B.thuringiensis* in reducing pod damage caused by lepidopteran pests.

2.2.6 Entomogenous fungi

2.2.6.1 Nomuraea rileyi Samson

Gopalakrishnan and Narayanan (1988) studied the pathogenicity of *N. rileyi* by spraying aqueous spore suspension of 1.8×10^9 spores/ml against all the instars of *H.armigera* and found that the fungus was highly virulent, inflicting 100 per cent

mortality for second to fourth instar larvae. The natural epizootic of *N. rileyi* on *H.armigera* was reported by Gopalakrishnan and Mohan (1997) in cabbage ecosystem. The fungus naturally controls the pest population to an extent of 56.1, 70.8 and 53.9 per cent in cabbage and 70 per cent in banana respectively.

The infectivity of *N. rileyi* to *H.armigera* at 8 x 10^6 spores/ml gave 60 to 77per cent mortality of first to second instar larvae. The LC_{50} of *N. rileyi* was 3.27x10^6/ml spore suspension for first instar larvae, 3.12 x10^6 for second instar larvae, and 5.02x10^6 for fourth instar larvae. In field trials, infection of *H.armigera* larvae six days after spraying a spore suspension of *N. rileyi* was 33.6 per cent (Lu and Yin, 1998). Tang and Hou (1998) reported that the entomopathogenic fungi *N. rileyi* caused 90-100 per cent mortality of fourth instar larvae of *H.armigera* when applied at 10^7 conidia/ml to silks of maize and leaves of soybean, tomato and chrysanthemum. The efficacy of *N. rileyi* against *H.armigera* was also reported by Ignacimuthu (2000).

Lingappa *et al.* (2003) studied the field performance of *N.rileyi* at 2 x 10^8 conidia/litre against *H.armigera* on soybean. Results revealed significant effect of mycopathogen on larval stages of *H.armigera.* Larval population was reduced to the extent of 28 and 62 per cent in ten days after first and second application respectively. Different isolates of entomopathogenic fungi *N. rileyi* (1x10^8 spores/ml) was tested against third instar larvae of *H.armigera.* The *N.rileyi* isolate obtained from *Achaea janata* cadavers caused significantly higher mortality (76.6 per cent) of *H. armigera* (Ramanujam *et al.*, 2003).

2.2.6.2 *Beauveria bassiana* Balsamo

Host pathogen relationship studies conducted with *B.bassiana* against *H.armigera* by spraying aqueous spore suspension at four different concentrations *viz.*, 1 x10^7, 1x10^8, 1x10^9 and 1x10^{10} spores/ml revealed that all the five instars were highly

susceptible to the first two concentrations tested recording 60 to100 per cent mortality with an incubation period ranging from two to 15 days (Gopalakrishnan and Narayanan, 1989 and 1990).

Devaprasad *et al.* (1990) bioassayed five entomogenous fungi for their infectivity to second instar larvae of *H.armigera*. Of them, *B.bassiana* (Bapatla isolate) was found to be the most virulent recording the lowest LC_{50} of $2.17x10^5$ conidia/ml.

Sandhu and Singh (1993) investigated different routes of infection of *H.armigera* by *B.bassiana*. A mortality of 100 per cent was obtained by dorsal and ventral infection with an LT_{50} of four days.

Spraying of *B.bassiana* spores @ $2.68x10^7$ spores/ml on chickpea minimized *H.armigera* infestation to six per cent and increased yield to 2377 kg/ha. Unsprayed plots showed 16.30 per cent pod damage and 1844 kg /ha yield (Saxena and Ahmad, 1997). The efficacy of *B.bassiana* against five instars of *H.armigera* was studied by Manjula and Padmavathamma (1999). They observed greater mortality in the early instars compared to late instars.

Sun *et al.* (2001) studied the pathogenicity of *B.bassiana* to *H.armigera* under different environmental conditions. The larvae died most quickly and mortality was the highest at 95 per cent relative humidity. Mortality decreased drastically below 70 per cent relative humidity. The mean mortality percentage observed was 36.60.

Rathod (2002) reported the use of *B. bassiana* (1.18×10^4, 10^6, 10^8 and 10^{10} spores/ml) to control *H. armigera* (eggs, instar I, II, III, IV and V) on groundnut. Instars II and III were more susceptible to the pathogen than other larval stages. This susceptibility decreased with age. The fungus was pathogenic to all stages of the pest

(two to 72 per cent mortality) at 1.18 x 10^{10} spores/ml. Ramanujam *et al.* (2003) reported the pathogenicity of *B.bassiana* against third instar larvae of *H.armigera.*

The pathogenicity of *B. bassiana* (5 x 10^6 conidia/ml) and *M. anisopliae* (5x10^7 conidia/ml) on eggs and pupae of *H. armigera* was investigated. *B. bassiana* and *M. anisopliae* caused mean egg mortalities of 44.0 and 38.1 per cent and pupal mortalities of 40.8 and 22.5 per cent. The eggs were more susceptible to *B. bassiana* than *M. anisopliae*. Pathogenicity to pupae and eggs of the pests was higher for *B. bassiana* than *M. anisopliae* (Pandey, 2003).

2.2.6.3 Metarhizium anisopliae Sorokin

Rengaswami *et al.* (1968) reported for the first time in India the feasibility of microbial control of *H. armigera* with *M. anisopliae.* The treatment with 1.8x10^9 conidia /ml of *M. anisopliae* caused 80 to 100 per cent mortality of all the five instars, prepupae and pupae of *H. armigera* within two to ten days (Gopalakrishnan and Narayanan, 1989).

Zhao *et al.* (2001) conducted an experiment with *M. anisopliae* at a dilution rate of 1:10 and 20 in a cabbage field. The application of *M. anisopliae* at a dilution rate of 1:20 gave a control efficiency of 77.48 per cent against diamond back moth (*P. xylostella*) and 77.73 per cent against common cabbage worm (*Pieris rapae*) on kohlrabi and 53.98 per cent against tobacco budworm (*H. armigera*) on tomato.

M. anisopliae in oil formulation (7:3 diesel : sunflower oil) using ULV spray (5x10^{12}conidia/ha) against *H. armigera* in pigeon pea ecosystem reduced pod infestation to 8.76 per cent and was found to be superior to other microbial pathogens and endosulfan spray (Chandelle *et al.*, 2003). In pathogenecity studies 100,90,76.67 and 56.67 per cent mortality was recorded by spraying 1x10^8 spores/ml of fungal

suspension of *M.anisopliae* against first, second, third and fourth instar larvae of *H. armigera* respectively. LC_{50} value on second instar larvae of *H. armigera* was $1.18x10^5$ spores/ml (Wadyalkar *et al.*, 2003).

Kulat *et al.* (2003) conducted an experiment to determine the conidial concentration of *M. anisopliae* to achieve 50 per cent mortality in laboratory-reared second instar larvae of *H. armigera*. The bioassay showed that the LC_{50} against *H. armigera* larvae was 1.47×10^5 conidia/ml. *M. anisopliae* at 2.28×10^{10} had the highest larval mortality (97.5 per cent). At 192 h, the LT_{50} value for the second instar was inversely proportional to the conidial concentration of the inoculum.

2.3 COMPATIBILITY OF ENTOMOPATHOGENS WITH CHEMICAL PESTICIDES AND BOTANICALS

Compatibility with chemical pesticides is important in the effective usage of entomopathogens in pest management

2.3.1 Compatibility of *Bacillus thuringiensis* with insecticides and botanicals

Most insecticides were compatible with *B. thuringiensis* having little or no effect on spore germination or cell multiplication. Low concentrations of carbamates (carbaryl and carbofuran) and organophosphates (diazinon, malathion and phorate) did not affect the bacterial growth, whereas others, especially the chlorinated hydrocarbons (DDT, aldrin and heptachlor) inhibited the growth (Sutter *et al.*, 1971).

Dougherty *et al.* (1971) evaluated the compatibility of *B. thuringiensis* with carbaryl and baygon. The results indicated that carbaryl and baygon had no effect on the bacterium. Compatibility of *B. thuringiensis* with 27 chemicals revealed that

carbamates were generally more compatible with *B. thuringiensis* than other insecticides *viz.*, acephate, trichlorphon, methomyl, carbaryl, mexacarbate and diflubenzuron and that technical formulations were less harmful to bacteria than wettable powders (Morris, 1977). However, chemicals like DDT and fenthion significantly reduced bacterial population at all concentrations (Baskaran and Sekar, 1976).

The neem preparations Neemazal–F and Neemazal–TS and their active neem ingredient Neemazalpulvar, were screened *in vitro* for the presence of antibacterial activity Neemazal–F and a one per cent Neemazalpulvar solution (3000 ppm azadirachtin) inhibited the growth of *B.cereus, B.mycoides, B.thuringiensis* and *B.subtilis* (Coventry *et al.*, 1997). Monocrotophos and azadirachtin were reported compatible and safer for sporulation of *B. thuringiensis* (Pramanik *et al.*, 1997). They also indicated that endosulfan significantly inhibited the growth of *B.thuringiensis* compared to other insecticides.

Bhattacharya *et al.* (1998) studied the compatibility of eight pesticides with *B.thuringiensis*. Quinalphos 25EC (0.05, 0.1 and 0.2 per cent), endosulfan 35EC (0.035 ,0.07 and 0.14 per cent), phosphamidon 85SL (0.25 and 0.5per cent) ethion 50EC (0.05 and 0.1per cent) and copper oxychloride 50WP (0.2, 0.4 and 0.8 per cent) inhibited the growth of the bacteria. Monocrotophos 36EC and carbendazim 50WP did not inhibit the growth of bacteria. The chemical insecticide cypermethrin (Patel and Vyas, 1999) and acephate (Sunildutt, 2000) did not affect the growth and sporulation of *B.thuringiensis.*

Maghodia and Vyas (2003) evaluated the compatibility of different insecticides against *B.thuringiensis*.The study indicated that among the insecticides tested, monocrotophos, methyl-o-demeton and azadirachtin were safe and compatible with

B.thuringiensis while chlorpyriphos and quinalphos were compatible at half the field recommended doses only.

2.3.2 Compatibility of entomogenous fungi with insecticides and botanicals

2.3.2.1 Nomuraea rileyi

Ignoffo (1981) studied the compatibility of 44 chemical pesticides with *N.rileyi.* The three most inhibiting insecticides were monocrotophos, phenthoate and methyl parathion under *in vitro* testing, the insecticides compatible with *N. rileyi* were pyroxychlor, acephate, carbaryl, carbofuran, DBCP, DDT, dimilin, endrin, methoxychlor, bentazone, chlorbromuron, chloroxuron, dalapon-Na, metribuzin, naptalam and trifluran.

Devi and Prasad (1996) conducted compatibility test of seed kernel extracts from *A. indica, Melia azadarach* and *P. pinnata,* whole plant extract from *Tephrosia purpurea, Parthenium hysterophorus* and *Cleome viscosa* and vegetable oils from sunflower, safflower, groundnut, rapeseed, sesame, coconut and cotton seed with the entomogenous fungi *N. rileyi.* They reported that none of the oils were detrimental to the fungus.

Monocrotophos, phosphamidon and dimethoate were safe to *N. rileyi* at all concentrations tested. Quinalphos (0.025), carbaryl (0.025), endosulfan (0.035) and fenvalerate (0.005) were safe at low concentrations and these were highly detrimental to the fungus at higher concentrations (0.075, 0.150, 0.106 and 0.015 respectively) (Gopalakrishnan and Mohan, 2000). Devi *et al.* (2002) reported that neem did not inhibit the growth and sporulation of *N. rileyi* whereas insecticides monocrotophos and endosulfan at recommended rate completely inhibited the growth of *N. rileyi.*

Cypermethrin and fenvalerate exhibited 98.27 and 96.97 per cent inhibition respectively.

2.3.2.2 Beauveria bassiana

Aguda *et al.* (1984) evaluated the effect of insecticides on the germination of *B.bassiana* spores. The insecticides *viz.*, monocrotophos, BPMC, carbosulfan and azinphos-ethyl inhibited spore germination of *B. bassiana.* The BPH resurgence causing insecticides *viz.,* deltamethrin and methyl parathion also greatly reduced spore germination.

The effect of carbaryl on growth and sporulation of *B.bassiana* was investigated by Aguda *et al.* (1988). They reported that all the five concentrations of the insecticide tried inhibited the germination of conidia. The insecticides *viz.*, diazinon, pirimicarb, cypermethrin and oxamyl did not affect the growth of *B.bassiana* (Hokkanen, 1988).

Devaprasad *et al.* (1989) studied the effect of certain botanicals viz., *O.sanctum, Allium sativum, A. calamus, Tribulus terrestris* as well as neem seed kernel extract and neem oil on the conidial germination of *B.bassiana.* Neem oil and neem seed kernel extract were deleterious to the spore germination. The per cent inhibition of conidial germination in other botanicals ranged from 21 to 48. Vyas *et al.* (1992) observed that *in vitro* application of nicotine sulphate, repellin (extracts of *A.indica, P.glabra* and *Madhuca indica* and Indiara (diallyl disulphide and allyl propyl disulphide) inhibited the growth of *B.bassiana.* The insecticides *viz.*, fenitrothion, pirimphos methyl, endosulfan and dicrotophos inhibited the germination and growth of *B.bassiana* (Malo, 1993).

Moino and Alves (1998) evaluated the effect of imidacloprid and fipronil on *B.bassiana*. Imidacloprid was less toxic to the fungi than fipronil. The effect of deltamethrin on the germination of conidia of *B. bassiana* was studied by Alzogaray *et al.* (1998). They found that there was significant decrease in the germination of conidia in proportion to the concentration of insecticides.

Almeida *et al.* (1998) reported that the colony diameter of *B.bassiana* was significantly reduced with deltamethrin, methamidophos, cyhalothrin and endosulfan.

In vitro fungitoxic effect of the neonicotinoid insecticides acetamiprid, imidacloprid and thiamethoxam to *B.bassiana* showed that use of insecticides in the recommended formulations had no negative effect on conidia germination, conidia production and vegetative growth of *B.bassiana* (Neves *et al.*, 2001).

Xu *et al.* (2002) reported that imidacloprid 10 WP, Yashiling 22 WP (a mixture of imidacloprid and buprofezin), methomyl 20 EC, triazophos 20 EC and fipronil 5 FF exhibited high compatibility with the fungus. The other two insecticides chlorfluazuron 5 EC and fenvalerate 20 EC greatly reduced the germination rate of *B.bassiana*. Abamectin 0.5 EC was highly incompatible with *B.bassiana*.

Sheila *et al.* (2003) evaluated the relative toxicity of the insecticides methyl-chlorpyriphos, disulfoton, ethion, methyl parathion and endosulfan to the fungus *B.bassiana*. Methyl-chlorpyriphos and methyl parathion were highly toxic to *B.bassiana*; endosulfan was moderately toxic; while ethion and disulfoton were selective to this fungi. Triflumuron reduced mycelial growth but not conidial germination of *B.bassiana* (Saenz *et al.*, 2003).

2.3.2.3 Metarhizium anisopliae

Carbaryl inhibited the conidial germination of *M.anisopliae* (Sundarababu *et al.*, 1983 and Aguda *et al.*, 1988). Aguda *et al.* (1984) reported that the insecticides *viz.*, monocrotophos, BPMC, carbosulfan and azinphos ethyl inhibited spore germination of *M.anisopliae*. The BPH resurgence causing insecticides *viz.*, deltamethrin and methyl parathion also greatly reduced spore germination. Neem oil at 5 per cent concentration inhibited germination and sporulation of *M.anisopliae* (Aguda and Rombach, 1986).

The insecticides *viz.*, diazinon, pirimicarb, cypermethrin and oxamyl (Vannien and Hokkanen, 1988) Dichlorvos and Hostathion (Moorhouse *et al.*, 1992) inhibited the growth of *M.anisopliae*. Nicotine sulphate, and repellin inhibited the growth of *M.anisopliae*. But Neemark did not inhibit the growth (Vyas *et al.*, 1992). Li and Holden (1994) reported that the insecticides carbofuran, aldicarb and 2,4-D amine were compatible with *M.anisopliae*. The fungitoxic effect of imidacloprid and fipronil on *M.anisopliae* was evaluated by Moino and Alves (1998). They found that imidacloprid was less toxic to the fungus than fipronil.

The insecticides *viz.*, diazinon, malathion, methamidophos, trichlorfon, carbaryl, endosulfan and permethrin were tested *in vitro* on *M.anisopliae*. The least growth inhibition was exhibited by trichlorfon and methamidophos. Malathion fully inhibited the growth of fungi (Ayala-zermeno *et al.*, 1999).

Pachamuthu *et al.* (1999) conducted an *in vitro* study to determine the compatibility of *M.anisopliae* with chlorpyriphos, propetamphos and cyfluthrin. Chlorpyriphos and propetamphos caused significantly reduced sporulation. The neonicotinoid insecticides acetamiprid, imidacloprid and thiamethoxam (Neves *et al.*,

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2001) monocrotophos, chlorpyrifos and azadirachtin (Gupta *et al.*, 2002) were well tolerated by the fungus *M.anisopliae.*

2.4 INTERACTION OF BIOCIDES AND SYNTHETIC CHEMICALS

2.4.1 Interaction of *Bacillus thuringiensis* and botanicals

Hellpap (1984) reported that a combination of the methanolic extract of neem seed kernel with *B.thuringiensis* increased the mortality of *S. frugiperda* larvae and reduced considerably the LT_{50} and LT_{100}. Hellpap and Zebitz (1986) evaluated the combined efficacy of neem seed extracts with *B.thuringiensis* products in the control of *S.frugiperda* and *Aedes toigoi* and found that the combinations had an additive effect in most cases, but in some, a synergistic effect was seen.

Justin *et al.* (1987) conducted experiments on third instar larvae of *P. xylostella* with *B.thuringiensis* alone and in combination with plant extracts and found that when *B.thuringiensis* was combined with neem seed kernel extract (5 per cent), LC_{50} value was reduced by 1.9 times. Similarly, the combination of *B.thuringiensis* with *Catharanthus roseus* (3 per cent) also resulted in a reduced LC_{50}.

Salama and Sharaby (1988) studied the feeding deterrency induced by some plants in *S. littoralis* and their potentiating effect on *B.thuringiensis*. A marked increase was observed in the potency of *B.thuringiensis* endotoxin preparation against *S.littoralis,* when combined with plant products like orange and pomegranate peel. Leaf extracts of *Tagetes patula* and *Argemone mexicana* did not increase the potency of *B. thuringiensis* against *H.armigera* and *S.litura* (Balasaraswathy, 1990).

Joshi *et al.* (1990) could not get enhanced control of *S.litura* larvae in tobacco nurseries by the addition of water extracts of neem seed kernel and pongamia cake to

B.thuringiensis. Combination of neem products Neemazal-F one per cent (1.26ml/l) and Neemazal–F five per cent (0.36ml/l) with *B.thuringiensis* products Delfin (0.25g/l) and Bioasp (0.23g/l) at sub lethal concentrations resulted in increased mortality from 50 to 70 per cent of *P.xylostella* (Jeyarani, 1995).

Murugan *et al.* (1998) studied the combined effect of *B.thuringiensis* subsp. *kurstaki* (Dipel) and certain botanical insecticides on growth, feeding and nutritional efficiency of *H.armigera*. Mortality was significantly increased by pongamia oil with *B.thuringiensis* subsp. *kurstaki*. The toxicity of neem and combinations of neem and *B.thuringiensis* was examined on second instar *Leptinotarsa decemlineata*. Combinations of sub lethal concentrations of *B.thuringiensis* to larvae of *L.decemlineata* yielded an additive effect in larval mortality (Andi and Mark, 1999).

Bioefficacy of commercial formulations of *B.thuringiensis* (Delfin, Spicturin and Agree) in combination with botanicals *viz., A. indica, Mentha spicata, A. calamus Aristolochia bracteata, P. juliflora, Aloe vera* and palmarosa oil was tested under laboratory against *S.litura* and *H.armigera*. Toxic effects of *B.thuringiensis* products and palmarosa oil in mixture were significantly superior to that of other combinations (Venkadasubramaniam and David, 1999).

Joint spraying of *B.thuringiensis* (0.05 per cent) + azadirachtin 1500ppm (0.15 per cent) recorded the best result of 87.43 and 84.85 per cent reduction in larval population of *H.armigera* after three and 14 days of spraying respectively (Chatterjee and Senapati, 2000).

2.4.2 Interaction of *B. thuringiensis* and insecticides

Commercial preparation of *B.thuringiensis* could be mixed with most insecticides, fungicides, spreaders and other adjuvants without serious loss in activity

(Benz, 1971). Combinations of DDT resulted in independent synergism when applied in field against *Zeiraphera diniana*. Benz (1971) also reported that sub lethal doses of DDT resulted in potentiating synergisms with *B.thuringiensis* against *Agrotis ipsilon* and *Hyphantria cunea*. Creighton and Mcfadden (1975) stated that spraying of chlordimeform hydrochloride and *B.thuringiensis* in a spray was needed for effective protection to cabbage against the diamond back moth.

Integrated control of aphid *Myzus persicae* and diamond back moth *P.xylostella* by applying a combination of dimethoate with *B. thuringiensis* could control both *M. persicae* and *P. xylostella* more effectively than either of them alone (Narayanan *et al.*, 1975). According to Narayanaswami *et al.* (1978), application of a mixture of endosulfan and quinalphos with Dipel controlled the insects and the little leaf of brinjal. Krishnaiah *et al.* (1981) reported that better control of diamond back moth was achieved when Dipel was sprayed in combination with chlordimeform.

The effectiveness of Dipel alone and in combination with sublethal doses of carbaryl (0.05 per cent), monocrotophos (0.02 per cent) and phenthoate (0.02 per cent) was determined in a laboratory experiment against third instar larvae of *H.armigera*. The combinations had a synergistic action, the most effective were Dipel + endosulfan and Dipel + monocrotophos (Dabi *et al.*, 1988).

Dibyantro and Siswajo (1988) obtained effective control of *H.armigera* when a mixture of *B.thuringiensis* subsp. *kurstaki* (0.05per cent) and acephate (0.05 per cent) was applied in tomato field. The highest yield of tomatoes was also obtained from this treatment. They also obtained effective control of *P.xylostella* on cabbage, when *B.thuringiensis* (0.1 per cent) and triazophos (0.05 per cent) were applied together in the cabbage field. Laboratory bioassays showed that treatment of second instar larvae of noctuids *H.armigera* and *S.litura* with Bactospiene (*B.thuringiensis*) increased their

susceptility to endosulfan, monocrotophos, fenvalerate and cypermethrin on leaves of chickpea and castor respectively (Justin *et al.*, 1989).

Hoy and Hall (1993) conducted laboratory studies to examine interactions between esfenvalerate and *B.thuringiensis* and their effects on the feeding behaviour of larvae of *P.xylostella* and *L.decemlineata.* It was observed that the bacteria significantly reduced feeding either alone or in combination with esfenvalerate for *P. xylostella.* Gu *et al.* (1993) studied various degrees of synergistic effects shown by an aqueous concentrate of *B.thuringiensis* and 25 per cent dimethyl ester disodium salt against *Cnaphalocrocis medinalis* in a laboratory bioassay. The optimal synergistic activity was at ratio of 3:1 and 1:1 with toxicity coefficients of 265 and 298 respectively.

Butter *et al.* (1995) recommended spraying of *B. thuringiensis* (750ml/ha) alternated with endosulfan 2.5 l/ha for the effective control of bollworms in cotton. The increase in seed cotton yield was 52.1 per cent. Combinations of insecticides quinalphos (310ppm) and acephate (302ppm) with *B.thuringiensis* products *viz.*, Delfin (0.25g/ l) and Bioasp (0.23g / l) at sublethal concentrations resulted in increased mortality from 50 to 70 per cent of *P.xylostella* (Jeyarani, 1995). Mathur *et al.* (1996) conducted field trials in Rajasthan and reported that *B.thuringiensis* var. *kurstaki* in combination with methomyl can protect the tomato crop from *H.armigera.*

Treatment with 1ml Dipel 8L + 0.80g methomyl / l water produced the lowest percentage of fruit damage by *L. orbonalis* and the highest fruit yield of 16.41 t / ha. (Qureshi *et al.*, 1998). Satpathy and Panda (1997) reported that both asporogenous and sporogenous formulations of the subsp. *kurstaki* showed effective control against okra borers, *Earias vitella* and *E.insulana* @1 kg ai /ha in combination with carbaryl @0.5 kg ai/ha.

Tomar (1998) tested the combinations of commercial formulation of *B. thuringiensis* (Dipel) with lower concentration of insecticide endosulfan, fenvalerate, Multineem, carbaryl and acephate against okra shoot and fruit borer *E.vitella*. He observed that the maximum yield of healthy fruits was obtained in Dipel (0.1 per cent) with fenvalerate (0.0025 per cent) followed by Dipel (0.1 per cent) with acephate (0.06 per cent). Singh *et al.* (1999) evaluated Dipel 8L(one litre / ha) Delfin WG (1 kg /ha) and NPV (250LE /ha) in combination with endosulfan for their effectiveness against *H.armigera* on chickpea in Bihar. The results indicated that when biopesticides were used in combination with endosulfan, they were more effective resulting in relatively low average pod damage of 4.21 (Delfin), 5.65 (Dipel) and 6.65 per cent (NPV) resulting in 49.7, 47.2 and 46.7 per cent increase in yield respectively.

Highest suppression of 88.85 and 90.54 per cent of diamond back moth larval population was achieved from combined spraying of *B. thuringiensis* (0.05) + avermectins (0.05 per cent) after three and 14days of spraying respectively. (Chatterjee and Senapati, 2000). Mehta *et al.* (2000) evaluated the effect of deltamethrin alone and in combination with *B. thuringiensis* against tomato fruit borer *H. armigera*. A mixture of Deltamethrin + *B. thuringiensis* application revealed a fruit damage of 5.58 per cent. The mean fruit damage was also highest in this combination.

The efficacy of commercial formulations of *B.thuringiensis* and diflubenzuron or chlorpyriphos was evaluated under field condition against *S. litura*. Dipel (0.05 per cent) + chlorpyriphos (0.0025 per cent) proved superior and significantly effective by reducing 71.86 per cent population over control (Obulapathi *et al.*, 2000).

Khaliqus and Ahmed (2001) reported the compatibility and synergism of *B.thuringiensis* and lambda-cyhalothrin against *H.armigera*. The evaluation of treatments in the control of *S.litura* on groundnut under glass house conditions revealed that combinations of *B.thuringiensis* $1x10^7$ spores/ml + fenvalerate 0.005 per

cent gave highest larval population reduction and lowest leaf damage (Jayanthi and Padmavathamma, 2001).

Rao and Singh (2003) evaluated the insecticides (Lamda- cyhalothrin and flufenoxuron) and *B.thuringiensis* (Biobit) combinations against rice leaf folder damage, and found that they adversely affected the population levels of natural enemies. The synthetic insecticides when combined with the biopesticide, Biobit, showed moderate effect on leaf folder damage as well as predator populations.

Athira (2003) conducted a field experiment to assess the combined efficacy of *B.thuringiensis kurstaki* (Delfin, Spicturin) and insecticides (spinosad, indoxacarb, quinalphos and thiocarb) against *P.xylostella*. Delfin + spinosad and Delfin + indoxacarb were superior in reducing the larval population of *P.xylostella*.

2.4.3 Interaction of nuclear polyhedrosis virus and botanicals

Nicotine sulphate and pyrethrum were found to produce additive to potentiation effect on the NPV in *S.litura* larva. (Choudhari and Ramakrishnan, 1983). Heish *et al.* (1984) reported the additive effect of NPV and rotenone in *Galleria mellonella.* Devaprasad (1989) found that the addition of methanol fractions of *O. sanctum* and *A. calamus* at 0.01per cent increased the efficacy of NPV against third instar larvae of *H.armigera* and *S.litura.*

Rabindra *et al.* (1991) reported that a combination of NPV + *V. negundo* was significantly more effective than virus alone in reducing the damage to flowers and pods by *H.armigera.* Aqueous leaf extract (10 per cent) of *V. negundo* when applied with *Ha*NPV @1.5 $x10^{12}$ POB/ha gave better control of *H.armigera* and increased the grain yield (Rabindra and Jayaraj, 1992).

Rabindra *et al.* (1994) found that aqueous leaf extracts of *T. patula* (10 per cent) and *C. gigantea* (10 per cent) when fed to apparently healthy *H.armigera* larvae, expressed latent NPV infections resulting in mortality ranging from 36 to 50 per cent.

Sarode *et al.* (1997) reported that NPV and NSKE were more effective when applied alone. *Ha*NPV at 500LE/ha +5 per cent NSKE recorded the highest per centage control. The NPV– neem bitter, crude sugar combination recorded the shortest LT_{50}. The enhanced action was seen even at a lower dose of neem bitter (0.025 per cent) with NPV ($1x10^5$POB / ml) and crude sugar (1 per cent). The larval weight and growth rate were significantly reduced in the NPV-neem combinations (Rabindra *et al.*, 1997).

Neem seed kernel extract at 2.5 per cent enhanced the activity of NPV at 10^2 polyhedral occlusion bodies /ml against *H.armigera* on cotton leaves suppressing overall consumption, fecundity and survival (Murugan *et al.*, 1998 and Murugan and Jeyabalan, 1998). The potential enhancement of NPV by azadirachtin and its impact on *H.armigera* was evaluated in the laboratory. The combined treatment of NPV by azadirachtin significantly reduced the consumption index and decreased the efficency of conversion of ingested food to 5.07% and digested food to 9.8 per cent (Kumar and Murugan, 1998; 1999).

Baskaran *et al.* (1999) reported that the combination of NPV with NSKE and neemoil increased the efficacy of NPV and the virus – induced mortality. NPV with 10 per cent *V.negundo* did not increase the efficacy of the virus appreciably, while *P.juliflora* and *I. carnea* had an antagonistic effect on the virus of *S.litura*. The biopesticides NPV and plant product Nimbitor were evaluated against *H.armigera*. Considering the efficacy, yield and economics, the sequences with NPV- Nimbitor-Nimbitor was the best treatment (Mote and Satpate, 2003).

2.4.4. Interaction of nuclear polyhedrosis virus and insecticides

Ignoffo and Montoya (1966) found that carbaryl slightly synergised the polyhedrosis virus of *H.zea*. Benz (1971) studied synergism of the NPV of *I.seriata* and the insecticides. Carbaryl, derris, DDD, DDT, Nirosan, imidazole, pyrethum, methoxychlor and toxaphene had only antagonistic properties, mainly because they inhibited uptake of virus treated food. None of the insecticides tested was able to potentiate non lethal doses of the virus.

The joint action of NPV of *S.litura* and sub lethal concentrations of DDT, lindane, malathion and pyrethrin was investigated on five day old larvae. NPV with DDT at five ppm produced supplemental effect, while pyrethrin at five ppm resulted in potentiation in addition to exhibiting supplemental effect at higher concentrations tested. Lindane produced additive effect only at 50ppm. Malathion was found to be antagonistic to virus (Komolpith and Ramakrishnan, 1978).

Luttrell *et al.* (1979) studied the efficacy of Elcar with permethrin, methomyl and methyl parathion against *H.zea*. No significant differences were detected between observed and expected mortality, of *H.zea* larvae in the laboratory. The efficacy of NPV of *H.armigera* and insecticides (BHC, carbaryl and endosulfan) on the control of gram pod borer on bengal gram, was investigated under field conditions. A single spray of the combination at reduced doses of carbaryl (0.05 per cent) and NPV (125 LE / ha) was at par with one spray of carbaryl 0.1 per cent (Santharam and Balasubramaniam, 1982)

Chaudhari and Ramakrishnan (1983) tested twelve insecticides for their compatability with NPV of *S.litura*. Aldrin, endosulfan and DDT were found to be most compatible with the virus. Organophosphates, in general, were antagonistic to the virus. Diazinon showed synergistic effect with the virus, the lower doses with virus

exhibiting potentiating effect. Carbaryl was most compatible at 100 and 50 ppm. The interaction was additive to potentiation with regard to nicotine sulphate and pyrethrum.

The effect of combining the NPV of *S.mauritia* with various insecticides on the mortality of the fourth instar larvae was studied by Mathai *et al.* (1986). NPV in combination with quinalphos, fenthion and permethrin showed synergistic reaction. Chaudhari (1987) evaluated the efficacy of carbaryl, DDT, pyrethrin and sumithion with *Diacrisia obliqua* NPV. Carbaryl, DDT and pyrethrin increased the mortality of *D. obliqua* larvae when applied along with NPV. In the case of sumithion the mixing of *D.obliqua* NPV resulted in antagonism.

A combination of NPV 250 LE + endosulfan was the most effective treatment in controlling *H.armigera* (Dhandapani *et al.*, 1987; Rabindra and Jayaraj, 1990; Rajasekahar *et al.*, 1996; Gopal and Senguttuvan, 1997; Ganguli and Dubey,1998; Sivapraksam,1998 ; Pokharkar and Chaudhary,1999; Sajjanar *et al* ., 1999; Satpathy *et al.*,1999; Balikai and Sattigi ,2000 ; Satpathy and Rai,2000 and Praisy,2002)

When larvae of *H.armigera* sub-lethally infected with NPV were exposed to insecticides, their susceptibility to fenvalerate, cypermethrin, endosulfan and monocrotophos was enhanced significantly (Rabindra and Jayaraj, 1990; Sathiah *et al.*, 1990; Rabindra *et al.*, 1991). Bhanukiran *et al.* (1997) conducted field experiment to determine the effect of triazophos 0.05 per cent and methomyl 0.05 per cent with *S.litura* NPV. A mixture of conventional insecticides at half the recommended concentrations with NPV had little effect on predatory coccinellids.

Third instar larvae of *H. armigera* were fed on artificial diet mixed with combinations of *Ha*NPV and insecticides including cyhalothrin, deltamethrin, methomyl, phoxim and acephate. The results showed that a combination of *Ha*NPV

and each of the insecticides was more effective than *Ha*NPV or insecticides alone (Wan *et al.*, 2000).

In toxicity tests under laboratory conditions, spinosad alone was found to be highly toxic to all instars of *H.armigera* at 48 h. *Ha*NPV +spinosad recorded cent per cent mortality at 72 h (Sathish, 2003). A laboratory experiment was conducted to determine the effect of NPV infection on the susceptibility of *H.armigera* larvae to fenvalerate, quinalphos, endosulfan, cypermethrin, monocrotophos, and chlorpyrifos using bioassay. NPV-inoculated larvae were more susceptible to the insecticides than the control (Vijaykumar *et al.*, 2003).

2.4.5 Interaction of fungi and botanicals

Patil *et al.* (2003) reported that combinations of *N. rileyi* with botanicals performed better than individual treatments. Among different combinations of botanicals with *N.rileyi*, NSKE (five per cent) + *N. rileyi* ($2x10^{11}$ conidia /ha) and *V. negundo* (five per cent) + *N. rileyi* ($2x10^{11}$ conidia /ha) proved to be as effective as recommended insecticides in reducing larval incidence of *S. litura* and defoliation and recorded higher yield of ground nut.

Azadirachtin and *Paecilomyces fumosoroseus* were tested against whitefly *Bemisia argentifolii.* Both tank mixes and separate sprays were tested. Up to 90 per cent nymphal mortality was obtained when both the fungus and azadirachtin were combined, a significant increase over the 70 per cent and less mortality obtained when only one agent was used. However, the combined effects were less than additive. Azadirachtin had moderate inhibitory effects on growth and germination of *P.fumosoroseus* (James, 2003).

2.4.6 Interaction of entomopathogenic fungi and synthetic chemicals

Malik *et al.* (1993) conducted an experiment to assess the efficacy of *B.bassiana* alone and in combination with insecticides *viz.*, monocrotophos and endosulfan. The combination of fungus and insecticides did not give effective control of *H. armigera*. The interaction between *B. bassiana* and mineral oil was evaluated by Batistafilho *et al.* (1995) in order to control the banana plant borer, *Cosmopolites sordidus* (Gem.). These investigators observed an additive effect of the combination, which caused about 98 per cent adult insect mortality comparaed to 70 per cent caused by the fungus alone and 33 per cent by mineral oil alone.

Boncias *et al.* (1996) reported the synergistic effect of imidacloprid and *B. bassiana* against subterranean termite species *Reticulitermes flavipes*. Sanyang *et al.* (1996) reported that the application of *M. flavoviridae* spores along with cypermethrin 10ppm advanced the onset of locust mortality by 48 h.

Combinations of direct contact to the conidia of *M.anisopliae* and feeding on imidacloprid bait indicated synergy when compared with the activity of the individual components. Cockroaches were killed significantly faster when fed on imidacloprid after a topical application of spore suspension of *M. anisopliae* than when fed on imidacloprid bait alone indicating possible synergistic interaction (Kaakeh *et al.*,1997).

Hiromori and Nishigaki (1998) studied the effect of *M.anisopliae* in combination with synthetic insecticides against first stadium larvae of *Anomala cuprea* and suggested that the joint action between *M. anisopliae* and synthetic insecticides resulted from easy penetration of this fungus under conditions of distribution or decline of the larval defence system caused by the existence of a small quantity of insecticides.

Quintela and McCoy (1998) studied the impact of sub lethal concentrations of imidacloprid and the entomopathogenic fungi *M. anisopliae* and *B. bassiana* alone and in combination on mobility, mortality and mycosis of first instar larvae of the citrus pest *Diaprepes abbreviatus*. The larval mortality and mycosis increased synergistically when a sub lethal concentration of imidacloprid was applied in combination with the fungus. Delgado *et al.* (1999) reported the synergistic effect of diflubenzuron with *B.bassiana* against *Manduca sexta*.

Diatomaceous earth, a dessicant insecticide, when applied in combination with *B.bassiana* conidia (300mg/kg of grains) for the control of stored grain pests *R. dominica* and *O. surinamensis*, revealed synergistic effects on adults at all doses (Lord, 2000).

Jayanthi and Padmavathamma (2001) reported that the recommended dose of insecticides fenvalerate 0.005 per cent and monocrotophos at 0.025 per cent in combination with *B. bassiana* was superior in conrolling *S.litura.* James and Elzen (2001) used imidacloprid and *B. bassiana* for the management of whitefly *B. argentifolii*. They found antagonism in that *B.bassiana* inhibited the effectiveness of imidacloprid, insect response was either less than or similar to that when imidacloprid was used alone.

Enhanced mortality due to the dual application of the fungus *M.anisopliae* and insecticides like teflubenzuron and diflubenzuron was observed in *S.gregaria* (Seyoum, 2001).

Neves *et al.* (2002) reported the synergistic interaction between entomopathogenic fungi *B.bassiana* and *M.anisopliae* and commercial insecticides *viz.*, DDT, triflumuron and its positive implications on integrated pest management. Enhanced mortality of *M.anisopliae* was observed in combination with boric acid

against *B. germanica* (Zurek *et al.*, 2002). The application of fungus *N.rileyi* at $1.6x10^8$ spores /ml along with endosulfan (0.035 per cent) gave good control of *H.armigera*, *S.litura* and *Trichoplusia ni* on cabbage. The treatment combination increased the yield also (Gopalakrishnan and Mohan, 2003).

2.5 IMPACT OF ENTOMOPATHOGENS AND PLANT EXTRACTS ON DIGESTIVE ENZYMES

When the earthworm *Pheretima posthuma* was exposed in moist soil to carbaryl and endosulfan at two-eight mg/kg for up to 24 h, the α-amylase activity in the intestine was reduced (Gupta and Sundararaman, 1988).

El-Saidy and Degheele (1990) studied the effects of sublethal concentrations of the insect growth regulator diflubenzuron on the digestive enzymes activity of sixth instar larvae of the noctuid *S. littoralis*. Diflubenzuron reduced amylase activity *in vivo*, reduction in activity being positively correlated with concentration, but invertase, trehalase and protease [proteinase] were not affected. In sixth instar larvae, diflubenzuron probably inhibited amylase indirectly by acting on a physiological system affecting amylase activity or secretion.

El-Saidy *et al.* (1992) investigated the effects of sublethal concentration of methomyl and profenofos on sixth instar larvae of *S. littoralis* under laboratory conditions. Of all the *in vitro* enzymes tested (amylase, invertase, trehalase and proteinase), the activity of amylase was the most affected by all methomyl treatments. They observed 68 and 78 per cent decrease in amylase activity at 10 and 15 ppm. The per cent mortality did not exceed nine and 33 per cent respectively. On the other hand, an increase in methomyl concentration resulted in an increase of protease activity in the midgut walls.

El-Ghar *et al.* (1995) studied the effect of abamectin and *B. thuringiensis* on digestive enzymes of *S.littoralis.* In enzyme assay abamectin caused a remarkable decrease in invertase, amylase and trehalase activities by 81, 76 and 54 per cent respectively, compared to those recorded in the control larvae. *B. thuringiensis* also caused a pronounced decrease in digestive enzyme activities. The effect of sub lethal dosages of the toxic crystal of *B. thuringiensis* subsp. *galleriae* on proteinase, trypsin and amylase in the gut of larvae of *G. mellonella* was investigated. The specific activities of proteinase and trypsin decreased and the activities of two amylase isoenzymes increased in treated larvae (Shen and Qian ,1995).

Semi-lethal doses of endosulfan (0.0022 ml/litre) and methyl parathion (parathion-methyl) (0.0012 ml/litre) initially induced hypersecretory activity in the midgut epithelial cells of *O. materna.* Endosulfan initially stimulated the activities of amylase, invertase, lipase and protease, but after 24 h, amylase, invertase and protease activities returned to normal levels, while lipase activity decreased. Parathion-methyl initially stimulated amylase, invertase, lipase and protease activities, but activities declined four h after treatment. After 24 h, the amylase and invertase activities had returned to normal, while lipase and protease activities remained static (Deshmukh and Tembhare, 1998).

The combined effect of *B. thuringiensis* and certain botanicals on digestive enzymes of *H.armigera* was investigated in the laboratory. The profiles of digestive enzymes were decreased by *B. thuringiensis* subsp. *kurstaki* treatment. The inhibition of digestive enzymes in the midgut of *H.armigera* suggested that *B. thuringiensis* and plant extracts greatly affect the gut homeostasis and suppressed the feeding physiology (Murugan *et al.*,1998).

Studies on the effect of *C. roseus* alkaloids on the biology of *Euproctis fraterna* showed that at a dose of ten ppm, only 19.25 per cent fertile eggs were laid. The hatchability was totally suppressed at higher doses. There was a considerable

reduction in total carbohydrate and protein content in treated larvae. *C. roseus* alkaloids inhibited the activity of digestive enzymes also (Sundari, 1998).

The activities of digestive enzymes such as amylase, invertase, trehalase and protease were analyzed in *Bm*NPV infected silkworm, *Bombyx mori* L. The activity of amylase, invertase and protease in the *Bm*NPV infected larva increased initially but as the disease progressed, it decreased significantly (Gururaj *et al.*, 1999). Hu *et al.*(1999) evaluated the toxic effects of *Myoporum bontioides* leaf extracts against *P. rapae*. Preliminary physiological reaction studies showed that the chloroform extract significantly reduced the protein content of haemolymph and esterase in the midgut and inhibited esterase activity in the midgut. Kumar and Murugan (1999) reported that the combined treatment of nuclear polyhedrosis virus and azadirachtin significantly reduced the digestive enzyme activity in the midgut of *H.armigera*. The protease activity was reduced to $1x10^{-4}$ mg / min, amylase activity to 3.1 $x10^{-4}$ mg / min and lipase activity to 0.4 $x10^{-4}$ mg / min at $1x10^{4}$ PIB /ml NPV +2.0 per cent azadirachtin treatment.

Ortego *et al.* (1999) studied the effects of toxic and deterrent terpenoids azadirone, F 18 (from *Trichilia havanensis*) and scutalpin-B (from *Scutellaria alpina*) on digestive proteases and detoxification enzymes in the larval midgut of Colorado potato beetle *L. decemlineata*. Larvae fed on the F18 mixture showed reduced digestive protease and esterase activity, whereas glutathione transferase and polysubstrate monooxygenase activity increased. The effects of pyrethroid pesticides (deltamethrin, permethrin and cypermethrin) and an organophosphate ester (methidathion) on the activities of carp trypsin, α-chymotrypsin, carboxypeptidase A and lipase were studied. These pesticides modified the triacylglycerol lipase activity to a lesser extent; the highest inhibition was measured with cypermethrin (Simon *et al.*, 1999).

The effects of neem leaf extracts and cyhalothrin were observed on adult *S. oryzae*. Biochemical estimation revealed that both the compounds decreased the alkaline phosphatase activity, by 18.39 per cent (neem leaf extract) and 20.22 per cent (cyhalothrin) (Ahmed *et al.*, 2001). The activities of the midgut digestive enzymes of the third, fourth and fifth instar larvae after feeding on *B.t.* transgenic cotton were lower than that of the control. The rate of enzyme activities decreased significantly as the instar larvae developed (Zhou *et al.*, 2001).

Loseva *et al.* (2002) investigated the protease activity profiles and toxin-binding capacities in the midgut of a strain of Colorado Potato Beetle (CPB) that has developed resistance to the Cry3A toxin of *B. thuringiensis* subsp. *tenebrionis*. The results indicate that resistance by the CPB to the Cry3A toxin correlated with specific alterations in protease activity in the midgut as well as with decreased toxin binding. Lavanya and Chitra (2002) studied the effects of sublethal concentrations of *Annona* seed extracts on the amylase and invertase (beta-fructofuranosidase) activities in the midgut of the fifth instar larvae of *S. litura.* The amylase and invertase activities in the midgut of the treated larvae were reduced to 20.14 and 15.53 per cent, respectively, compared to the control. The reduction in enzyme activities can be attributed to the direct action of the extract on the enzyme-secreting walls of the midgut wall, rupturing the tissues that can induce subsequent disruption in enzyme secretion.

The changes in intracellular proteases following DDT application at LD_5 and LD_{20} level in a DDT-resistant strain of *M. domestica* showed that at three h of the treatment, the cytoplasmic enzymes except proline endopeptidase showed a non-significant increase in activities. The activities of all cytoplasmic enzymes further increased at LD_{20} level, suggesting the dose-dependence of activities, but protease activities at 24 h with both doses showed non-significant difference with control activities (Ahmed *et al.*, 2003).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study was undertaken in the Department of Entomology, College of Horticulture, Vellanikkara, Thrissur during 2002-2004. The details of the materials utilized and methods followed for the present investigation are elaborated hereunder with the following objectives

1. Survey of *Helicoverpa armigera* and its natural enemies in vegetable ecosystem during 2002-2004.
2. Screening of plant extracts and essential oils at different concentration against third instar larvae of *H.armigera.*
3. Compatibility of botanicals, insecticides and biocides.
4. Bio efficacy of botanicals, insecticides and biocides alone and in combination.
5. Effect of botanicals and biocides on digestive enzymes activity in the host insect.
6. Evaluation of field efficacy of successful biocide combinations in managing *H. armigera.*

3.1 SURVEY OF NATURAL ENEMIES OF *H. armigera*

Field collection of natural enemies of *H.armigera* was done from vegetable ecosystem in and around the College of Horticulture, Vellanikkara during 2002-2004. Weekly sampling of egg and larvae of *H.armigera* was carried out in different host plants *viz.*, tomato, bhendi, cowpea and bittergourd to record the seasonal trend of parasitism and to evaluate their efficacy as a natural mortality agent of *H.armigera.* Ten randomly selected plants were observed every week for the

presence of *H.armigera* and its natural enemies in each ecosystem during each season.

3.1.1 Parasitoids

Eggs of *H.armigera* were collected along with the leaf or stem and kept in separate glass vials in the laboratory and observed daily. Moist cotton swab was placed inside the vial to maintain humidity. Various instars of *H. armigera* larvae were placed in individual vial containing semi synthetic diet as per Sathiah (2001). The diet was changed regularly as and when required until the larvae attained pupation. The parasitoids that emerged were preserved in card mount and pinned with micro-pins. These were then identified with the help of taxonomic keys and by sending to the taxonomists.

3.1.2 Predators

Spiders, coccinellids and other predators (Ants, *Chrysoperla*) on the vegetable canopy were counted and collected in specimen tubes. These were brought to the laboratory and identified with the help of taxonomic keys.

3.1.3 Pathogens

Dead larvae found hanging from or remaining on the leaves or cadavers were collected from the field and brought to the laboratory for examination. For general diagnostic work, wet mount and smear of the specimen were prepared. The specimens/mounts/smears were then observed under a research microscope to confirm the preliminary diagnosis and the pathogens involved were identified.

3.1.3.1 *Isolation of fungi from diseased cadavers*

The mycosed cadavers were identified and surface sterilized with 0.1 per cent sodium hypochlorite solution for three minutes and then washed three times with sterile distilled water. After drying, the cadavers were carefully picked up with needle and kept in Potato Dextrose Agar (PDA) plates. The petridishes were incubated at room temperature and examined daily for the growth of the fungus. The pure culture of the fungus was maintained on PDA slants. The identification was done based on the basis of external symptoms, morphology of spores and sporulating structures. Pathogenicity test was conducted by spraying the aqueous suspension of the fungus on healthy third instar larvae. After five days of incubation, dead larvae were collected and the fungus was reisolated from the cadavers, thus satisfying Koch's postulates.

3.2 MASS CULTURING OF *H.armigera*

Field collected populations of *H.armigera* were cultured in the laboratory using chickpea based diet standardized by Sathiah (2001).

3.2.1 Larval rearing

The plastic rearing trays of size 30 x 15 x 15 cm were used for rearing first and second instar larvae. The later instars were reared in five ml penicillin vials. The larvae were supported by a semi-synthetic diet. It was prepared by blending and cooling with the following ingredients.

1. Chickpea seeds	- 100 g
2. Agar agar	- 12.8 g
3. Yeast	- 30 g
4. Methyl-para-hydroxy benzoate	- 2 g
5. Sorbic acid	- 1 g
6. Ascorbic acid	- 3.2 g
7. Streptomycin sulphate	- 40 mg
8. Vitamin supplement	- 2 ml
9. Formaldehyde 40 per cent	- 1 ml
10. Carbendazim	- 500 mg
11. Water	- 750 ml

Larvae of *H. armigera* collected from different locations from the campus of College of Horticulture, Vellanikkara were reared in semi synthetic diet till pupation. Chickpea seeds were soaked overnight, boiled in water and blended with yeast 30 g and 375 ml of water. Agar 12.8g was melted simultaneously in 375 ml of water. After fine grinding of chickpea seeds, molten agar and other ingredients were added into the blender and mixed thoroughly. The hot diet was poured into five ml penicillin vials well before solidification using plastic wash bottle. The trays containing the diet were kept at room temperature for an hour. Upon solidification, they were covered, inverted and placed inside the refrigerator at 15°C and used whenever required.

In the plastic rearing trays newly hatched larvae were transferred and confined properly with the help of muslin cloth reinforcement beneath the lid. The trays were kept inverted to facilitate the positively phototropic and negatively geotactic larvae to feed unhindered on the diet. When the larvae reach second instar

premoult stage they were transferred to glass vials (five ml) with diet and bottles were cleansed with water and kept in 0.5 per cent sodium hypochlorite solution overnight, rinsed thrice in clean water and allowed to sun dry for 30 to 60 min before reuse.

3.2.2 Adult rearing

The pupae were collected from the culture and placed in adult emergence cages of size 30x30x45 cm made of wooden frame to which glass panels were fitted on the two sides and metallic wire netting at the backside. The bottom was provided with wooden plank of one cm thickness and top with plain glass. Front door was of metallic netting fitted on wooden frame. Five pairs (sex ratio 1:1) of newly emerged adults were transferred to a plastic bucket of five litre capacity for mating and oviposition. Adults were fed with ten per cent sugar solution enriched with ABDEC© multivitamin solution. The solution was provided in a five ml glass vial with a cotton wool wick filling the mouth to prevent the moths from drowning. The mouth of the bucket was covered with sterile muslin cloth, which served as an oviposition substrate.

3.2.3 Collection of eggs

The adults in the bucket oviposited on the sterile muslin cloth. This muslin cloth with eggs was collected from third day onwards and was replaced with fresh cloth on every succeeding day. The egg cloth was placed in plastic containers covered with lid and was in turn kept inside another container with a layer of water to maintain humidity. After formation of distinct germ band in the eggs, the egg clothes were surface sterilized with ten per cent formalin for ten minutes and washed in running water for 20 minutes. This prevented the incidence of *Ha*NPV in the culture. Egg clothes were shade dried at room temperature and brought back to

another sterile humidified chamber for hatching. Newly hatched larvae were transferred using a camel hair brush to plastic trays containing three mm layer of semi synthetic diet. When the larvae attained second instar premoult stage, they were transferred to individual glass vial and used for the bioassay.

3.3 SCREENING OF PLANT EXTRACTS AND ESSENTIAL OILS AGAINST *H.armigera*

Kerala is famous for its floral diversity. The rich tropical flora seems to possess enormous potential as insecticides. The locally available three insecticidal plants and four essential oils were assayed at different concentrations (one, two and half and five per cent) against third instar larvae of *H.armigera.*

The details of botanicals used in the study are given in the following table

Sl.No.	Botanicals	Scientific name	Part used
1	Sweet Flag (Vayambu)	*Acorus calamus* (Linn.)	Rhizome
2	The Creat (Kiriyathu)	*Andrographis paniculata* (Wall.)	Leaves
3	Indian privet (Karinotchi)	*Vitex negundo* (Linn.)	Leaves
4	Citronella (Theruvapullu)	*Cymbopogon winterianus* (Jowitt.)	Oil
5	Lemon grass (Inchipullu)	*Cymbopogon flexuosus* (Steud.)	Oil
6	Galanga (Kacholam)	*Kaempferia galanga* (Linn.)	Oil
7	Palmarosa (Palmarosa)	*Cymbopogon martinii*(Roxb.)	Oil

3.3.1 Method of extraction of plant materials

Fresh plant materials were collected from Medicinal plants garden, College of Horticulture, Vellanikkara. Fresh leaves of *A. paniculata* and *V. negundo* (ten g

each) were taken every time when required, chopped into small pieces and macerated with 100 ml water in a pestle and mortar thoroughly. The macerated slurry was strained through muslin cloth and Whatman No. 1 filter paper. The volume was made up to 100 ml to form the primary stock extract (ten per cent). The desired concentrations were prepared by adding distilled water containing 0.1 per cent teepol. Teepol is a commercially available spreading agent, used as liquid soap.

Dried rhizomes of *A. calamus* procured from the local market were used to prepare the extracts. The rhizomes were powdered coarsely in a mortar and then finely powdered in a blender. Ten grams of finely powdered rhizomes were transferred to a 100 ml volumetric flask and the volume was made up with distilled water. The flask was shaken vigorously for 15 minutes and kept in a cool dark place for 24 h. During this period, the flask was shaken periodically. At the end of the period, the contents were filtered and the extract was immediately used for bio efficacy studies. The stock solution was prepared afresh for each subsequent experiment in order to avoid fermentation. The required concentrations were prepared by suitably diluting the primary stock with distilled water containing 0.1 per cent teepol.

3.3.2 Method of extraction of essential oils

The essential oils were obtained from Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali, KAU. The required concentrations (One, two and half and five per cent) were prepared by diluting with distilled water containing teepol 0.1 per cent.

3.3.3 Evaluation of insecticidal action of different plant materials and essential oils

Freshly emerged third instar larvae of uniform size and age were collected from mass culture maintained in the laboratory. The different concentrations of the plant extracts/ oils were directly sprayed on the test insects released in clean petridishes using an atomizer. Ten insects formed one replication. Three replications were maintained for each treatment. The larvae sprayed with distilled water containing teepol 0.1 per cent served as control. After a lapse of five minutes when the spray of the extracts/oils got dried, the larvae were transferred to vials containing semi synthetic diet. Mortality counts were taken at the end of 24 h. The per cent mortalities in treatments were corrected for mortality in control using Abbott's formula (Abbott, 1925).

3.4 COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI WITH INSECTICIDES AND BOTANICALS

In vitro studies were conducted to find out the compatibility of three species of entomopathogenic fungi with the selected insecticides and botanicals, which were used in the interaction studies.

The entomopathogenic fungi used in the compatibility tests were as follows

Sl. No.	Common name	Scientific name	Place
1	Muscardine	*Nomuraea rileyi* (Farlow) Samson	Vellanikkara Isolate
2	White muscardine	*Beauveria bassiana* (Bals-Criv.) Yuill	Sugarcane Breeding Institute,Coimbatore
3	Green muscardine	*Metarhizium anisopliae* (Metsch.)Sorokin	-do-

The genera *Nomuraea, Beauveria* and *Metarhizium* belong to taxa Deuteromycotina – Hyphomycetes – Moniliales

The fungi were grown in two media, PDA and Sabouraud's Maltose Agar +Yeast (SMA+Y). PDA was used as culturing media for *M. anisopliae* and *B. bassiana*. *N. rileyi* was grown in SMA+Y. The compositions of the media are given below and were prepared by the procedure given by Lomer and Lomer (1995).

1. Potato Dextrose Agar (PDA)

Potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000ml

2. Sabouraud's Maltose Agar +Yeast (SMA+Y)

Maltose	40 g
Yeast Extract	10 g
Peptone	10 g
Agar	15 g
Distilled water	1000ml

3.4.1 Compatibility of the fungi with insecticides / essential oils

The entomopathogenic fungi mentioned in the section 3.4 were tested for their compatibility with four insecticides and four essential oils by following poison food technique (Falck, 1907). The essential oils and insecticides used in the study are presented in the following table.

3.4.1.1 *Essential oil*

Sl. No.	Common Name	Scientific name	Dose (%)
1	Citronella oil	*Citronella winterianus*	5.0
2	Kacholam oil	*Kaempferia galanga*	5.0
3	Lemon grass oil	*Cymbopogon flexuosus*	5.0
4	Palmarosa oil	*Cymbopogon martinii*	5.0

3.4.1.2 *Insecticides*

Sl. No.	Common Name	Trade name	Dose (%)
1	Carbosulfan	Marshal 25 EC	0.05
2	Profenofos	Curacron 50EC	0.10
3	Spinosad	Tracer 45 SC	0.02
4	Triazophos	Hostathion 40 EC	0.10

One hundred ml of the medium (PDA/SMA+Y) was prepared in 250 ml conical flasks and sterilized. The required quantities of the insecticides/essential oils were added aseptically to 100 ml medium to get the required concentration. After adding the insecticides/essential oils to the medium, it was mixed well and transferred to sterilized petridishes. For each treatment, three replications were kept. Fungal growth of four mm disc taken from ten days old culture using sterilized cork borer and was kept at the center of the above petridishes using a sterilized needle. The dishes were incubated at room temperature and observed daily for the growth. Control was also maintained without adding the insecticides. The per cent inhibition was evaluated using the formula suggested by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

Where

I = Per cent inhibition of growth

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

3.4.1.3 Compatibility of the fungi with botanicals

The botanicals evaluated for their compatibility with entomopathogenic fungi are given in the following table.

Sl. No	Common name	Scientific name	Dose (%)
1	Vayambu	*Acorus calamus*	5
2	Kiriyathu	*Andrographis paniculata*	5
3	Karinotchi	*Vitex negundo*	5

Five grams of these plant materials were surface sterilized with 70 per cent alcohol. They were washed and kept for air-drying (10 min). After drying they were kept under UV light in a laminar air- flow for about 45 minutes for further sterilization. Then the leaves were chopped into small pieces and macerated with 100 ml of sterile water in a sterilized pestle and mortar. The extract was filtered through muslin cloth. The extract was added to the conical flask containing the sterilized medium. It was mixed well and transferred to the petridishes aseptically. Actively growing ten days old fungal growth of four mm diameter disc was taken using sterilized cork borer and was transferred aseptically to the center of the dishes using a sterilized needle. The dishes were incubated at room temperature and observed daily for growth of the fungus. The diameter of the fungal growth was recorded daily. Three replications were kept for each botanical. Un treated control was also maintained without adding the botanicals and the per cent inhibition over control was calculated as mentioned in section 3.4.1.2

3.4 COMPATIBILITY OF COMMERCIAL FORMULATIONS OF *Bacillus thuringiensis* WITH BOTANICALS AND INSECTICIDES

Three formulations of *B. thuringiensis* (Halt, Delfin and Dipel) were used in combination with botanicals and insecticides to understand their synergism or antagonism or otherwise.

The details of commercial formulations of *B. thuringiensis* are given below

Commercial formulation	Variety/Subspecies	Formulation	Active ingredient	Source
Delfin(0.2 %)	*Bacillus thuringiensis* var. *kurstaki*, serotype, 3a, 3b	Water soluble microgranules	53,000 SU/mg	Margo Biocontrols (P) Limited, Bangalore.
Dipel (0.2%)	*Bacillus thuringiensis* var. *kurstaki*, serotype, 3a, 3b	Suspension 8 L	-	Chemnova (India) Limited, Mumbai.
Halt (0.2%)	*Bacillus thuringiensis* var. *kurstaki*, serotype, 3a, 3b, 3c	Wettable powder	56,000 SU/mg	Wockhardt Life Science Ltd, Mumbai.

One hundred ml of the nutrient agar medium (peptone ten g, beef extract five g, agar 20g and distilled water one litre) was prepared in 250 ml conical flask and sterilized.

The required quantities of *B. thuringiensis* formulations were added aseptically to 100 ml medium to get the required concentration. The *B. thuringiensis* formulation and the media was mixed well uniformly and transferred to sterilized petridishes. The dishes were incubated for about 24 to 48 h. For each concentration three replications were kept. Sterile filter paper of four mm diameter was dipped in respective insecticides / botanicals (Section 3.4.1.1, 3.4.1.2 and 3.4.1.3) solution and dried over a wire net. The dried filter paper disc was placed on the nutrient agar medium inoculated with pure culture of *B. thuringiensis* using sterile forceps. The petridishes were incubated at room temperature. Filter paper disc dipped in sterile distilled water served as control. The diameter of the inhibition zone developed around the filter paper disc dipped in test chemical was recorded. Observations were taken for one week.

3.6 STUDIES ON INTERACTION OF BIOCIDES AND INSECTICIDES

The bio-efficacy of botanicals and insecticides individually and in combination with biocides was evaluated under laboratory condition. This study was conducted to assess the synergistic/antagonistic response of combined application of insecticides and biocides in the management of *H. armigera*.

3.6.1 Joint action of entomopathogenic fungi with botanicals, essential oils and insecticides

Three different entomopathogenic fungi, namely *N. rileyi* ($2x10^8$ spores/ml), *B. bassiana* ($1x10^8$spores/ml) *and M. anisopliae* ($1x10^8$spores/ml) were assayed for their pathogenicity against third instar larvae of *H.armigera*. This was done to ascertain the virulence of the fungi on the test insect. Ten larvae of *H.armigera* were first inoculated with different isolates and fungi reisolated in pure form from the

diseased cadavers showing typical mycosis. After reisolation from the cadavers, the isolates were purified by sub culturing on PDA. The conidia for the bioassay were harvested from the ten day-old cultures just before use by washing from the surface of the plates. For this, 100 ml of sterile distilled water containing 0.1 per cent teepol was used. Conidial suspensions were standardized for each isolate with haemocytometer using the following formula (Mark and Douglas, 1997)

$$\text{Number of spores /ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where

X = Number of spores counted totally

Y = Number of smaller (1/400) squares checked

10 = Depth factor

1000 = Conversion factor for mm^3 to cm^3

D = Dilution factor

Newly moulted third instar larvae of *H.armigera* were bioassayed for their susceptibility to the different fungal isolates. Ten larvae taken in a petridish lined by a filter paper were directly sprayed with conidial suspension using a hand atomizer. Three such replicates were maintained for each concentration. Control insects received a spray of only teepol in sterile distilled water. After air-drying, the treated larvae were carefully transferred to individual glass vials containing freshly prepared semi synthetic diet (without formalin). The larvae were reared till pupation. The per cent mortality was calculated at 24 h interval.

3.6.1.1 Entomopathogenic fungi and plant extracts

The aqueous extracts of plant materials *viz., A. calamus* (vayambu), *A. paniculata* (kiriyathu) *and V. negundo* (karinotchi) at five per cent concentration were assessed for their synergistic/antagonistic/other interaction with the fungi in producing mycosis in the test insect. For this a low rate of fungi *N. rileyi* ($1x10^8$spores/ml), *B. bassiana* ($0.5x10^8$spores/ml) and *M. anisopliae* ($0.5x10^8$spores / ml) and two and half per cent plant extracts were used. The combination of fungi and plant extracts according to the above treatments were directly sprayed on the larvae kept in the petridishes. The treated larvae were then reared on the semi synthetic diet. Ten insects formed one replication. Three replications were maintained for each treatment. Mortality counts were taken at 24 h interval and mortality due to fungus was determined through microscopic examination of the dead larvae. The per cent mortalities in treatments were calculated as in 3.6.1.

3.6.1.2 Entomopathogenic fungi and essential oils

The essential oils *viz., C. winterianus* (Theruvapullu), *C. flexuosus* (Inchipullu), *C.martinii* (Palmarosa) and *K. galanga* (Kacholam) at five per cent level were tested alone and in combination with entomopathogenic fungi. A lower rate of fungi *N. rileyi* ($1x10^8$spores/ml), *B. bassiana* ($0.5x10^8$spores/ml) and *M. anisopliae* ($0.5x10^8$spores /ml) and sub lethal concentration of essential oils (two and half per cent) were used in combination studies. The treatments were applied as mentioned in section 3.6.1.1.

3.6.1.3 Entomopathogenic fungi and insecticides

The insecticides namely, carbosulfan (0.05 per cent), profenofos (0.1 per cent), spinosad (0.02 per cent) and triazophos (0.1 per cent) were tested against third instar larvae of *H. armigera.* These insecticides were tested alone and in combination with entomopathogenic fungi. In the case of combination studies, the larvae were treated with sub lethal concentrations of fungi *N. rileyi* ($1x10^8$spores / ml), *B. bassiana* ($0.5x10^8$spores / ml) and *M. anisopliae* ($0.5x10^8$spores / ml) and half the rate of insecticides viz., carbosulfan 0.025 per cent, profenofos 0.05 per cent, spinosad 0.01 per cent and triazophos 0.05 per cent. The treatments were applied as mentioned in section 3.6.1.1. The treated larvae were transferred to the semi synthetic diet after the spray got dried up. For each treatment, ten larvae were used and the treatments were replicated thrice. Larval mortality was recorded at 24 h interval until eighth day of treatment as in section 3.6.1.1.The percent mortalities calculated for all the above experiments were corrected for mortality in control using Abbott's formula (Abbott, 1925).

3.6.2 Joint action of B. thuringiensis with plant extracts, essential oils and insecticides

Three commercial formulations of *B. thuringiensis* var. *kurstaki (B.t.k)* (Halt, Delfin and Dipel) were tested against the third instar larvae of *H.armigera.* The details of the products are mentioned in Para 3.4.3. The desired concentration was obtained by diluting with distilled water containing 0.1 per cent teepol. Ten µl of the suspension was applied on the diet surface (without formalin). The distribution of the suspension was ensured by smearing it with a blunt end of polished glass rod. Third

instar larvae of uniform size were released on to the diet 15 minutes after surface treatment. Mortality counts were taken at 24 h intervals.

3.6.2.1 Bacillus thuringiensis and plant extracts

The aqueous extracts of plant materials *viz.*, *A. calamus* (Vayambu), *A. paniculata* (Kiriyathu) *and V. negundo* (Karinotchi) at five per cent concentration were tested against third instar larvae of *H. armigera*. In the case of interaction studies, the third instar larvae were treated with a lower rate of different commercial formulation *viz.*, Halt, Delfin and Dipel at 0.1 per cent and sub lethal concentration of botanicals (2.5 per cent). The dilutions were prepared with distilled water containing teepol 0.1 per cent. The botanicals were directly sprayed on the larvae kept in the petridishes using an atomizer. The treated larvae were transferred to the semi synthetic diet. The diet was already toxicated with a lower rate of *B. thuringiensis* commercial products as mentioned in 3.6.2. For each treatment, ten larvae were used and the treatments were replicated thrice. Observations were recorded at 24 h intervals.

3.6.2.2 Bacillus thuringiensis and essential oils

The different essential oils (*C. winterianus, K. galanga, C. flexuosus* and *C.martini*) at five per cent level were tested against third instar larvae of *H. armigera*. A lower rate of *B. thuringiensis* (0.1per cent) and sub lethal concentration of essential oils (two and half per cent) were used in the combination studies. *B. thuringiensis* and essential oils were applied using the same procedure as in 3.6.2.1.

3.6.2.3 Bacillus thuringiensis and insecticides

The insecticides namely, carbosulfan (0.05 per cent), profenofos (0.1 per cent), spinosad (0.02 per cent) and triazophos (0.1 per cent) mentioned in section 3.4.1.2 were tested against third instar larvae of *H. armigera*. A lower rate of *B. thuringiensis* (0.1 per cent) and insecticides at sub lethal concentration *viz*., carbosulfan 0.025 per cent, profenofos 0.05 per cent, spinosad 0.01 per cent and triazophos 0.05 per cent were used in interaction studies. The treatments were applied as mentioned in section 3.6.2.1.

3.6.3 Joint action of Nuclear Polyhedrosis Virus (NPV) with plant extracts, essential oils and insecticides

*Ha*NPV isolate used in the study was supplied by the Margo Biocontrols Private Limited, Bangalore, Karnataka, India (Tumkur isolate – Tumkur HaNPV). The isolate Tumkur *Ha*NPV was formulated in water and supplied by the company as Heligard© containing $1x10^9$ POB/ml. The enumeration of the POB was done using a standard haemocytometer of depth factor 0.1 mm. The required concentration of $1.5x10^{12}$ POB/ha was prepared after enumeration. Ten µl of the viral suspension was dispensed on to the semi synthetic diet. The viral suspension was uniformly smeared with a blunt end of polished glass rod. Third instar larvae of uniform size were released on to the diet 15 minutes after surface treatment. For each treatment, ten larvae were used and the treatments were replicated thrice. The larval mortalities were recorded at 24 h intervals.

3.6.3.1 Nuclear polyhedrosis virus and plant extracts

The third instar larvae were treated with the aqueous extracts of plant materials *viz., A. calamus* (vayambu), *A. paniculata* (kiriyathu) *and V. negundo* (karinotchi) at 5 per cent concentration. The sub lethal concentrations of nuclear polyhedrosis virus (0.75 $x10^{12}$ POB/ha) and plant extracts (two and half per cent) were used in interaction of studies. The treatments were applied as mentioned in section 3.6.2.1. The treated larvae were then reared on the semi synthetic diet. The diet was already contaminated with a lower rate of nuclear polyhedrosis virus as per the methodology detailed in 3.6.3. For each concentration 30 larvae in three replications were used. Observation was taken at 24 h intervals.

3.6.3.2 Nuclear polyhedrosis virus and essential oils

Third instar larvae of *H. armigera* were tested against different essential oils (*C. winterianus, K. galanga, C. flexuosus* and *C.martinii*) at five per cent level. In combination studies, a lower rate of nuclear polyhedrosis virus (0.75 $x10^{12}$ POB / ha) and sub lethal concentration of essential oils at two and half per cent concentration was used. The nuclear polyhedrosis virus and essential oils were applied as mentioned in section 3.6.3.1.

3.6.3.3 Nuclear polyhedrosis virus and insecticides

The insecticides, namely, carbosulfan (0.05 per cent), profenofos (0.1 per cent), spinosad (0.02 per cent) and triazophos (0.1 per cent) were tested against third instar larvae of *H. armigera*. A lower rate of nuclear polyhedrosis virus (0.75 $x10^{12}$ POB / ml) and insecticides at sub lethal concentration *viz.*, carbosulfan 0.025 per

cent, profenofos 0.05 per cent, spinosad 0.01 per cent and triazophos 0.05 per cent were used in interaction studies. The nuclear polyhedrosis virus and insecticides were applied as per the methodology described in section 3.6.3.1.

3.6.4 Determination of synergism between biocides and insecticide molecules

Based on the larval mortality data generated from the experiments conducted, the types of synergism between biocides and insecticide molecules were assessed following the method of Benz (1971).

3.6.4.1 Independent synergism

Independent synergism is a system of two components acting independently and not interfering with each other. If P_M is the probability of death due to microorganisms taken alone, and Pi the corresponding value for insecticide, the probability of death by combined action is

$P_{M+i} = P_M + Pi(1-PM)$

On the other hand, if the corresponding value of mortality in percentage are used,

$M_{M+i} = M_M + Mi\ (1-M_M / 100)$

Where

M_{M+i} = Per cent mortality due to microorganism

Mi = Per cent mortality due to insecticides

M_{M+i} = Per cent combined mortality

3.6.4.2 Sub additive synergism

Sub additive synergism is a system of two components, which together produce an effect greater than the independent synergism, but less than algebraic

sum of the two single effects. A weak potentiating effect is necessary to produce such a result.

3.6.4.3 Supplemental synergism

Supplemental synergism is a system of two effective components, which together produce an effect greater than the algebraic sum of the single effect (M_{M+i}> M_M+Mi).

3.6.4.4 Potentiating Synergism

Potentiating synergism is a system of component A causing the effect M_A (mortality due to component A) and a synergist S which alone cause no effect (Mortality due to synergist S, M_S=O), but which in combination produce an effect which is significantly greater than M_A. This type of synergism may be found when non-lethal concentration of an insecticide is combined with a microorganism.

3.6.4.5 Temporal synergism

Temporal synergism is a system, which occurs when two components together kill insects quicker than either component alone.

3.6.4.6 Economic synergism

Economic synergism is a system of two components, which together reduce damage more than each component alone. Two types comes under this are interspecific and intraspecific economical synergism. Temporal synergism may be included in economic synergism.

3.6.4.7 Synergistic co efficient

It is an index of multiplicative effect. Synergistic co efficient is calculated by

$Y = x + y + \lambda xy$

$Y = Mm+i$ (Mm+i mortality of micro organism and insecticides)

$x = Mm$ (Mm mortality of micro organism)

$y = Mi$ (Mi mortality of insecticides)

λ = Synergistic co efficient

λ is estimated by non-linear regression programme using STATISTICA package.

$\lambda = 1$ perfect synergism

$\lambda \geq 1$ higher order synergism

$0 \leq \lambda \geq 1$ lower order synergism

$\lambda \leq 0$ sub multiplicative synergism

3.7 ESTIMATION OF DIGESTIVE ENZYMES ACTIVITY

The combined effect of biocides (*B. thuringiensis* and NPV) and certain botanicals on digestive enzymes of *H.armigera* was estimated in the laboratory. The digestive enzymes *viz.*, the proteases, amylases and lipases were assayed for their activity. The third instar larvae were treated with sub lethal doses of *B. thuringiensis* and NPV in combination with botanicals (*A. calamus, A. paniculata* and *V. negundo*) as per the methodology detailed in 3.6.2 and 3.6.3.

3.7.1 Preparation of enzyme extract

The live larvae were removed from the treatment after 24 and 48 h. The larvae were dissected and midgut tissues removed under ice-cold condition. Then the

midgut tissues were homogenized with phosphate buffer (pH 6.8) in a pre chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 10 min at 4^0C. The supernatant was used as enzyme source.

3.7.2 Protease activity

The protease activity was estimated by incubating enzyme extract with casein as a protein source. The reaction mixture was prepared by adding 0.5 ml casein solution (one per cent in, 0.1M, pH 6.0) and 0.1 ml enzyme extract. This was incubated at 45^0 C for one hour. The reaction was terminated by adding 0.1ml tricarboxylicacid (TCA) 40 per cent. The reaction mixture was centrifuged at 10,000 rpm for ten minutes. TCA soluble component (precipitate) was retained in the micro centrifuge tube. The amino acid content of the TCA soluble component was measured after reaction with folin-phenol reagent. The blue colour developed was read at 660 nm using a spectrophotometer. The enzyme activity was expressed in terms of μg amino acids released per unit time (Malik and Singh, 1980).

3.7.3 Amylase activity

The α -amylase activity was assessed by preparing the following reaction mixture. One ml of starch solution was prepared by boiling 150 mg of potato starch, 600 mg KH_2PO_4 and 20 mg of anhydrous $CaCl_2$ in 100 ml distilled water for one minute. Then the solution was filtered through Whatman No.1 filter paper. The clear supernatant was used as starch solution in the assay. One ml of starch solution was mixed with 0.5 ml of enzyme extract. At zero time, 0.2 ml aliquot was removed from the reaction mixture and three ml of IKI solution (254 mg I_2 and 4.0 g KI in one litre distilled water) was added. The optical density was recorded immediately at 620 nm. Then after every 30 minutes the aliquot was removed and the optical density was

recorded. The enzyme activity was expressed in terms of decrease in optical density at 620 nm per unit time (Malik and Singh, 1980).

The activity of β - amylase was estimated by measuring the increase in reducing groups in the presence of chelating compound Ethylene Di amino Tetra Acetic acid (EDTA).The reaction mixture was prepared by adding 0.7 ml starch solution (0.2 per cent in 0.067M phosphate buffer,pH6.0),0.1 ml EDTA solution and 0.1 ml enzyme extract. This mixture was incubated for 30 minutes at 30^0C. The reaction was stopped by the addition of one ml dinitrosalicylic acid reagent. The reaction mixture tubes were kept in the water bath for 20 minutes. After cooling, three ml of distilled water was added to each tube. The optical density was recorded at 560 nm. A reference curve was prepared by heating known amounts of maltose sugar with dinitrosalicylic acid reagent. Enzyme activity was expressed in terms of maltose released per unit time (Malik and Singh, 1980).

3.7.4 Lipase activity

Lipase activity was determined by following the method of Sadasivam and Manickam (1997). The substrate was prepared by stirring two ml of coconut oil with 25 ml of water in the presence of 100 mg bile salts. The substrate was mixed thoroughly till an emulsion is formed. Twenty ml of substrate with five ml of phosphate buffer (pH 7.0) was taken in a beaker. The beaker was kept on the magnetic stirrer cum hot plate. The temperature was maintained at 35^0 C. The electrodes of the pH meter were dipped in the reaction mixture to note pH. Initially the pH was adjusted to 7.0. Then enzyme extract was added to the reaction mixture and the pH was recorded immediately. This was pH at zero time. At ten minutes interval 0.1 N NaOH was added and pH was brought to the initial value. The titration was continued for 30 minutes. The volume of alkali consumed was noted.

The enzyme activity was expressed in terms of milli equivalents $min^{-1}mg^{-1}$ protein

Activity m eq / min / mg sample = $\frac{\text{Volume of alkali consumed x strength of alkali}}{\text{Weight of sample in mg x time in minutes}}$

3.9 FIELD EVALUATION OF PROMISING BIOCIDES

The promising biocides were tested under field condition in pot culture. The experiment was carried out during December 2003 – April 2004 to evaluate the efficacy of biocides and synthetic chemicals against *H. armigera* on tomato variety Anagha. Application of fertilizer and other crop husbandry practices were adopted as per the package of practices (KAU, 2003) excluding the plant protection measures. There were 15 treatments with three plants per pots per treatment, in a completely randomized design and replicated twice.

Larval implantation was done to build up the larval population. Three-third instar larva per plant was released on potted plants 45 days after transplanting. The pots were covered with iron insect cages size of 100 x 70cm (Plate 6.) to prevent the migration of larvae. Establishment of released larvae on the plants was ascertained the next day. The different treatments were then applied with water containing 0.5 per cent teepol as a diluent.

The treatment details are given below.

T1 - *Bacillus thuringiensis* (Delfin) 0.2 per cent

T2 – Nuclear polyhedrosis virus $1.5x10^{12}$ POB / ha

T3 - *Nomuraea rileyi* $2x10^{8}$ conidia / ml

T4 - *Vitex negundo* 5 per cent

T5 - *Cymbopogon martinii* 5 per cent

T6 - Spinosad 75g ai / ha

T7 - *B.thuringiensis* (Delfin) (0.1 per cent) + *V. negundo* (2.5 per cent)

T8 - *B. thuringiensis* (Delfin) (0.1 per cent) + *C.martini* (2.5 per cent)

T9 - *B.thuringiensis* (Delfin) (0.1 per cent) + Spinosad (37.5g ai/ha)

T10 - NPV ($0.75x10^{12}$POB / ha) + *V. negundo* (2.5 per cent)

T11 - NPV ($0.75x10^{12}$POB / ha) ++ *C. martinii* (2.5 per cent)

T12 - NPV ($0.75x10^{12}$POB / ha) + Spinosad (37.5g ai/ha)

T13 – *N. rileyi* ($1x10^{8}$ conidia / ml) + *V. negundo* (2.5 per cent)

T14 – *N. rileyi* ($1x10^{8}$ conidia / ml) + Spinosad (37.5g ai / ha)

T15 - Control

The treatments were sprayed with hand sprayer. Two applications were given at one-month interval. Mortality of the larvae was recorded up to pre pupal stage periodically. Surviving insects were transferred to plastic containers containing moist soil at bottom and maintained undisturbed for pupation. Observations on adult emergence were recorded. The deformed pupae and adults also were considered dead. The total yield and marketable yield in individual treatments were recorded and the percentage mortality was calculated. Percentage loss due to borer infestation on the fruit number and fruit weight basis was also calculated.

3.10 ECONOMICS OF THE DATA

Economic analysis of the treatment was undertaken by working out benefit: cost ration for all insecticidal treatment.

$$\text{BCR} = \frac{\text{Value of increased yield over control} - \text{Cost of Application of biocide combinations}}{\text{Cost of application of biocide combinations}}$$

(Benefit: Cost Ratio)

3.11 STATISTICAL ANALYSIS

Statistical analysis was done using MSTATC package

RESULTS

4. RESULTS

In the present investigation, laboratory and field experiments were carried out to assess the effect of joint action potential of biocides with botanicals and insecticides for the management of tomato fruit borer *Helicoverpa armigera*. The results of the experiments are presented in this chapter.

4.1 SURVEY OF *H. armigera* AND ITS NATURAL ENEMIES IN VEGETABLE ECOSYSTEM

The occurrence of *H.armigera* and its natural enemies were recorded in vegetable ecosystem at college of Horticulture, Vellanikkara during Oct - 2002 to Mar - 2004.

4.1.1 Occurrence of *H. armigera* and its natural enemies in tomato ecosystem

The incidence of fruit borer *H.armigera* and its natural enemies in tomato ecosystem are presented in Table 1.The population of *H. armigera* was high during Oct 2002 (67 larvae/10 plants). The number of larva per 10 plants was 48 and 41 during Apr - 2003 and Nov - 2002 respectively. There was no incidence of *H. armigera* during July to Sept '03. The other months in the survey period recorded only 13 to 26 numbers of larvae per 10 plants. Various natural enemies recorded from these field-collected larvae are given below

4.1.1.1 Parasitoids - *Carcelia illota* Curran (Tachinidae: Diptera)

Medium sized, black flies characterized by well-developed hypoplueral and ptero plueral bristles and short and long bristles covering the abdomen (Plate 1.). The compound eyes were separated by two white stripes on either side of a median black

Table 1. Occurrence of natural enemies of *Helicoverpa armigera* in tomato ecosystem during Oct. 2002- Mar. 2004

Month	Total no. of larvae/ 10 plants	Number of Natural enemies			Name of the Natural enemies
		Parasites	Predators	Pathogens	
Oct - 2002	67	0.0	0.0	3.0	*Nosema* sp.
Nov - 2002	41	0.0	0.0	2.0	*Nosema* sp.
Dec - 2002	Off season	-	-	-	-
Jan - 2003	17	0.0	2.0	0.0	*Chrysoperla* adults
Feb - 2003	26	0.0	2.0	0.0	*Chrysoperla* eggs
Mar - 2003	13	4.0	0.0	0.0	*Carcelia illota*
Apr - 2003	48	4.0	0.0	1.0	*C. illota & Nomuraea rileyi*
May - 2003	21	6.0	0.0	1.0	-do-
Jun - 2003	14	0.0	0.0	0.0	Nil
Jul - 2003	0	0.0	0.0	0.0	Nil
Aug - 2003	0	0.0	0.0	0.0	Nil
Sep - 2003	0	0.0	0.0	0.0	Nil
Oct - 2003	15	0.0	0.0	0.0	Nil
Nov - 2003	21	2.0	0.0	0.0	*Carcelia illota*
Dec - 2003	13	3.0	0.0	0.0	-do-
Jan - 2004	15	2.0	0.0	0.0	-do-
Feb - 2004	24	1.0	0.0	0.0	-do-
Mar - 2004	16	2.0	0.0	0.0	-do-

stripe that cover the ocellar and frontal areas of the head. It is a larval pupal parasitoid and was collected from field-collected larva of *H. armigera*. The maggot of the parasitoid fed on the internal contents. When the parasitic maggot completed its development, it killed the host larva/pupa and came out within a couple of minutes. The maggots were observed to move around for about one to one and half hours before changing into pupation. Fresh puparia were soft and white or cream yellow in colour, which gradually become hard and brown within few hours.

This tachinid was found to parasitise late instars of *H.armigera*. The number of *C. illota* emerged from *H.armigera* larva and pupa during the survey period was 24. Only single parasitoid emerged from each larva / pupa. The percentage parasitisation was 6.18.

4.1.1.2 Predators

4.1.1.2.1 Spiders

The spiders found to be predating on young larvae of *H.armigera* are given in Table 2. Out of nine species (Plate 3.) observed *Oxyopes sunandae* Tikader was present throughout the season. The maximum number of spiders belonged to the *O. sunandae* (75/10 plants) followed by *O. shweta* Tikader (40/ 10 plants). These spiders tracked and killed the larvae but did not consume them. Even in the laboratory under confinement, the spiders only paralysed and killed the larvae with a single stinging, but did not devour them. The other species of spiders recorded more frequently in tomato ecosystem were *Cyrtophora citricola* Forskar, *Neoscona mukherji* Tikader and *Lycosa* sp. The spider species namely, *Pardosa lugerbris* Linnaeus, *Plexipus paykulli* Tikader, *Theridion* sp., and *C. feae* Thorell were observed only in few numbers during the survey period.

Table 2. Occurrence of predatory spiders in tomato ecosystem during Oct. 2002 - Mar. 2004

Month	Number of spiders per 10 plants								
	Cyrtophora citricola	*Cyrtophora feae*	*Lycosa* sp.	*Neoscona mukherji*	*Oxyopes sunandae*	*Oxyopes shweta*	*Pardosa lubgerbris*	*Plexippus paykullii*	*Theridion* sp.
Oct - 2002	5	0	2	0	7	3	0	0	0
Nov - 2002	2	2	3	0	6	2	0	0	2
Dec - 2002	0	0	0	0	4	7	0	0	0
Jan - 2003	2	0	2	0	2	0	3	2	0
Feb - 2003	3	0	1	0	3	2	0	1	0
Mar - 2003	3	0	1	0	3	0	2	0	1
Apr - 2003	0	0	2	0	1	3	0	0	0
May - 2003	0	0	2	2	3	4	0	0	0
Jun - 2003	3	0	0	3	2	1	2	2	1
Jul - 2003	0	0	1	6	4	0	0	0	0
Aug - 2003	0	1	1	5	10	2	0	0	0
Sep - 2003	2	0	0	2	7	2	2	0	2
Oct - 2003	2	0	3	0	3	3	0	0	0
Nov - 2003	0	1	2	0	6	5	0	0	0
Dec - 2003	3	0	1	0	4	2	0	0	0
Jan - 2004	0	0	0	0	3	0	0	2	0
Feb - 2004	3	0	0	0	3	4	0	1	0
Mar - 2004	0	0	0	0	4	0	0	0	0
Total	28	4	21	18	75	40	9	8	6

4.1.1.2.2 Chrysoperla carnea Stephens (Chrysopidae: Neuroptera)

Adults and pedicellate eggs were observed in tomato ecosystem. The eggs were laid in silken stalks of about one to one and half cm in length. They were arranged in groups (30-32 eggs) on tomato fruits (Plate 2.). The larva fed on the eggs and neonate larva of *H. armigera.*

4.1.1.3 Pathogens

4.1.1.3.1 Fungus - *Nomuraea rileyi* Samson

The diseased *H. armigera* larvae found clinging to the leaf were brought to the laboratory and kept on moist filter paper in petridishes for fungal growth and sporulation. Initially, the fungal growth was noticed on the intersegmental region of the body and later the entire body surface was covered with white mycelial growth. At the final phase of growth the diseased larvae were characterized by light dirty green sporulation.

The fungus from *H. armigera* was isolated into pure culture on SMA+Y. Colonies were convex, shining, initially white and later turning light green with profuse sporulation. Slide cultures were prepared and viewed under microscope. The fungus showed the following morphological characters: mycelium sub- hyaline, septate and highly branched. Conidiophores bearing dense whorls of branches and phialides. Conidia broadly ellipsoidal to cylindrical smooth and pale green measuring 3.0 to 4.5 µm (Plate 4.).

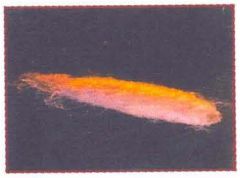
Based on the cultural and morphological characters it was identified as *N. rileyi*. Koch's postulate was proved on third instar larvae of *H.armigera*. The fungus was re-isolated and was used for further study.

Plate 1. PARASITOIDS ON *Helicoverpa armigera*

Apanteles taragamae (Braconidae)

ADULT

PUPA



Carcelia illota (Tachinidae)

Larva with parasitoid pupa

Host larva after emergence of parasitoid pupa

ADULT

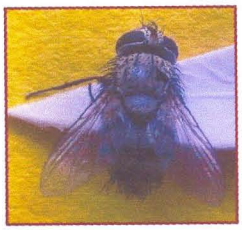
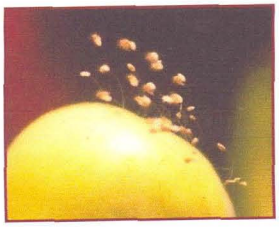


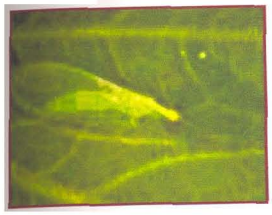
Plate 2. PREDATORS ON *Helicoverpa armigera*

Chrysoperla carnea (Chrysopidae)

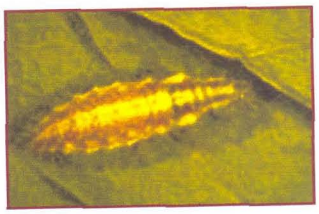
EGG



ADULT



LARVA



Cheilomenus sexmaculatus (Coccinellidae)

ADULT

GRUB

The fungus *N. rileyi* infection was rare under tomato ecosystem. Only two diseased larvae were collected from this ecosystem.

4.1.1.3.2 Protozoa - Nosema sp.

Nosema infection was observed in the field-collected larvae. The larvae were found dead or moribund. Whitish discharge from rectum and mouth of the dead larvae was seen. In some cases the larvae were found inactive, sluggish in movement and exhibited loss of appetite. This resulted in malformed pupae and adults or adults with reduced vigour, fecundity, longevity and locking of copulating organ. Spores of *Nosema* were ovoid, with one pole more pointed than the other. The artificial inoculation of the *Nosema* sp. on the larvae of *H.armigera* did not give any positive results.

The disease incidence was observed in the field–collected larvae during October and December 2002 period. The protozoan infection was not severe in *H.armigera* in tomato ecosystem.

4.1.2 Occurrence of *H. armigera* and its natural enemies in bhendi ecosystem

The results relating to these studies are presented in Table 3. The maximum number of *H.armigera* larvae was observed during Nov- 02 (62) followed by Feb - 03 where the population per ten plants was 54. The number of larvae per ten plants was found to be less during the same period in the next year. The population of *H.armigera* was least during Oct-03 and the population recorded was seven per ten plants. The population was nil during Jul- Sep'03 period. The other months of the survey period recorded only 13 to 31 larvae per ten plants. The population was nil

Table 3. Occurrence of natural enemies of *Helicoverpa armigera* in bhendi ecosystem during Oct. 2002- Mar. 2004

Months	Total no. of larvae/ 10 plants	Number of Natural enemies			Name of the Natural enemies
		Parasites	Predators	Pathogens	
Oct - 2002	24	0.0	0.0	0.0	-
Nov - 2002	62	0.0	7.0	0.0	*Cheilomenes sexmaculata*
Dec - 2002	22	0.0	9.0	0.0	*Cheilomenes sexmaculata*
Jan - 2003	31	2.0	5.0	16.0	*C. illota, C. sexmaculata & Nosema* sp.
Feb - 2003	54	6.0	8.0	21.0	-do-
Mar - 2003	26	3.0	12.0	7.0	-do-
Apr - 2003	Off season	-	-	-	-
May - 2003	-do-	-	-	-	-
Jun - 2003	-do-	-	-	-	-
Jul - 2003	0	0.0	0.0	0.0	Nil
Aug - 2003	0	0.0	0.0	0.0	Nil
Sep - 2003	0	0.0	0.0	0.0	Nil
Oct - 2003	7	0.0	0.0	0.0	Nil
Nov - 2003	13	0.0	2.0	0.0	*Cheilomenes sexmaculata*
Dec - 2003	16	0.0	3.0	0.0	-do-
Jan - 2004	19	0.0	4.0	0.0	-do-
Feb - 2004	25	4.0	5.0	0.0	*C. illota & C. sexmaculata*
Mar - 2004	21	2.0	6.0	0.0	-do-

during the other seasons. The natural enemies recorded in this ecosystem are given below.

4.1.2.1 Parasitoids – C. illota

The parasitoid recorded in this ecosystem was same as tomato ecosystem. The description and the characteristic features were detailed in Para 4.1.1.1. The maximum number of tachinid parasitoids (11) was recorded from field collected *H.armigera* larvae during the period Jan - Feb'03 followed by Feb - Mar'04 (six). The occurrence of *C. illota* from field-collected larvae was not noticed in the other months.

4.1.2.2 Predators

4.1.2.2.1 Coccinellids

Coccinellid recorded in bhendi ecosystem was *Cheilomenes sexmaculata.* The grubs black in colour fed on the eggs of *H.armigera* and young larvae. The beetles were yellowish orange, oval shaped with three wavy markings on each elytra and with the pronotum yellow with median half moon shaped marking connected to posterior marginal stripe. The maximum population of *C. sexmaculata* was recorded during Mar '03 and the number of grubs per 10 plants was 12. The population was least during Nov '03 and the number recorded was two.

4.1.2.2.2 Spiders

In bhendi, seven species of spiders were recorded. The observations are presented in the Table 4.The most predominant species of spider were *O. sunandae and O. shweta.* The other species of spider recorded were *C. citricola, Lycosa* sp., *N.mukherji, N. nautica* and *N. elliptica* (Plate 3.).

Table 4. Occurrence of predatory spiders in bhendi ecosystem during Oct. 2002 - Mar. 2004

Month	Number of spiders per 10 plants						
	Cyrtophora citricola	*Lycosa* sp.	*Neoscona mukherji*	*Neoscona nautica*	*Neoscona elliptica*	*Oxyopes sunandae*	*Oxyopes shweta*
Oct - 2002	3	2	0	1	0	2	3
Nov - 2002	2	3	0	3	0	6	2
Dec - 2002	0	0	0	0	0	4	3
Jan - 2003	2	2	0	0	2	2	0
Feb - 2003	1	1	1	1	1	3	2
Mar - 2003	3	1	0	1	1	3	0
Apr - 2003	0	2	0	0	0	1	3
May - 2003	0	2	2	0	1	3	4
Jun - 2003	0	0	3	0	1	2	1
Jul - 2003	0	1	0	1	0	1	0
Aug - 2003	0	1	5	2	0	3	2
Sep - 2003	0	0	2	1	0	7	2
Oct - 2003	2	3	0	0	3	3	3
Nov - 2003	0	2	0	0	0	1	1
Dec - 2003	1	1	0	0	0	2	2
Jan - 2004	0	0	1	0	0	3	0
Feb - 2004	3	0	0	0	2	3	2
Mar - 2004	0	0	0	0	1	4	1
Total	17	21	14	10	12	53	31

4.1.2.3 Pathogens - Nosema sp.

The disease incidence was observed during Jan- Mar '03 period. The number of larvae infected with the *Nosema* sp. during this period was 44.The detailed characteristic features were mentioned in 4.1.1.3.2. There was no *Nosema* infection in the other months of survey period.

4.1.3 Occurrence of *H. armigera* and its natural enemies in bittergourd ecosystem

The results are presented in Table 5. The early instars of *H.armigera* larvae were found feeding on the flower buds. Later instars were found boring the fruits. The maximum population was recorded during Dec '02 period and the number of larvae per ten plants was 15. The population of *H. armigera* was least during Oct - 03 and the number of larvae observed were only two. The occurrence of *H.armigera* in the other months of the study ranged from three to ten per ten plants. The population of *H. armigera* was absent during Jul – Sept '03. Parasitoid recorded in this ecosystem is given below.

4.1.3.1 *Apanteles taragamae* Wilk. (Braconidae)

It is a solitary endoparasitoid parasitising the early instars of *H. armigera*. These are small, black hymenopterans. The field-collected larva was very sluggish. The movement of the parasitoid larva inside the host could be seen. The larval parasitoid completed its development inside the host larva. When the growth was completed it came out of the host's body and started spinning cocoon and pupated outside the host. The cocoons were white, elongated and cylindrical in shape. The adult emerged on the ninth day of pupation (Plate 1.).

Table 5. Occurrence of natural enemies of *Helicoverpa armigera* in bittergourd ecosystem during Oct. 2002- Mar. 2004

Months	Total no. of larvae/ 10 plants	Number of Natural enemies			Name of the Natural enemies
		Parasites	Predators	Pathogens	
Oct - 2002	3	0.0	0.0	0.0	Nil
Nov - 2002	10	0.0	0.0	0.0	Nil
Dec - 2002	15	0.0	0.0	0.0	Nil
Jan - 2003	7	1.0	3.0	0.0	*Apanteles taragamae* & *Cheilomenes sexmaculata*
Feb - 2003	10	0.0	2.0	0.0	*Cheilomenes sexmaculata*
Mar - 2003	7	1.0	0.0	0.0	*Apanteles taragamae*
Apr - 2003	9	0.0	0.0	0.0	Nil
May - 2003	Off season	-	-	-	-
Jun - 2003	-do-	-	-	-	-
Jul - 2003	0	0.0	0.0	0.0	Nil
Aug - 2003	0	0.0	0.0	0.0	Nil
Sep - 2003	0	0.0	0.0	0.0	Nil
Oct - 2003	2	0.0	0.0	0.0	Nil
Nov - 2003	6	0.0	0.0	0.0	Nil
Dec - 2003	9	0.0	0.0	0.0	Nil
Jan - 2004	3	0.0	0.0	0.0	Nil
Feb - 2004	8	0.0	0.0	0.0	Nil
Mar - 2004	0	0.0	0.0	0.0	Nil

Only two hymenopteran parasitoids emerged from field-collected larvae. This was not a major parasitoid and the incidence was rare.

4.1.3.2 Coccinellid - Cheilomenes sexmaculata

The detailed characteristic features were mentioned in 4.1.1.3.2. The grubs fed on the eggs and young larvae of *H.armigera.*

4.1.4 Occurrence of *H. armigera* and its natural enemies in cowpea ecosystem

The occurrence of *H.armigera* as pod borer in cowpea ecosystem was observed during Oct – Nov '02, Jan – Mar '03 and Jan – Feb '04. The maximum population of nine larvae was recorded during Nov '02. The larvae of *H. armigera* were absent during the other months of study. There was no incidence of parasitoids and pathogens from field-collected larvae. Few grubs of the coccinellid *C. sexmaculata* were recorded during this period.

Among the four vegetable ecosystems observed for occurrence of *H. armigera* and its natural enemies, tomato recorded the maximum number (388) of *H. armigera* larvae (Table7.). This was followed by bhendi ecosystem (320). Bittergourd and cowpea ecosystems recorded 89 and 33 number of larvae per ten plants throughout the survey period. Regarding the per cent parasitism, the highest was recorded in tomato (6.18 per cent). The maximum percentage of predation was noticed in cowpea ecosystem (36.60 per cent). Bhendi ecosystem recorded 13.75 per cent infection by pathogens.

Table 6. Occurrence of natural enemies of *Helicoverpa armigera* in cowpea ecosystem during Oct. 2002- Mar. 2004

Months	Total no. of larvae/ 10 plants	Number of Natural enemies			Name of the Natural enemies
		Parasites	Predators	Pathogens	
Oct - 2002	7	0.0	0.0	0.0	Nil
Nov - 2002	9	0.0	0.0	0.0	Nil
Dec - 2002	0	0.0	0.0	0.0	Nil
Jan - 2003	4	0.0	4.0	0.0	*Cheilomenus sexmaculata*
Feb - 2003	1	0.0	8.0	0.0	-do-
Mar - 2003	3	0.0	0.0	0.0	Nil
Apr - 2003	Off season	-	-	-	-
May - 2003	-do-	-	-	-	-
Jun - 2003	0	0.0	0.0	0.0	Nil
Jul - 2003	0	0.0	0.0	0.0	Nil
Aug - 2003	0	0.0	0.0	0.0	Nil
Sep - 2003	0	0.0	0.0	0.0	Nil
Oct - 2003	0	0.0	0.0	0.0	Nil
Nov - 2003	0	0.0	0.0	0.0	Nil
Dec - 2003	2	0.0	0.0	0.0	Nil
Jan - 2004	3	0.0	0.0	0.0	Nil
Feb - 2004	2	0.0	0.0	0.0	Nil
Mar - 2004	Off season	-	-	-	-

Table 7. Occurrence of *Helicoverpa armigera* and its natural enemies in vegetable ecosystem during 2002-2004

Crops	Total number of larva /plant				Percentage of		
	H.armigera	Parasitoids	Predators	Pathogen	Parasitoids	Predator	Pathoger
Tomato	388	24[#]	4[$]	6*+2[@]	6.18	1.03	2.06
Bhendi	320	17[#]	69[β]	44*	5.30	5.31	13.75
Cowpea	33	0	12[β]	0	0.00	36.60	0.00
Bittergourd	89	2[€]	5[β]	0	2.24	5.61	0.00

- *Carcelia sp,* $ - *Chrysoperla sp,* * - *Nosema sp,* [@] - *Nomuraea rileyi,* [€] - *Apanteles taragamae,* [β] – Coccinellids – *C.sexmaculatus*

Plate 3. Spiders of Vegetable Ecosystem

Neoscona mukherjei

Neoscona elliptica

Neoscona nautica

Oxyopus sunandae

Oxyopus shweta

Plexippus paykulli

Cyrtophora citricola

Cyrtophora feae

Phidippins sp

Pardosa lugerbris

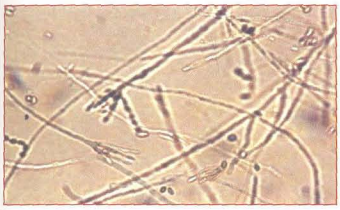
Lycosa tista

Plate 4. Pahogens

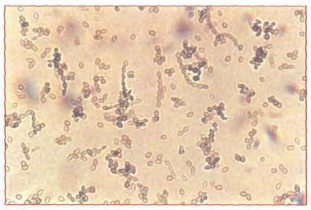
Fungus : *Nomuraea rileyi*

N. rileyi infected larva

N. rileyi mycelium

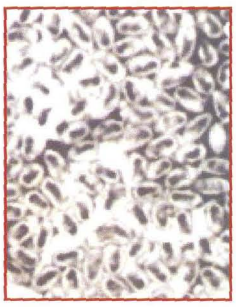


N.rileyi spores



Protozoa : *Nosema* sp

Spores of *Nosema*



4.2 SCREENING OF BOTANICALS AGAINST THIRD INSTAR LARVAE OF *H.armigera*

4.2.1 Plant extracts

The plant extracts *viz., A. calamus, A. paniculata and V. negundo* at one, two and half and five per cent concentrations were evaluated against third instar larvae of *H.armigera.* The results of this experiment (Table 8.) indicated that 96 h after treatment, *V. negundo* at five per cent concentration recorded the highest mortality per cent of 39.27 and was significantly superior to other treatments. The per cent mortality observed in *A. calamus* and *A. paniculata* was 32.14 and 28.56 respectively. Both the treatments were on par with each other. At 2.5 per cent concentration, the larval mortality percentage observed in *V. negundo, A. calamus* and *A. paniculata* were 24.99, 21.42 and 17.85 respectively. Plant extracts showed a range of 3.56 to 14.28 per cent mortality at one per cent strength.

At 72 h after treatment, the maximum mortality of 32.14 per cent was recorded in *V. negundo* (Five per cent) and it was significantly superior to other treatments. The lowest mortality per cent of 3.56 per cent was observed in *A. calamus* (one per cent). Other plant extracts induced less than 25 per cent mortality only.

4.2.2 Essential oils

Mortality per cent of *H. armigera* larvae treated with essential oils are presented in Table 9. The essential oils (*C. winterianus, C. flexuosus, K. galanga* and *C. martini*) were evaluated at different concentrations *viz.*, one, two and half and five per cent.

Table 8.Efficacy of different plant extracts against third instar larvae of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Corrected per cent mortality	
			72 h	96 h
1.	*Acorus calamus*	1.0	3.56 (0.190)	3.56 (0.190)
		2.5	21.42 (0.481)	21.42 (0.481)
		5.0	24.99 (0.523)	32.14 (0.603)
2.	*Andrographis paniculata*	1.0	10.71 (0.333)	10.71 (0.333)
		2.5	14.28 (0.387)	17.85 (0.436)
		5.0	24.99 (0.523)	28.56 (0.564)
3.	*Vitex negundo*	1.0	10.71 (0.333)	14.28 (0.387)
		2.5	17.85 (0.436)	24.99 (0.523)
		5.0	32.14 (0.603)	39.27 (0.677)
	CD (5 %)	-	0.04	0.05

Figures in the parenthesis indicate arc-sine transformed value

At one per cent concentration, the highest mortality of 32.14 per cent was recorded in *C. martini* and *K. galanga* whereas, *C. flexuosus* and *C. winterianus* recorded 28.56 and 24.99 per cent respectively at 96 h after treatment. The mortality per cent varied from 17.85 to 28.56 at 72 h after treatment.

Treatment with *C. flexuosus, C. martini, K. galanga* and *C. winterianus* induced 46.42, 42.85, 39.27 and 32.14 per cent larval mortality respectively at 2.5 per cent level at 96 h after treatment. The larval mortality percentage observed in *C. flexuosus, K. galanga* and *C. martini* at 72 h after treatment were 35.71,32.14 and 39.27 respectively. The lowest larval mortality of 24.99 per cent was recorded in *C. winterianus.*

At five per cent concentration, *C. martini* and *C. flexuosus* treatments were on par and recorded 71.42 and 67.85 per cent larval mortality respectively at 96 h after treatment. *K. galanga* and *C. winterianus* treatments gave 56.67 and 46.42 per cent larval mortality respectively. *C. martini* caused highest mortality percentage of 67.85 and was significantly superior to other treatments at 72 h after treatment. Oils of *C. flexuosus, K. galanga* and *C. winterianus* recorded 60.70, 46.42 and 42.85 per cent larval mortality at the above concentration.

All the plant extracts and essential oils induced higher larval mortality at five per cent concentration, which was the highest level tested.

Table 9.Efficacy of different essential oils against third instar larvae of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Corrected per cent mortality	
			72 h	96 h
1.	*Citronella winterianus*	1.0	17.85 (0.436)	24.99 (0.523)
		2.5	24.99 (0.523)	32.14 (0.603)
		5.0	42.85 (0.714)	46.42 (0.750)
2.	*Kaempferia galanga*	1.0	21.42 (0.481)	32.14 (0.602)
		2.5	32.14 (0.603)	39.27 (0.677)
		5.0	46.42 (0.750)	56.67 (0.852)
3.	*Cymbopogon flexuosus*	1.0	28.56 (0.564)	28.56 (0.564)
		2.5	35.71 (0.640)	46.42 (0.750)
		5.0	60.70 (0.893)	67.85 (0.968)
4.	*Cymbopogon martini*	1.0	24.99 (0.523)	32.14 (0.602)
		2.5	39.27 (0.677)	42.85 (0.714)
		5.0	67.85 (0.968)	71.42 (1.007)
	CD (5%)	-	0.02	0.04

Figures in the parenthesis indicate arc-sine transformed value

4.3 COMPATIBILITY OF BIOCIDES AND PESTICIDES

4.3.2 Compatibility of entomopathogenic fungi with botanicals and insecticides

In vitro evaluation was carried out to know the compatibility of the fungus with selected insecticides and botanicals. Per cent inhibition on fungal growth due to the effect of these insecticides and botanicals was recorded.

4.3.2.1 Compatibility of Nomuraea rileyi with botanicals and insecticides

4.3.2.1.1 Insecticides

Among the insecticides tested, spinosad recorded the maximum compatibility with the fungus (Table 10.). Here the fungal growth started on the very first Day After Inoculation (DAI) and recorded a colony diameter of 81 mm on 8 DAI, which was on par with the growth recorded in control (82mm). In this case the inhibition of growth over control was 1.2 per cent (Table 10 a.), which was on par with control. The radial growth of the fungus in medium incorporated with imidacloprid, triazophos and carbosulfan was also found statistically on par with spinosad at 8 DAI. The least radial growth was observed in profenofos (39mm), which recorded maximum per cent inhibition of growth (52.40) over control. The insecticides triazophos and profenofos completely inhibited the sporulation of the fungus. The decreasing order of compatibility of the fungus with selected insecticides was spinosad, imidacloprid, triazophos, carbosulfan and profenofos.

4.3.2.1.2 Botanicals

The essential oils *viz.*, *C. winterianus, C. flexuosus, K. galanga* and *C. martini* at five per cent were found to completely inhibit the growth of the fungus (Table 11.). There was no growth of the fungus in the media treated with these

Table 10.*In vitro* evaluation on compatibility of *Nomuraea rileyi* with insecticides

Sl. No	Insecticides	Mean Colony Diameter (mm) DAI							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1	Carbosulfan (0.05%)	8	16	25	32	41	52	63	71
2	Imidacloprid (0.04 %)	7	18	29	43	52	61	70	80
3	Profenofos (0.10 %)	4	9	13	19	23	27	33	39*
4	Spinosad (0.02 %)	11	20	30	46	53	64	70	81
5	Triazophos (0.10 %)	8	19	25	37	43	55	66	79*
6	Control	10	21	31	48	55	66	71	82
7	CD (5%)	3.0	3.8	1.8	3.1	2.8	2.2	2.5	2.2

* - No sporulation
DAI – Days After Inoculation

Table 10a. Per cent inhibition of growth of *Nomuraea rileyi* with insecticides

Sl.no	Insecticides	Per cent inhibition of mycelial growth over control
1	Carbosulfan (0.05%)	13.4 (0.375)
2	Imidacloprid (0.04 %)	2.4 (0.155)
3	Profenofos (0.10 %)	52.4 (0.809)
4	Spinosad (0.02 %)	1.2 (0.110)
5	Triazophos (0.10 %)	3.7 (0.193)
6	CD (5%)	0.03

Figures in the parenthesis indicate arc-sine transformed value

Table 11. *In vitro* evaluation on compatibility of *Nomuraea rileyi* with botanicals

Sr No	Botanicals	Mean Colony Diameter (mm) DAI							
		1st	2nd	3rd	4th	5th	6th	7th	8th
1	*Acorus calamus* (5 %)	6	10	17	21	24	26	30	33
2	*Andrographis paniculata* (5 %)	7	11	17	23	29	33	39	48
3	*Vitex negundo* (5 %)	8	14	20	28	36	49	58	61
4	*Citronella winterianus* (5 %)	0	0	0	0	0	0	0	0
5	*Kaempferia galanga* (5 %)	0	0	0	0	0	0	0	0
6	*Cymbopogon flexuosus* (5 %)	0	0	0	0	0	0	0	0
7	*Cymbopogon martini* (5 %)	0	0	0	0	0	0	0	0
8	Control	11	19	30	47	54	65	72	80
	CD (5%)	4.4	3.3	5.0	4.0	5.0	3.3	3.8	5.4

DAI – Days After Inoculation

Table 11a. Per cent inhibition of growth of *Nomuraea rileyi* by botanicals

Sl.no	Botanicals	Per cent inhibition of mycelial growth over control
1	*Acorus calamus* (5 %)	58.8 (0.874)
2	*Andrographis paniculata* (5 %)	40.0 (0.685)
3	*Vitex negundo* (5 %)	23.8 (0.510)
4	*Citronella winterianus* (5 %)	100.0
5	*Kaempferia galanga* (5 %)	100.0
6	*Cymbopogon flexuosus* (5 %)	100.0
7	*Cymbopogon martini* (5 %)	100.0
	CD (5%)	0.10

Figures in the parenthesis indicate arc-sine transformed value

essential oils throughout the incubation period and resulted in cent per cent inhibition over the growth in control (Table 11 a.). The inhibition by *A. calamus* (58.80 per cent) was significantly different from that of *A. paniculata* (40.0 per cent), which resulted in a mean colony diameter of 33 mm and 48 mm respectively at 8 DAI. The mean colony diameter in *V. negundo* at 8 DAI was 61 mm, which was superior to the growth noted in other botanicals except control. It recorded the lowest per cent of inhibition over control (23.8).

4.3.1.2 Compatibility of Beauveria bassiana with insecticides and botanicals

4.3.1.2.1 Insecticides

The results on compatibility of *B. bassiana* with insecticides are presented in Table 12. The growth of the fungus in the medium incorporated with spinosad recorded the maximum mean colony diameter of 25 mm, followed by imidacloprid where the mean colony diameter was 24 mm. Both the treatments were on par with each other but significantly different from control (30mm). The radial growth of fungus recorded in the medium incorporated with profenofos and triazophos were 14 and 13 mm respectively. They recorded the per cent inhibition of growth 53.3 and 56.7 respectively (Table 12a). The least radial growth was observed in carbosulfan (12 mm), which recorded the maximum per cent inhibition of growth (60.0) over control.

4.3.1.2.2 Botanicals

The results given in Table 13 revealed that the medium incorporated with *A. paniculata* recorded the maximum growth of *B. bassiana* 15 mm among the botanicals. At 18 DAI, this treatment recorded 51.6 per cent inhibition of growth over control (Table 13a). The mean colony diameters recorded in *V. negundo* and *A.*

Table 12. *In vitro* evaluation on compatibility of *Beauveria bassiana* with insecticides

Sl. No	Insecticides	Mean Colony Diameter (mm) DAI					
		3rd	6th	9th	12th	15th	18th
1	Carbosulfan (0.05%)	1	3	5	7	10	12
2	Imidacloprid (0.04 %)	4	8	10	13	20	24
3	Profenofos (0.10 %)	1	4	6	8	10	14
4	Spinosad (0.02 %)	2	7	10	14	19	25
5	Triazophos (0.10 %)	2	4	6	8	10	13
6	Control	5	8	14	19	26	30
	CD (5%)	1.3	1.8	1.6	1.8	2.0	2.2

DAI – Days After Inoculation

Table 12a. Per cent inhibition of growth of *Beauveria bassiana* by insecticides

Sl.no	Insecticides	Per cent inhibition of mycelial growth over control
1	Carbosulfan (0.05%)	60.0 (0.886)
2	Imidacloprid (0.04 %)	16.8 (0.422)
3	Profenofos (0.10 %)	53.3 (0.818)
4	Spinosad (0.02 %)	16.6 (0.420)
5	Triazophos (0.10 %)	56.7 (0.852)
	CD (5%)	0.08

Figures in the parenthesis indicate arc-sine transformed value

Table 13. *In vitro* evaluation on compatibility of *Beauveria bassiana* with botanicals

Sr No	Botanicals	Mean Colony Diameter (mm) DAI					
		3rd	6th	9th	12th	15th	18th
1	*Acorus calamus* (5 %)	1	3	5	7	9	10
2	*Andrographis paniculata* (5 %)	2	4	6	9	11	15
3	*Vitex negundo* (5 %)	1	2	4	6	9	12
4	*Citronella winterianus* (5 %)	0	0	0	0	0	0
5	*Kaempferia galanga* (5 %)	0	0	0	0	0	0
6	*Cymbopogon flexuosus* (5 %)	0	0	0	0	0	0
7	*Cymbopogon martini* (5 %)	0	0	0	0	0	0
8	Control	4	9	14	18	25	31
	CD (5%)	0.9	1.3	1.6	2.4	1.6	1.9

DAI – Days After Inoculation

Table 13a. Per cent inhibition of growth of *Beauveria bassiana* by botanicals

Sr.no	Botanicals	Per cent inhibition of mycelial growth over control
1	*Acorus calamus* (5 %)	67.7 (0.966)
2	*Andrographis paniculata* (5 %)	51.6 (0.789)
3	*Vitex negundo* (5 %)	61.3 (0.900)
4	*Citronella winterianus* (5 %)	100.0
5	*Kaempferia galanga* (5 %)	100.0
6	*Cymbopogon flexuosus* (5 %)	100.0
7	*Cymbopogon martini* (5 %)	100.0
	CD (5%)	0.05

Figures in the parenthesis indicate arc-sine transformed value

calamus were 12 and 10 mm at 18 DAI and the percentages of growth inhibition observed were 61.30 and 67.70 respectively. So among the plant extracts, *A. calamus* recorded the maximum per cent inhibition of growth. The essential oils *viz.*, *C. winterianus, C. flexuosus, K. galanga* and *C. martinii*, completely inhibited the growth of white muscardine fungus *B. bassiana* and recorded cent per cent inhibition over control.

4.3.1.3 Compatibility of Metarhizium anisopliae with insecticides and botanicals

4.3.1.3.1 Insecticides

Table 14 reveals that, out of the five insecticides; imidacloprid recorded the maximum compatibility with the fungus *M. anisopliae*. The fungus recorded a mean colony diameter of 26 mm, which was significantly different from control where the colony diameter was 41 mm. The radial growth of fungus in the medium incorporated with spinosad (25mm) was statistically on par with imidacloprid. The per cent inhibition recorded in imidacloprid and spinosad were 36.6 and 38.3 respectively (Table 14 a.). The mean colony diameter recorded in carbosulfan and triazophos were 21 and 16 mm respectively. The per cent inhibition of growth over control in carbosulfan and triazophos were 48.80 and 60.90 respectively. The least radial growth was observed in profenofos 14 mm, which recorded the maximum per cent growth inhibition of 65.80.

4.3.1.3.2 Botanicals

From the Table 15, it was evident that the essential oils *viz., C. winterianus, C. flexuosus, K. galanga* and *C. martini* completely inhibited the growth of the fungus. There was no growth of the fungus *M. anisopliae* in the media treated with these essential oils throughout the incubation period and resulted in cent per cent

Table 14. *In vitro* evaluation on compatibility of *Metarhizium anisopliae* with insecticides

Sl. No	Insecticides	Mean Colony Diameter (mm) DAI					
		3rd	6th	9th	12th	15th	18th
1	Carbosulfan (0.05%)	3	8	11	15	18	21
2	Imidacloprid (0.04 %)	4	10	15	19	23	26
3	Profenofos (0.10 %)	1	5	8	9	12	14
4	Spinosad (0.02 %)	4	9	13	16	22	25
5	Triazophos (0.10 %)	4	6	8	11	13	16
6	Control	8	15	21	31	35	41
	CD	1.3	1.6	1.6	1.8	2.2	1.8

DAI – Days After Inoculation

Table 14a. Per cent inhibition of growth of *Metarhizium anisopliae* by insecticides

Sr. no	Insecticides	Per cent inhibition of mycelial growth over control
1	Carbosulfan (0.05%)	48.8 (0.773)
2	Imidacloprid (0.04 %)	36.6 (0.650)
3	Profenofos (0.10 %)	65.8 (0.946)
4	Spinosad (0.02 %)	38.3 (0.683)
5	Triazophos (0.10 %)	60.9 (0.895)
	CD (5%)	(0.05)

Figures in the parenthesis indicate arc-sine transformed value

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Table 15. *In vitro* evaluation on compatibility of *Metarhizium anisopliae* with botanicals

Sr No	Botanicals	Mean Colony Diameter (mm) DAI					
		3rd	6th	9th	12th	15th	18th
1	*Acorus calamus* (5 %)	2	5	8	11	15	20
2	*Andrographis paniculata* (5 %)	1	3	5	8	15	19
3	*Vitex negundo* (5 %)	5	8	12	19	22	26
4	*Citronella winterianus* (5 %)	0	0	0	0	0	0
5	*Kaempferia galanga* (5 %)	0	0	0	0	0	0
6	*Cymbopogon flexuosus* (5 %)	0	0	0	0	0	0
7	*Cymbopogon martini* (5 %)	0	0	0	0	0	0
8	Control	5	12	18	29	34	42
	CD (5%)	0.9	1.3	2.1	1.9	2.3	1.9

DAI – Days After Inoculation

Table 15a. Per cent inhibition of growth of *Metarhizium anisopliae* by botanicals

Sl.no	Botanicals	Per cent inhibition of mycelial growth over control
1	*Acorus calamus* (5 %)	52.4 (0.809)
2	*Andrographis paniculata* (5 %)	54.7 (0.832)
3	*Vitex negundo* (5 %)	38.0 (0.664)
4	*Citronella winterianus* (5 %)	100.0
5	*Kaempferia galanga* (5 %)	100.0
6	*Cymbopogon flexuosus* (5 %)	100.0
7	*Cymbopogon martini* (5 %)	100.0
	CD (5%)	(0.10)

Figures in the parenthesis indicate arc-sine transformed value

inhibition over the growth in control (Table 15a.). The maximum mean colony diameter was recorded in *V. negundo* (26 mm), which was significantly different from *A. calamus* (20mm) and *A. paniculata* (19mm) at 18 DAI. The least per cent inhibition of growth over control was observed in *V. negundo* (38). The per cent inhibition by *A. calamus* (52.4) was on par with that by *A. paniculata* (54.7).

4.3.2 Compatibility of *Bacillus thuringiensis* with botanicals and insecticides

Different botanicals and insecticides were evaluated at different concentrations as described in 3.4 to know their compatibility with the commercial formulation of *B. thuringiensis* (Halt, Delfin and Dipel). The results are reported herein.

4.3.2.1 Insecticides

The inhibitory effect of insecticides, namely, carbosulfan, imidacloprid, profenofos, spinosad and triazophos on the growth and sporulation of *B. thuringiensis* was studied by poison food technique using nutrient agar medium. There was no inhibition zone developed around the filter paper impregnated with these insecticides (Plate 5.)

4.3.2.1 Botanicals

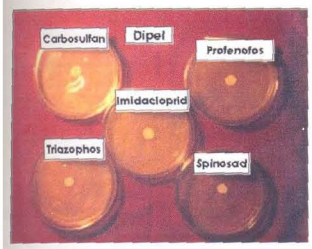
Botanicals *viz., A. calamus, A. paniculata, V. negundo, C. winterianus, C. flexuosus, K. galanga* and *C. martini* were evaluated to study the compatibility with *B. thuringiensis*. It was observed that all the three commercial formulations (Halt, Delfin and Dipel) proved to be compatible with botanicals, as they showed no inhibition zone around the filter paper disc impregnated with botanicals (Plate 5.).

Plate 5. Compatibility of *Bacillus thuringiensis* with botanicals and insecticides

Halt and Insecticides

Delfin and insecticides

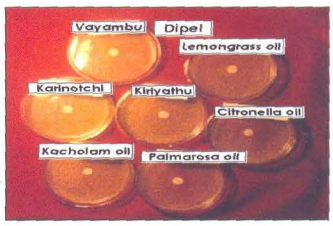
Dipel and insecticides



Halt and botanicals

Delfin and botanicals

Dipel and botanicals



4.4 JOINT ACTION OF BIOCIDES WITH PLANT EXTRACTS, ESSENTIAL OILS AND INSECTICIDES

4.4.1 Efficacy of entomopathogenic fungi with plant extracts and insecticides

4.4.1.1 *Nomuraea rileyi* and plant extracts

The efficacy of combinations of *N. rileyi* and plant extracts on third instar larvae of *H. armigera* is presented in Table 16. It could be seen that the *N. rileyi* and plant extract combinations gave mortalities from the first day onwards. The highest mortality of 76.6 per cent was recorded in the combination of *N. rileyi* + *V. negundo* and it was significantly superior to other treatments. This was followed by *N. rileyi* + *A. paniculata* (70.0 per cent) and *N. rileyi* + *A. calamus* (66.6 per cent). Both the treatments were on par with each other. The plant extracts alone recorded 30.0 to 40.0 per cent mortality only. The fungus *N. rileyi* recorded 60.0 per cent mortality at eighth day after treatment. There was steady increase in mortality from the fifth day onwards.

The LT_{50} for the combined treatments ranged from 5.1 to 6.3 days. In the case of plant extracts the mean time for larval death ranged from 8.5 to 10.1days. The fungus *N. rileyi* when used alone recorded an LT_{50} of 6.9 days.

The results are presented in Table 16 a. Supplemental synergism was noticed in all the *N. rileyi* and plant extract combinations. The mortality of larvae caused due to these set of treatments was 66.6, 70.0 and 76.6 per cent respectively. This was greater than the algebraic sum of the mortality caused individually by *N. rileyi* and plant extracts resulting 54.2, 52.1 and 56.1 per cent mortality. The combinations viz., *N. rileyi* + *A. calamus*, *N. rileyi* + *A. paniculata* and *N. rileyi* + *V. negundo* produced synergistic coefficient of 0.032, 0.039 and 0.047 showing lower order synergism.

Table 16. Efficacy of *Nomuraea rileyi* with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Nomuraea rileyi	$2x10^8$spores/ml	0.0	3.3	16.7	20.0	36.6	43.3	56.6	60.0	60.0[c]	6.9
Acorus calamus	5	3.3	13.3	20.0	23.3	26.6	30.0	33.3	33.3	33.3[e]	9.8
Andrographis paniculata	5	3.3	6.6	16.6	20.0	26.6	30.0	30.0	30.0	30.0[e]	10.1
Vitex negundo	5	6.6	9.9	16.6	23.3	30.0	36.6	40.0	40.0	40.0[d]	8.5
N. rileyi + *A. calamus*	$1x10^8$spores/ml + 2.5	13.3	20.0	30.0	33.3	43.3	56.6	56.6	66.6	66.6[b]	6.2
N. rileyi + *A. paniculata*	$1x10^8$spores/ml + 2.5	9.9	13.3	20.0	36.6	40.0	53.33	56.6	70.0	70.0[b]	6.3
N. rileyi + *V. negundo*	$1x10^8$spores/ml + 2.5	16.6	23.3	33.3	43.3	60.0	63.3	66.6	76.6	76.6[a]	5.1

* Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 16 a. Effect of the combination of *Nomuraea rileyi* and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co - efficient and effect
	Mp	Mf	Mf+p		
Acorus calamus	33.3 (21.4)	**60.0 (32.8)**	66.6	Supplemental synergism	0.032 (LOS)
Andrographis paniculata	30.0 (19.3)		70.0	Supplemental synergism	0.039 (LOS)
Vitex negundo	40.0 (23.3)		76.6	Supplemental synergism	0.047 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mf - Mortality per cent of fungus (*Nomuraea rileyi*)
M f+p - Mortality per cent of fungus + plant extracts

4.4.1.2 *Nomuraea rileyi* and insecticides

Data on the joint action of *N. rileyi* and sub lethal doses of insecticides as compared to their individual actions on the larvae of *H.armigera* are presented in Table 17. Mortality of larvae treated with *N. rileyi* + insecticides was observed from the first day of treatment. A steady increase in the mortality of the larvae under all the combinations was noticed from the second day onwards. The highest mortality per cent was recorded in *N. rileyi*+ spinosad treatment (96.6), which was significantly superior to other treatments. This was followed by spinosad alone, which recorded 90.0 per cent mortality. *N. rileyi* + triazophos, *N. rileyi* + carbosulfan and *N. rileyi* + profenofos recorded 76.6, 70.0 and 66.6 per cent mortality, respectively, and these are on par with each other. The per cent mortalities ranged from 63.3 to 90.0 in the treatments involving the different insecticides individually.

The lowest LT_{50} of 0.5 days was noticed when *N. rileyi* and spinosad were combined. The highest LT_{50} was observed in *N. rileyi* (7.5 days) followed by carbosulfan (5.9days). The drastic reduction in LT_{50} was observed in *N. rileyi* and insecticides mixture treatments (Table 17.).

The effect of combining *N. rileyi* with insecticides together with the categorization of their combined effects is presented in Table 17a. Supplemental synergism was observed when the larvae were exposed to *N. rileyi* and insecticides such as profenofos, spinosad and triazophos treatments. The per cent combined mortality due to the above treatments recorded was 66.6, 96.6 and 76.6 respectively, which were more than the algebraic sum of mortality caused individually by *N. rileyi* and insecticides resulting 66.2, 76.3 and 63.3 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.007, 0.027 and 0.009 exhibiting lower order synergism. The combination *N. rileyi* + carbosulfan produced sub

Table 17. Efficacy of *Nomuraea rileyi* with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Nomuraea rileyi	$2x10^8$spores/ml	3.3	16.6	20.0	23.3	23.3	33.3	50.0	56.6	56.6^{e}	7.5
Carbosulfan	0.05	20.0	26.6	33.3	46.6	50.0	60.0	63.3	63.3	63.3^{d}	5.9
Profenofos	0.10	26.6	33.3	46.6	56.6	66.6	66.6	70.0	70.0	70.0^{c}	4.2
Spinosad	0.02	43.3	50.0	80.0	83.3	86.6	90.0	90.0	90.0	90.0^{b}	1.0
Triazophos	0.10	33.3	53.3	63.3	63.3	66.6	66.6	66.6	66.6	66.6^{cd}	1.4
N. rileyi + carbosulfan	$1x10^8$spores/ml + 0.025	26.6	30.0	43.3	50.0	60.0	63.3	66.6	70.0	70.0^{c}	4.9
N. rileyi + profenofos	$1x10^8$spores/ml + 0.05	30.0	36.6	50.0	63.3	66.6	66.6	66.6	66.6	66.6^{cd}	3.7
N. rileyi + spinosad	$1x10^8$spores/ml + 0.01	50.0	60.0	83.3	90.0	96.6	96.6	96.6	96.6	96.6^{a}	0.5
N. rileyi + triazophos	$1x10^8$spores/ml + 0.05	40.0	56.6	60.0	63.3	66.6	66.6	73.3	76.6	76.6^{c}	0.8

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 17 a. Effect of the combination of *Nomuraea rileyi* and insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic Coefficient and effect
	Mi	Mf	Mf+i		
Carbosulfan	63.3 (38.0)	**56.6 (32.6)**	70.0	Sub additive synergism	-0.002 (SMS)
Profenofos	70.0 (33.6)		66.6	Supplemental synergism	0.007 (LOS)
Spinosad	90.0 (43.7)		96.6	Supplemental synergism	0.027 (LOS)
Triazophos	66.6 (30.7)		76.6	Supplemental synergism	0.009 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and insecticides

SMS – Sub Multiplicative Synergism
LOS – Lower Order Synergism

Mi – Mortality per cent of insecticide
Mf - Mortality per cent of fungus (*Nomuraea rileyi*)
M f+i - Mortality per cent of fungus + insecticide

additive synergism. The mortality of larvae caused due to this set of treatment was 70.0 per cent which was less than the algebraic sum of mortality caused individually by *N. rileyi* and carbosulfan (70.6 per cent), but it was greater than independent synergism (58.4 per cent). The combination *N. rileyi* + carbosulfan recorded synergistic co efficient of – 0.002 showing sub multiplicative synergism.

4.4.1.3 *Metarhizium anisopliae* and plant extracts

The data on mortality of third instar larvae of *H.armigera* caused by the combination of *M. anisopliae* and a lower concentration of plant extracts are presented in Table 18. The plant extracts alone caused only 3.3 to 13.3 per cent mortality on the first day of treatment. But it was between 20.0 and 30.0 per cent in combination on the same day of treatment. The highest corrected mortality of 62.0 per cent was observed in *M. anisopliae* + *V. negundo* treatment on the eighth day and was significantly superior to other combinations. The mortality per cent recorded in *M. anisopliae* + *A. paniculata* and *M. anisopliae* + *A. calamus* were 55.1 and 51.7 respectively. Both the treatments were on par with each other. The plant extracts caused 27.6 to 34.4 per cent mortality by them at eighth day after treatment. The fungus *M. anisopliae* recorded only 37.9 per cent mortality.

The lowest LT_{50} of 5.9 days was recorded in *M. anisopliae* + *V. negundo* treatment. The plant extracts recorded highest LT_{50} of 11.5 – 10.6 days. The fungus *M. anisopliae* produced the time mortality response (LT_{50}) of 8.5 days.

The effects of combining *M. anisopliae* and plant extracts in comparison with the effect of the individual components are presented in Table 18 a. The combinations *M. anisopliae* + *A. calamus,* and *M. anisopliae* + *V. negundo* produced supplemental synergism. The mortality of the larvae caused due to these set of treatments were 51.7, 55.1 and 62.0 per cent which were more than the algebraic sum of the mortality caused individually by *M. anisopliae* and plant extracts such as

Table 18. Efficacy of *Metarhizium anisopliae* with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Metarhizium anisopliae	$1x10^8$spores/ ml	3.3	3.3	6.6	10.0	16.6	30.0	33.3	40.0	37.9^{c}	8.5
Acorus calamus	5	9.9	20.0	23.3	30.0	33.3	33.3	33.3	33.3	31.0^{de}	11.5
Andrographis paniculata	5	3.3	16.6	20.0	23.3	26.6	30.0	30.0	30.0	27.6^{e}	10.9
Vitex negundo	5	13.3	20.0	23.3	30.0	30.0	33.3	36.6	36.6	34.4^{cd}	10.6
M. anisopliae+ *A. calamus*	$0.5x10^8$spores /ml + 2.5	20.0	26.6	33.3	36.6	40.0	43.3	50.0	53.3	51.7^{b}	7.4
M. anisopliae + *A. paniculata*	$0.5x10^8$spores /ml + 2.5	26.6	30.0	40.0	43.3	46.6	46.6	50.0	56.6	55.1^{b}	7.0
M. anisopliae + *V. negundo*	$0.5x10^8$spores /ml + 2.5	30.0	36.6	43.3	43.3	46.6	50.0	53.3	63.3	62.0^{a}	5.9

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 18a. Effect of the combination of *Metarhizium anisopliae* and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic coefficient and effect
	Mp	Mf	M f+p		
Acorus calamus	31.0 (18.1)	**37.9 (28.1)**	51.7	Supplemental synergism	0.016 (LOS)
Andrographis paniculata	27.6 (15.2)		55.1	Supplemental synergism	0.027 (LOS)
Vitex negundo	34.4 (22.6)		62.0	Supplemental synergism	0.035 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mf - Mortality per cent of fungus (*Metarhizium anisopliae*)
M f+p - Mortality per cent of fungus + plant extracts

A. calamus, *A. paniculata* and *V. negundo* resulting in 46.2, 43.3 and 50.7 per cent mortality respectively. These treatments recorded synergistic co efficient of 0.016, 0.027 and 0.035 and exhibited lower order synergism.

4.4.1.4 *Metarhizium anisopliae* and insecticides

Results of the experiment on the combined efficacy of *M. anisopliae* and insecticides showed that there was significant increase in mortality due to addition of insecticides (Table 19.). It could be seen that mortality of the third instar larvae of *H.armigera* started from first day onwards. The combinations involving *M. anisopliae* and spinosad recorded highest mortality of 93.0 per cent, which was on par with spinosad alone (89.6 per cent). The other combinations *viz.*, *M. anisopliae* + carbosulfan, *M. anisopliae* + triazophos and *M. anisopliae* + profenofos recorded 72.30, 68.90 and 62.0 per cent mortality respectively. All these treatments were on par. The fungus *M. anisopliae* caused 44.7 per cent mortality at eighth day after treatment.

The lowest LT_{50} of 0.6 days was observed in *M. anisopliae* + spinosad followed by spinosad alone (1.6 days). The time mortality response (LT_{50}) recorded in *M. anisopliae* and insecticide mixtures ranged from 5.5 to 2.2 days. The highest LT_{50} was recorded in *M. anisopliae* treatment (8.0 days).

The interaction effects of combining *M. anisopliae* and insecticides together with the categorization of the different effects are presented in Table 19 a. Supplemental synergism was observed when *M. anisopliae* was mixed with insecticides *viz.*, profenofos, triazophos, carbosulfan and spinosad. The synergistic co efficient observed were 0.008, 0.009 0.012 and 0.021 respectively, showing lower order synergism.

Table 19. Efficacies of *Metarhizium anisopliae* with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Metarhizium anisopliae	$1x10^8$spores/ml	0.0	3.3	9.9	13.3	20.0	33.3	40.0	46.6	44.7^{e}	8.0
Carbosulfan	0.05	16.6	30.0	36.6	43.3	50.0	56.6	56.6	56.6	55.1^{d}	6.3
Profenofos	0.10	20.0	36.6	40.0	46.6	53.3	60.0	60.0	60.0	58.6^{cd}	5.5
Spinosad	0.02	43.3	50.0	76.6	80.0	86.6	90.0	90.0	90.0	89.6^{a}	1.6
Triazophos	0.10	23.3	43.3	50.0	56.6	60.0	66.6	66.6	66.6	65.4^{b}	4.3
M. anisopliae + carbosulfan	$0.5x10^8$spores/ ml + 0.025	40.0	46.6	50.0	60.0	63.3	66.6	73.3	73.3	72.3^{b}	3.0
M. anisopliae + profenofos	$0.5x10^8$spores/ ml + 0.05	30.0	36.6	40.0	50.0	53.3	56.6	60.0	63.3	62.0^{bc}	5.5
M. anisopliae + spinosad	$0.5x10^8$spores/ ml + 0.01	50.0	66.6	76.6	80.0	86.6	90.0	93.3	93.3	93.0^{a}	0.6
M. anisopliae + triazophos	$0.5x10^8$spores/ ml + 0.05	40.0	50.0	56.6	60.0	63.3	66.6	66.6	70.0	68.9^{b}	2.2

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 19 a. Effect of the combination of *Metarhizium anisopliae* and insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic coefficient and effect
	Mi	Mf	Mf + i		
Carbosulfan	55.1 (28.4)	**44.7 (34.6)**	72.3	Supplemental synergism	0.012 (LOS)
Profenofos	58.6 (25.1)		62.0	Supplemental synergism	0.008 (LOS)
Spinosad	89.6 (43.7)		93.0	Supplemental synergism	0.021 (LOS)
Triazophos	65.4 (25.7)		68.9	Supplemental synergism	0.009 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and insecticides

LOS – Lower Order Synergism

Mi – Mortality per cent of insecticide
Mf - Mortality per cent of fungus (*Metarhizium anisopliae*)
M f+i - Mortality per cent of fungus + insecticide

4.4.1.5 *Beauveria bassiana* and plant extracts

The experiment conducted to evaluate the influence of plant extracts (*A. calamus*, *A. paniculata* and *V. negundo*) in combination with *B. bassiana* showed that the mortality per cent in the different treatments ranged from 3.30 to 20.0 only on the first day (Table 20.). There was steady increase in mortality of larvae from the fifth day onwards. The highest mortality per cent was observed in *B. bassiana* + *V. negundo* (56.6) combination followed by *B. bassiana* + *A. calamus* (53.3). Both were on par with each other. The fungus *B. bassiana* + *A. paniculata* gave 46.6 per cent mortality and was on par with *B. bassiana* alone (43.3 per cent). The mortality recorded in plant extracts ranged from 30.0 to 40.0 per cent.

The combination *B. bassiana* + *V. negundo* produced the lowest LT_{50} of 6.4 days followed by *B. bassiana* + *A. calamus* (7.0 days). The highest time mortality response (LT_{50}) was recorded in *A. paniculata* (11.4 days). The time mortality response observed in *B. bassiana* was 8.5 days.

The interaction effect of *B. bassiana* and plant extracts is presented in Table 20 a. All combinations of *B. bassiana* and plant extract showed supplemental synergism. The mortality of the larvae caused due to these set of treatments were 53.3, 46.6 and 56.6 per cent which were more than the algebraic sum of the mortality caused individually by *B. bassiana* and plant extracts such as *A. calamus*, *A. paniculata* and *V. negundo* resulting in 47.8, 45.7 and 51.6 per cent mortality respectively. Synergistic coefficient recorded in different treatments were *B. bassiana* + *A. calamus* (0.015), *B. bassiana* + *A. paniculata* (0.019) and *B. bassiana* + *V. negundo* (0.035) showing lower order synergism

Table 20. Efficacy of *Beauveria bassiana* with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Beauveria bassiana	$1x10^8$spores/ ml	3.3	3.3	9.9	20.0	26.6	30.0	36.6	43.3	43.3^{cd}	8.5
Acorus calamus	5	9.9	16.6	20.0	23.3	30.0	33.3	33.3	33.3	33.3^{e}	10.7
Andrographis paniculata	5	6.6	9.9	23.3	26.6	26.6	30.0	30.0	30.0	30.0^{e}	11.4
Vitex negundo	5	16.6	20.0	26.6	36.6	36.6	40.0	40.0	40.0	40.0^{d}	9.9
B. bassiana+ *A. calamus*	$0.5x10^8$spores /ml + 2.5	13.3	20.0	26.6	26.6	33.3	36.6	50.0	53.3	53.3^{ab}	7.0
B. bassiana+ *A. paniculata*	$0.5x10^8$spores /ml + 2.5	16.6	26.6	33.3	36.6	40.0	43.3	46.6	46.6	46.6^{c}	7.9
B. bassiana+ *V. negundo*	$0.5x10^8$spores /ml + 2.5	20.0	30.0	43.3	46.6	50.0	53.3	56.6	56.6	56.6^{a}	6.4

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 20 a. Effect of the combination of *Beauveria bassiana* and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mp	Mf	Mf+p		
Acorus calamus	33.3 (21.4)	**43.3 (26.4)**	53.3	Supplemental synergism	0.015 (LOS)
Andrographis paniculata	30.0 (19.3)		46.6	Supplemental synergism	0.019 (LOS)
Vitex negundo	40.0 (25.2)		56.6	Supplemental synergism	0.035 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mf - Mortality per cent of fungus (*Beauveria bassiana)*
M f+p - Mortality per cent of fungus + plant extracts

4.4.1.6 *Beauveria bassiana* and insecticides

Data on the combined efficacy of *B. bassiana* and sub lethal doses of insecticides as compared to their individual action on the third instar larvae of *H.armigera* are presented in Table 21. Mortality of larvae was observed in all the treatments from the first day onwards except *B. bassiana* treatment. Combination *B. b*assiana + spinosad recorded the highest mortality percentage of 93.3 followed by spinosad alone (90.0 per cent). Both the treatments were on par and significantly different from other treatments. Combination of *B. bassiana* + profenofos and *B. bassiana* + triazophos recorded 76.6 and 70.0 per cent mortality respectively. The lowest mortality was observed in *B. bassiana* alone (46.6 per cent).

The time mortality response ranged from 3.9 to 1.0 days in *B. bassiana* and insecticide mixtures. The insecticide alone treatment recorded 5.8 to 1.4 days. The highest time mortality response (LT_{50}) of 8.1 days was observed in *B. bassiana.*

The interaction effects of combining *B. bassiana* with sub lethal doses insecticides are presented in Table 21a. The combinations *viz.*, *B. bassiana* + triazophos, *B. bassiana* + profenofos and *B. bassiana* + spinosad caused supplemental synergism. These treatments recorded synergistic co efficient of 0.010, 0.011 and 0.012 exhibiting lower order synergism. Sub additive synergism was observed in *B. bassiana* + carbosulfan treatment. The mortality of the larvae caused due to this set of treatment was 63.3 per cent, which was less than the algebraic sum of the mortality caused individually by *B. bassiana* and carbosulfan (63.8 per cent), but it was greater than independent synergism 59.2 per cent mortality. The combination *B. bassiana* + carbosulfan recorded synergistic coefficient of –0.002 showing sub multiplicative synergism.

Table 21. Efficacy of *Beauveria bassiana* with insecticides against third instars larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Beauveria bassiana	$1x10^8$spores/ml	0.0	3.3	13.3	20.0	26.6	36.6	40.0	46.6	46.6[f]	8.1
Carbosulfan	0.05	20.0	33.3	46.6	50.0	53.3	56.6	56.6	56.6	56.6[e]	5.8
Profenofos	0.10	33.3	46.6	56.6	63.3	70.0	70.0	70.0	70.0	70.0[c]	2.0
Spinosad	0.02	43.3	53.3	83.3	86.6	86.6	90.0	90.0	90.0	90.0[a]	1.4
Triazophos	0.10	30.0	46.6	56.6	63.3	66.6	66.6	66.6	66.6	66.6[c]	2.1
B. bassiana + carbosulfan	$0.5x10^8$spores/ ml + 0.025	36.6	46.6	46.6	50.0	50.0	63.3	63.3	63.3	63.3[d]	3.9
B. bassiana + profenofos	$0.5x10^8$spores/ ml + 0.05	40.0	63.3	66.6	70.0	70.0	73.3	76.6	76.6	76.6[b]	1.5
B. bassiana + spinosad	$0.5x10^8$spores/ ml + 0.01	46.6	66.6	70.0	83.3	86.6	90.0	93.3	93.3	93.3[a]	1.0
B. bassiana + triazophos	$0.5x10^8$spores/ ml + 0.05	43.3	60.0	63.3	66.6	66.6	70.0	70.0	70.0	70.0[c]	1.3

*Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 21a. Effect of the combination of *Beauveria bassiana* and insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic coefficient and effect
	Mi	Mf	Mf+i		
Carbosulfan	56.6 (28.9)	**46.6 (34.9)**	63.3	Sub additive synergism	-0.002 (SMS)
Profenofos	70.0 (30.6)		76.6	Supplemental synergism	0.011 (LOS)
Spinosad	90.0 (40.7)		93.3	Supplemental synergism	0.012 (LOS)
Triazophos	66.6 (31.2)		70.0	Supplemental synergism	0.010 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of fungus and insecticides

SMS – Sub Multiplicative Synergism
LOS – Lower Order Synergism

Mi – Mortality per cent of insecticide
Mf - Mortality per cent of fungus (*Beauveria bassiana*)
M f+i - Mortality per cent of fungus + insecticide

4.4.2 Efficacy of commercial formulations of *Bacillus thuringiensis* with plant extracts, essential oils and insecticides

4.4.2.1 Bacillus thuringiensis (Halt) and plant extracts

The combined efficacy of combination of *B. thuringiensis* (Halt) with plant extracts of *A. calamus, A. paniculata* and *V. negundo* was evaluated and results showed that these extracts could increase mortality of third instar larvae of *H. armigera* individually (Table 22.). The mortality observed ranged from 3.3 to 20.0 per cent on the first day of treatment. Steady increase in mortality was noticed in Halt + plant extract treatments from the third day onwards. The highest mortality of 76.6 per cent was recorded in Halt + *V. negundo*. This treatment was closely followed by Halt + *A. calamus* (70.0 per cent). The combinations Halt + *A. paniculata* recorded 63.3 per cent mortality and was on par with Halt + *A. calamus*. Halt alone recorded 56.6 per cent mortality. The mortality in plant extracts ranged from 30.0 to 40.0 per cent only.

The Lowest LT_{50} of 3.6 days was recorded in combinations involving Halt + *V. negundo* followed by Halt + *A. paniculata* (3.8 days) and Halt + *A. calamus* (4.8 days). The highest LT_{50} of 7.6 days was observed in *A. calamus* alone.

The interaction effects of combination of Halt + plant extracts on the third instar larvae of *H.armigera* are presented in Table 22 a. Supplemental synergism was observed when the larvae were exposed to Halt and plant extracts of *A. calamus, A. paniculata* and *V. negundo*. The per cent combined mortality due to the above treatments recorded was 70.0, 63.3 and 76.6 respectively, which were more than the algebraic sum of mortality caused individually by Halt and plant extracts resulting in 52.4, 54.5 and 58.4 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.023, 0.020 and 0.040 exhibiting lower order synergism.

Table 22. Efficacy of *Bacillus thuringiensis* (Halt) with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Halt	0.2	6.6	20.0	33.3	50.0	53.3	56.6	56.6[c]	5.1
Acorus calamus	5	3.3	9.9	16.6	20.0	26.6	30.0	30.0[e]	7.6
Andrographis paniculata	5	6.6	6.6	20.0	23.3	26.6	33.3	33.3[e]	7.4
Vitex negundo	5	13.3	20.0	26.6	33.3	40.0	40.0	40.0[d]	6.8
Halt+ *A. calamus*	0.1+ 2.5	20.0	26.6	43.3	53.3	66.6	70.0	70.0[ab]	4.8
Halt+ *A. paniculata*	0.1+ 2.5	16.6	30.0	46.6	60.0	63.3	63.3	63.3[b]	3.8
Halt + *V. negundo*	0.1+ 2.5	20.0	33.3	53.3	66.6	76.6	76.6	76.6[a]	3.6

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 22 a. Effect of the combination of *Bacillus thuringiensis* (Halt) and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mp	Mb	Mb+p		
Acorus calamus	30.0 (19.3)	**56.6 (33.1)**	70.0	Supplemental synergism	0.023 (LOS)
Andrographis paniculata	33.3 (21.4)		63.3	Supplemental synergism	0.020 (LOS)
Vitex negundo	40.0 (25.3)		76.6	Supplemental synergism	0.040 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mb - Mortality per cent of bacteria (Halt)
M b+p - Mortality per cent of bacteria + plant extracts

4.4.2.2 *Bacillus thuringiensis* (Delfin) and plant extracts

Data on the joint action of Delfin and a lower concentration of plant extracts on the larvae of *H.armigera* are presented in Table 23. The mortality of larvae treated with Delfin and plant extract mixtures was observed from the first day of treatment. The mortality per cent increased steadily from the second day onwards. The combination Delfin + *V. negundo* recorded the highest mortality per cent of 82.7 and was significantly superior to other treatments. The mortality per cent observed in Delfin + *A. paniculata* and Delfin + *A. calamus* were 68.9 and 65.4 respectively. The mortality recorded in Delfin was 62.0 per cent and was on par with Delfin + *A. calamus*. Plant extracts alone gave 27.6 to 41.3 per cent mortality only.

The lowest LT_{50} of 1.7 days was noticed in Delfin + *V. negundo*. The LT_{50} recorded in Delfin + *A. paniculata*, Delfin + *A. calamus* and Delfin alone were 2.9, 4.3 and 4.6 days respectively. The plant extracts exhibited the highest LT_{50} values and ranged from 6.4 to 7.7 days.

The interaction effects of Delfin with plant extracts are presented in Table 23 a. The combinations Delfin + *A. calamus*, Delfin + *A. paniculata* and Delfin + *V. negundo* exhibited supplemental synergism. The mortality of the larvae caused due to these set of treatments was 65.4, 68.9 and 82.7 per cent, which was more than the algebraic sum of the mortality caused individually by Delfin and plant extracts viz., *A. calamus*, *A. paniculata* and *V. negundo* resulting 53.2, 53.6 and 60.8 per cent mortality. These treatments recorded synergistic coefficient of 0.036, 0.033 and 0.046 respectively showing lower order synergism.

Table 23. Efficacy of *Bacillus thuringiensis* (Delfin) with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Delfin	0.2	20.0	30.0	43.3	60.0	63.3	63.3	62.0[c]	4.6
Acorus calamus	5	3.3	10.3	20.0	26.6	30.0	30.0	27.6[e]	7.7
Andrographis paniculata	5	6.6	9.9	13.3	23.3	26.6	33.3	31.0[e]	7.4
Vitex negundo	5	9.9	16.6	20.0	33.3	36.6	43.3	41.3[d]	6.4
Delfin + *A. calamus*	0.1+ 2.5	20.0	40.0	46.6	56.6	63.3	66.6	65.4[bc]	4.3
Delfin + *A. paniculata*	0.1+ 2.5	26.6	46.6	56.6	70.0	70.0	70.0	68.9[b]	2.9
Delfin + *V. negundo*	0.1+ 2.5	33.3	53.3	66.6	80.0	83.3	83.3	82.7[a]	1.7

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 23 a. Effect of the combination of *Bacillus thuringiensis* (Delfin) and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mp	Mb	Mb+p		
Acorus calamus	27.6 (19.1)	**62.0 (34.1)**	65.4	Supplemental synergism	0.036 (LOS)
Andrographis paniculata	31.0 (19.5)		68.9	Supplemental synergism	0.033 (LOS)
Vitex negundo	41.3 (26.7)		82.7	Supplemental synergism	0.046 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mb - Mortality per cent of Bacteria (Delfin)
M b+p - Mortality per cent of bacteria + plant extracts

4.4.2.3 *Bacillus thuringiensis* (Dipel) and plant extracts

The combined efficacy of combination of Dipel and a lower concentration of plant extracts on third instar larvae of *H.armigera* is presented in Table 24. The mortality per cent observed in Dipel and plant extract combination was found to be more from the first day onwards. The highest mortality was recorded in Dipel + *V. negundo* (73.3 per cent). This treatment was significantly superior to other combinations. The mortality per cent recorded in Dipel + *A. calamus* and Dipel + *A. paniculata* were 56.6 and 53.3 respectively. Both the treatments were on par with each other. The mortality per cent recorded in Dipel was 50.0. The lowest mortality per cent of 36.6 was recorded in *A. calamus*.

The combination of Dipel + *V. negundo* recorded the lowest LT_{50} of 3.0 days followed by Dipel + *A. calamus* (4.8 days) and Dipel + *A. paniculata* (5.0 days). LT_{50} recorded in Dipel alone was 5.6 days. The plant extracts recorded the time mortality response (LT_{50}) of 7.1 to 7.6 days.

The interaction effect of Dipel and plant extracts is presented in Table 24 a. Supplemental synergism was observed when the larvae were exposed to Dipel and plant extracts such as *A. calamus*, *A. paniculata* and *V. negundo*. The per cent combined mortality due to the above treatments recorded was 56.6, 53.3 and 73.3 respectively, which were more than the algebraic sum of mortality caused individually by Dipel and plant extracts resulting in 52.2, 53.1 and 57.1 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.025, 0.015 and 0.037 exhibiting lower order synergism

Table 24. Efficacy of *Bacillus thuringiensis* (Dipel) with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Dipel	0.2	16.6	23.3	40.0	46.6	50.0	50.0	50.0[c]	5.6
Acorus calamus	5	9.9	16.6	23.3	30.0	33.3	36.6	36.6[de]	7.3
Andrographis paniculata	5	6.6	23.3	30.0	33.3	36.6	40.0	40.0[e]	7.6
Vitex negundo	5	13.3	26.6	33.3	36.6	40.0	40.0	40.0[e]	7.1
Dipel + *A. calamus*	0.1+ 2.5	26.6	36.6	43.3	50.0	56.6	56.6	56.6[b]	4.8
Dipel + *A. paniculata*	0.1+ 2.5	33.3	36.3	43.3	46.6	53.3	53.3	53.3[bc]	5.0
Dipel + *V. negundo*	0.1+ 2.5	30.0	46.6	56.6	63.3	66.6	73.3	73.3[a]	3.0

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 24 a. Effect of the combination of *Bacillus thuringiensis* (Dipel) and plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mp	Mb	Mb+p		
Acorus calamus	36.6 (21.8)	**50.0 (30.1)**	56.6	Supplemental synergism	0.025 (LOS)
Andrographis paniculata	40.0 (23.0)		53.3	Supplemental synergism	0.015 (LOS)
Vitex negundo	43.3 (26.7)		73.3	Supplemental synergism	0.037 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extracts
Mb - Mortality per cent of Bacteria (Dipel)
M b+p - Mortality per cent of bacteria + plant extracts

4.4.2.4 *Bacillus thuringiensis* (Halt) and essential oils

The experiment conducted to evaluate the combined efficacy of *B. thuringiensis* (Halt) with essential oils (*C. flexuosus, C. martini, K. galanga* and *C. winterianus*) revealed that the combination of Halt + *C. martini* recorded the highest mortality of 76.6 per cent (Table 25.). This was followed by Halt + *C. flexuosus* (73.3 per cent). Both were on par and the latter was on par with Halt + *K. galanga* (70.0 per cent). The combination Halt + *C. winterianus* recorded 63.3 per cent mortality. The essential oils namely, *C. martinii, C. winterianus, C. flexuosus* and *K. galanga* recorded 56.6, 46.6, 43.3 and 40.0 per cent mortality respectively. The treatment Halt alone gave 50.0 per cent mortality.

The lowest LT_{50} of 1.7 days was recorded in Halt + *C. martinii* followed by Halt + *C. flexuosus* (2.0 days). The LT_{50} recorded in Halt + *K. galanga* and Halt + *C. winterianus* were 2.5 and 3.7 days respectively. The highest LT_{50} of 6.9 days was recorded in *K. galanga.*

The interaction effects of Halt and essential oils are presented in Table 25 a. Supplemental synergism was observed when the larvae were exposed to Halt and essential oils such as C. *winterianus, K. galanga, C. flexuosus* and *C. martinii*. The per cent combined mortality due to the above treatments recorded was 63.3, 70.0, 73.3 and 76.6 respectively, which were more than the algebraic sum of mortality caused individually by Halt and essential oils resulting in 61.3, 59.7, 60.0 and 63.8 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.005, 0.013, 0.016 and 0.018 exhibiting lower order synergism.

Table 25. Efficacy of *Bacillus thuringiensis* (Halt) with essential oils against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Halt	0.2	20.0	26.6	40.0	46.6	50.0	50.0	50.0^{e}	5.5
Citronella winterianus	5	9.9	16.6	23.3	36.6	40.0	46.6	46.6^{ef}	6.1
Kaempferia galanga	5	13.3	26.6	26.6	36.6	40.0	40.0	40.0^{g}	6.9
Cymbopogon flexuosus	5	16.6	23.3	40.0	43.3	43.3	43.3	43.3^{fg}	6.4
Cymbopogon martini	5	20.0	36.6	43.3	53.3	56.6	56.6	56.6^{d}	4.5
Halt+ *C. winterianus*	0.1+ 2.5	30.0	36.6	50.0	60.0	63.3	63.3	63.3^{c}	3.7
Halt+ *K. galanga*	0.1+ 2.5	36.6	50.0	66.6	70.0	70.0	70.0	70.0^{b}	2.5
Halt + *C. flexuosus*	0.1+ 2.5	36.6	56.6	70.0	73.3	73.3	73.3	73.3^{ab}	2.0
Halt + *C. martini*	0.1+ 2.5	40.0	60.0	66.6	73.3	76.6	76.6	76.6^{a}	1.7

* Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 25 a. Effect of the combination of *Bacillus thuringiensis* (Halt) and essential oils on *Helicoverpa armigera*

Essential oils	Corrected percentage mortality			Effect	Synergistic co - efficient and effect
	Me	Mb	Mb+e		
Citronella winterianus	46.6 (29.6)	**50.0 (31.7)**	63.3	Supplemental synergism	0.005 (LOS)
Kaempferia galanga	40.0 (28.0)		70.0	Supplemental synergism	0.013 (LOS)
Cymbopogon flexuosus	43.3 (28.3)		73.3	Supplemental synergism	0.016 (LOS)
Cymbopogon martini	56.6 (32.1)		76.6	Supplemental synergism	0.018 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and essential oils

LOS – Lower Order Synergism

Me – Mortality per cent of essential oil
Mb - Mortality per cent of bacteria (Halt)
M b+e - Mortality per cent of bacteria + essential oil

4.4.2.5 *Bacillus thuringiensis* (Delfin) and essential oils

Results of the experiment involving the effectiveness of *B. thuringiensis* in combination with essential oils are presented in Table 26. The joint action of Delfin and essential oils recorded mortality on the third instar larvae of *H. armigera* from the first day onwards. The combination of Delfin and *C. martini* recorded the highest mortality per cent of 83.3. This treatment was significantly superior to the other treatments. This was followed by Delfin+ *C. winterianus* (73.3 per cent). The treatments Delfin + *K. galanga* (66.6 per cent), *C. martini* (63.3 per cent) Delfin alone (60.0 per cent) and Delfin + *C. flexuosus* (60.0 per cent) were on par. The lowest mortality of 40.0 per cent was observed in *C. winterianus.*

The lowest LT_{50} was recorded in Delfin + *C. martini* (1.5 days) followed by Delfin + *C. winterianus* (2.6 days). The combinations Delfin + *K. galanga* and Delfin + *C. flexuosus* caused time mortality response of 3.5 and 4.7 days respectively. The essential oils alone recorded LT_{50} of 4.1 to 7.0 days. The bacterial formulation Delfin alone recorded LT_{50} of 4.5 days.

The interaction effect of combination of Delfin and essential oils are presented in Table 26 a. The combinations *viz.*, Delfin + *C. winterianus*, Delfin + *K. galanga* and Delfin + *C. martinii* resulted in supplemental synergism. The per cent combined mortality due to the above treatments recorded was 73.3, 66.6 and 83.3 respectively, which were more than the algebraic sum of mortality caused individually by Delfin and essential oils resulting in 60.8, 64.0 and 68.0 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.0143, 0.0059, and 0.0220 exhibiting lower order synergism. Sub additive synergism was recorded when larvae were exposed to Delfin and *C. flexuosus* combinations and recorded synergistic coefficient of –0.0003 exhibiting sub multiplicative synergism.

Table 26. Efficacy of *Bacillus thuringiensis* (Delfin) with essential oils against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Delfin	0.2	23.3	30.0	40.0	56.6	56.6	60.0	60.0[d]	4.5
Citronella winterianus	5	9.9	16.6	23.3	30.0	33.3	40.0	40.0[f]	7.0
Kaempferia galanga	5	13.3	20.0	40.0	50.0	50.0	50.0	50.0[e]	5.5
Cymbopogon flexuosus	5	16.6	23.3	36.6	46.6	46.6	46.6	46.6[e]	5.9
Cymbopogon martini	5	26.6	33.3	50.0	60.0	63.3	63.3	63.3[cd]	4.1
Delfin+ *C. winterianus*	0.1+ 2.5	30.0	46.6	60.0	70.0	73.3	73.3	73.3[b]	2.6
Delfin+ *K. galanga*	0.1+ 2.5	26.6	40.0	53.3	60.0	66.6	66.6	66.6[c]	3.5
Delfin + *C. flexuosus*	0.1+ 2.5	23.3	30.0	43.3	50.0	53.3	60.0	60.0[d]	4.7
Delfin + *C. martini*	0.1+ 2.5	40.0	63.3	66.6	76.6	80.0	83.3	83.3[a]	1.5

* Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 26 a. Effect of the combination of *Bacillus thuringiensis* (Delfin) and essential oils on *Helicoverpa armigera*

Essential oils	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Me	Mb	Mb+e		
Citronella winterianus	40.0 (28.0)	**60.0 (32.8)**	73.3	Supplemental synergism	0.0143 (LOS)
Kaempferia galanga	50.0 (31.2)		66.6	Supplemental synergism	0.0059 (LOS)
Cymbopogon flexuosus	46.6 (29.5)		60.0	Sub additive synergism	-0.0003 (SMS)
Cymbopogon martini	63.3 (35.2)		83.3	Supplemental synergism	0.0220 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and essential oils

SMS – Sub Multiplicative Synergism
LOS – Lower Order Synergism

Me – Mortality per cent of essential oil
Mb - Mortality per cent of bacteria (Delfin)
M b+e - Mortality per cent of bacteria + essential oil

4.4.2.6 *Bacillus thuringiensis* (Dipel) and essential oils

The combined efficacy of Dipel with essential oils were evaluated and the results showed that all the essential oils could increase the mortality of third instar larvae of *H.armigera* (Table 27.). The highest mortality per cent was observed in Dipel + *C. martini* (76.6) and Dipel + *K. galanga* (76.6). These treatments were on par with Dipel + *C. winterianus* (70.0 per cent). The combination Dipel + *C. flexuosus* gave 60.0 per cent mortality and was on par with *C. flexuosus* (56.6 per cent) and *C. martini* (53.3 per cent). These treatments were significantly different from *K. galanga* (50.0 per cent), Dipel (46.6 per cent) and *C. winterianus* (40.0 per cent).

The lowest time mortality response (LT_{50}) was observed in Dipel + *K. galanga* (1.5 days). This was followed by Dipel + *C. martini* (1.7 days), Dipel + *C. flexuosus* (2.5 days) and Dipel + *C. winterianus* (2.7 days). The highest LT_{50} was recorded in *C. winterianus* (6.9 days).

The effect of combination of Dipel and essential oils are presented in Table 27 a. Supplemental synergism was observed when the larvae were exposed to Dipel and essential oils such as C. *winterianus, K. galanga* and *C. martini*. The per cent combined mortality due to the above treatments recorded was 70.0, 76.6 and 76.6 respectively, which were more than the algebraic sum of mortality caused individually by Dipel and essential oils resulting in 59.4, 62.6 and 63.7 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.012, 0.018 and 0.017 exhibiting lower order synergism. The combination Dipel + *C. flexuosus* exhibited sub additive synergism. This treatment recorded synergistic coefficient of – 0.007 showing sub multiplicative synergism.

Table 27. Efficacy of *Bacillus thuringiensis* (Dipel) with essential oils against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Dipel	0.2	20.0	33.3	40.0	43.3	46.6	46.6	46.6[d]	6.2
Citronella winterianus	5	16.6	23.3	30.0	36.6	40.0	40.0	40.0[e]	6.9
Kaempferia galanga	5	23.3	26.6	40.0	46.6	46.6	50.0	50.0[d]	5.9
Cymbopogon flexuosus	5	26.6	33.3	50.0	53.3	56.6	56.6	56.6[c]	4.5
Cymbopogon martini	5	30.0	36.6	46.6	50.0	53.3	53.3	53.3[c]	5.0
Dipel + *C. winterianus*	0.1+ 2.5	36.6	43.3	56.6	66.6	70.0	70.0	70.0[ab]	2.7
Dipel + *K. galanga*	0.1+ 2.5	40.0	63.3	66.6	70.0	76.6	76.6	76.6[a]	1.5
Dipel + *C. flexuosus*	0.1+ 2.5	36.6	50.0	56.6	60.0	60.0	60.0	60.0[c]	2.5
Dipel + *C. martini*	0.1+ 2.5	43.3	63.3	63.3	73.3	76.6	76.6	76.6[a]	1.7

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 27 a. Effect of the combination of *Bacillus thuringiensis* (Dipel) and essential oil on *Helicoverpa armigera*

Essential oils	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Me	Mb	Mb+e		
Citronella winterianus	40.0 (28.0)	**46.6 (31.4)**	70.0	Supplemental synergism	0.012 (LOS)
Kaempferia galanga	50.0 (31.2)		76.6	Supplemental synergism	0.018 (LOS)
Cymbopogon flexuosus	56.6 (35.2)		60.0	Sub additive synergism	-0.007 (SMS)
Cymbopogon martini	53.3 (32.3)		76.6	Supplemental synergism	0.017 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and essential oils

SMS – Sub Multiplicative Synergism
LOS – Lower Order Synergism

Me – Mortality per cent of essential oil
Mb - Mortality per cent of bacteria (Dipel)
M b+e - Mortality per cent of bacteria + essential oil

4.4.2.7 *Bacillus thuringiensis* (Halt) and insecticides

The results relating to these studies are presented in Table 28. Halt + insecticides mixtures recorded mortality from the first day onwards. The highest mortality was recorded in Halt + spinosad (96.2 per cent) and significantly superior to other treatments. This was followed by spinosad alone (89.6 per cent) and on par with Halt + profenofos (86.1 per cent). The combinations Halt + triazophos and Halt + carbosulfan gave 79.3 and 75.8 per cent mortality respectively. The mortality per cent observed in insecticide treatment alone ranged from 55.1 to 68.9.The lowest mortality was noticed in Halt alone, with 48.2 per cent death of larvae.

The lower LT_{50} values of 0.4, 1.0, 1.2, and 1.5 days were observed in Halt + spinosad, Halt + profenofos, Halt + triazophos and Halt + carbosulfan treatments respectively. The time mortality responses in insecticide alone treatments ranged from 4.2 to 1.0 days. The lowest LT_{50} was recorded in Halt + spinosad. The treatment Halt alone recorded the highest LT_{50} of 5.8 days.

The effect of combination of Halt and insecticides are presented in Table 28 a. Supplemental synergism was observed when larvae were exposed to Halt and insecticides such as carbosulfan, profenofos, spinosad and triazophos. These treatments recorded synergistic co efficient of 0.016, 0.021, 0.033 and 0.031 exhibiting lower order synergism.

4.4.2.8 *Bacillus thuringiensis* (Delfin) and insecticides

The combined efficacy of Delfin with sub lethal concentration of insecticides showed that there was significant increase in mortality of third instar larvae of *H.armigera* due to the addition of insecticides (Table 29.). The mortality per cent in all the treatments ranged from 16.6 to 50.0 in the first day. The combination of

Table 28. Efficacy of *Bacillus thuringiensis* (Halt) with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Halt	0.2	10.0	26.6	40.0	46.6	46.6	50.0	48.2[f]	5.8
Carbosulfan	0.05	26.6	46.6	50.0	53.3	56.6	56.6	55.1[e]	4.2
Profenofos	0.10	40.0	60.0	63.3	66.6	70.0	70.0	68.9[d]	1.7
Spinosad	0.02	40.0	70.0	80.0	83.3	90.0	90.0	89.6[b]	1.0
Triazophos	0.10	36.6	56.6	60.0	66.6	66.6	66.6	65.4[d]	2.0
Halt+ carbosulfan	0.1+ 0.025	36.6	70.0	73.3	76.6	76.6	76.6	75.8[c]	1.5
Halt+ profenofos	0.1+ 0.05	40.0	70.0	83.3	86.6	86.6	86.6	86.1[b]	1.0
Halt + spinosad	0.1+ 0.01	50.0	86.6	90.0	93.3	96.6	96.6	96.2[a]	0.4
Halt +triazophos	0.1+ 0.05	40.0	66.6	73.3	76.6	80.0	80.0	79.3[c]	1.2

*Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

Table 28 a. Effect of the combination of *Bacillus thuringiensis* (Halt) with insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mi	Mb	Mb+i		
Carbosulfan	55.1 (30.5)	**48.2 (30.2)**	75.8	Supplemental synergism	0.016 (LOS)
Profenofos	65.4 (33.7)		86.1	Supplemental synergism	0.021 (LOS)
Spinosad	89.6 (43.0)		96.2	Supplemental synergism	0.033 (LOS)
Triazophos	68.9 (35.2)		79.3	Supplemental synergism	0.031 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and insecticides

LOS – Lower Order Synergism

Mi – Mortality per cent of Insecticide
Mb - Mortality per cent of bacteria (Halt)
M b+i - Mortality per cent of bacteria + Insecticide

Delfin + spinosad recorded the highest mortality per cent of 96.6 and was significantly superior to other treatments. This was followed by Delfin + triazophos (90.0 per cent) and spinosad (86.6 per cent). These treatments were on par. The combinations Delfin + carbosulfan and Delfin + profenofos induced 80.0 and 76.6 per cent mortality. The latter was on par with the treatment triazophos alone (73.3 per cent). The lowest mortality per cent was observed in carbosulfan (53.3). The bacterial formulation Delfin recorded 60.0 per cent mortality.

The lowest LT_{50} of 0.5days was observed in Delfin + spinosad followed by Delfin + triazophos (1.2 days), Delfin + profenofos (1.5days) and Delfin + carbosulfan (2.0 days). The time mortality response recorded in insecticide alone ranged from 4.8 to 1.2 days. The treatment Delfin recorded LT_{50} of 4.6 days.

The interaction effect of Delfin and insecticide mixtures are presented in Table 29 a. The combinations *viz.*, Delfin+ carbosulfan, Delfin + profenofos, Delfin + spinosad and Delfin+ triazophos caused supplemental synergism. These treatments recorded synergistic coefficient of 0.018, 0.023, 0.027and 0.020 respectively exhibiting lower order synergism.

4.4.2.9 *Bacillus thuringiensis* (Dipel) and insecticides

The results are presented in Table 30. The highest mortality was observed in Dipel + spinosad (93.3 per cent) and was significantly superior to other treatments. This was followed by Dipel + carbosulfan (80.0 per cent) and spinosad alone (76.6 per cent). The combination Dipel + triazophos and Dipel + profenofos gave 73.3 and 70.0 per cent mortality respectively and was on par with each other. The other insecticides *viz.*, triazophos (66.6 per cent), profenofos (63.3 per cent) and

Table 29. Efficacy of *Bacillus thuringiensis* (Delfin) with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Delfin	0.2	16.6	23.3	36.6	53.3	60.0	60.0	60.0[f]	4.6
Carbosulfan	0.05	30.0	40.0	46.6	50.0	53.3	53.3	53.3[g]	4.8
Profenofos	0.10	36.6	50.0	60.0	66.6	70.0	70.0	70.0[e]	2.5
Spinosad	0.02	40.0	66.6	83.3	86.6	86.6	86.6	86.6[b]	1.2
Triazophos	0.10	30.0	56.6	60.0	70.0	73.3	73.3	73.3[df]	2.8
Delfin+ carbosulfan	0.1+ 0.025	36.6	53.3	76.6	80.0	80.0	80.0	80.0[c]	2.0
Delfin+ profenofos	0.1+ 0.05	40.0	63.3	70.0	70.0	73.3	76.6	76.6[cd]	1.5
Delfin + spinosad	0.1+ 0.01	43.3	80.0	93.3	96.6	96.6	96.6	96.6[a]	0.5
Delfin + triazophos	0.1+ 0.05	40.0	66.6	73.3	86.6	86.6	90.0	90.0[b]	1.2

*Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 29a. Effect of the combination of *Bacillus thuringiensis* (Delfin) with insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic coefficient and effect
	Mi	Mb	Mb+i		
Carbosulfan	53.3 (29.5)	**60.0 (32.7)**	80.0	Supplemental synergism	0.018 (LOS)
Profenofos	70.0 (35.3)		76.6	Supplemental synergism	0.023 (LOS)
Spinosad	86.6 (43.7)		96.6	Supplemental synergism	0.027 (LOS)
Triazophos	73.3 (37.9)		90.0	Supplemental synergism	0.020 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and insecticides

LOS – Lower Order Synergism

Mi – Mortality per cent of Insecticide
Mb - Mortality per cent of Bacteria (Delfin)
M b+i - Mortality per cent of bacteria + Insecticide

carbosulfan (60.0 per cent) were on par in their efficiency. The mortality recorded in Dipel alone was only 46.6 per cent.

The time mortality response (LT_{50}) observed in Dipel insecticide mixtures were ranged from 0.7 to 3.1 days. The highest LT_{50} was observed in Dipel (5.9 days).

The effects of joint action of Dipel and insecticide combinations are presented in Table 30 a. Supplemental synergism was observed in all the Dipel – insecticide combinations. Dipel + carbosulfan (0.011), Dipel + profenofos (0.023), Dipel + spinosad (0.029) and Dipel + triazophos (0.027) had supplemental synergism showing lower order synergism.

4.4.3 Efficacy of Nuclear Polyhedrosis Virus with plant extracts, essential oils and insecticides

4.4.3.1 Nuclear polyhedrosis virus and plant extracts

Data relating to the efficacy of nuclear polyhedrosis virus and plant extracts on third instar larvae of *H.armigera* are presented in Table 31. The mortality of the larvae treated with NPV and plant extracts ranged from 3.3 to 30.0 per cent on the first day. A steady increase in the mortality of the larvae under all the combinations was noticed from the third day of application. The highest mortality per cent was observed in NPV + *V. negundo* (80.0) and followed by NPV + *A. calamus* (76.6 per cent). Both were on par with each other. The NPV alone treatment recorded 63.3 per cent mortality on the eighth day after treatment. The mortality in plant extracts alone ranged from 30.0 to 40.0 per cent.

Table 30. Efficacy of *Bacillus thuringiensis* (Dipel) with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)							
		1	2	3	4	5	6	Total*	LT_{50}
Dipel	0.2	13.3	26.6	36.6	40.0	43.3	46.6	46.6[e]	5.9
Carbosulfan	0.05	26.6	40.0	56.6	60.0	60.0	60.0	60.0[d]	3.3
Profenofos	0.10	26.6	43.3	50.0	53.3	63.3	63.3	63.3[d]	3.5
Spinosad	0.02	36.6	53.3	70.0	73.3	76.6	76.6	76.6[c]	2.2
Triazophos	0.10	36.6	56.6	60.0	66.6	66.6	66.6	66.6[cd]	2.0
Dipel+ carbosulfan	0.1+ 0.025	40.0	70.0	76.6	80.0	80.0	80.0	80.0[b]	1.0
Dipel+ profenofos	0.1+ 0.05	33.3	46.6	46.6	53.3	60.0	70.0	70.0[c]	3.1
Dipel + spinosad	0.1+ 0.01	50.0	73.3	86.6	93.3	93.3	93.3	93.3[a]	0.7
Dipel + triazophos	0.1+ 0.05	40.0	70.0	70.0	73.3	73.3	73.3	73.3[cd]	1.0

*Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 30 a. Effect of the combination of *Bacillus thuringiensis* (Dipel) with insecticides on *Helicoverpa armigera*

Insecticides	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mi	Mb	Mb+i		
Carbosulfan	60.0 (28.0)	**46.6 (31.2)**	80.0	Supplemental synergism	0.011 (LOS)
Profenofos	63.3 (30.8)		70.0	Supplemental synergism	0.023 (LOS)
Spinosad	76.6 (42.1)		93.3	Supplemental synergism	0.029 (LOS)
Triazophos	66.6 (31.5)		73.3	Supplemental synergism	0.027 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of bacteria and insecticides

LOS – Lower Order Synergism

Mi – Mortality per cent of Insecticide
Mb - Mortality per cent of bacteria (Dipel)
M b+i - Mortality per cent of bacteria + Insecticide

The lowest LT_{50} was observed in NPV + *V. negundo* (3.0 days). The highest LT_{50} of 14.3 days was recorded in *A. paniculata*.

The effects of combining NPV and plant extracts together with the categorization of the different effects are presented in Table 31 a. The treatments NPV + *A. calamus*, NPV + *A. paniculata* and NPV + *V. negundo* produced supplemental synergism. The per cent combined mortality due to the above treatments recorded was 76.6, 66.6 and 80.0 respectively, which were more than the algebraic sum of mortality caused individually by NPV and plant extracts resulting in 53.4, 60.5 and 59.3 per cent mortality respectively. These treatments recorded synergistic coefficient of 0.033, 0.027 and 0.053 exhibiting lower order synergism.

4.4.3.2 Nuclear polyhedrosis virus and essential oils

The results are presented in Table 32. The mortality per cent recorded ranged from 6.6 to 36.6 in all the treatments on the first day of treatment. The combination involving NPV and *K. galanga* recorded the highest mortality per cent of 83.3. This was closely followed by NPV + *C. martinii* (80.0 per cent). The mortality per cent of 66.6 and 63.3 were recorded in NPV + *C. flexuosus* and NPV + *C. winterianus* respectively. The mortality per cent induced by essential oil treatments ranged from 50.0 to 60.0 per cent. The NPV alone caused 60.0 per cent mortality.

The lowest time mortality response was observed in NPV + *K. galanga* (2.5 days). The LT_{50} observed in other treatments ranged from 3.0 to 6.4 days.

The interaction effects of NPV and essential oil are presented in Table 32 a. The combinations *viz.*, NPV + *C. winterianus*, NPV + *K. galanga*, NPV + *C. flexuosus* and NPV + *C. martinii* produced supplemental synergism. The synergistic

Table 31. Efficacy of nuclear polyhedrosis virus with plant extracts against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Nuclear poly hedrosis virus	$1.5x10^{12}$ POB /ml	3.3	16.6	30.0	46.6	53.3	60.0	63.3	63.3	63.3[c]	6.5
Acorus calamus	5	9.9	16.6	20.0	26.6	26.6	30.0	30.0	30.0	30.0[e]	11.0
Andrographis paniculata	5	13.3	20.0	26.6	30.0	33.3	33.3	33.3	33.3	33.3[e]	14.3
Vitex negundo	5	16.6	23.3	30.0	33.3	36.6	40.0	40.0	40.0	40.0[d]	12.6
NPV + *A. calamus*	$0.75x10^{12}$ POB /ml+ 2.5	20.0	30.0	30.0	46.6	53.3	66.6	73.3	76.6	76.6[ab]	5.0
NPV + *A. paniculata*	$0.75x10^{12}$ POB /ml+ 2.5	23.3	30.0	43.3	53.3	60.0	60.0	66.6	66.6	66.6[b]	4.8
NPV + *V. negundo*	$0.75x10^{12}$ POB /ml+ 2.5	30.0	43.3	50.0	70.0	73.3	80.0	80.0	80.0	80.0[a]	3.0

*Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 31a. Effect of the combination nuclear polyhedrosis virus with plant extracts on *Helicoverpa armigera*

Plant extracts	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mp	Mv	Mv+p		
Acorus calamus	30.0 (19.3)	**63.3 (34.1)**	76.6	Supplemental synergism	0.033 (LOS)
Andrographis paniculata	33.3 (21.4)		66.6	Supplemental synergism	0.027 (LOS)
Vitex negundo	40.0 (25.2)		80.0	Supplemental synergism	0.053 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of virus and plant extracts

LOS – Lower Order Synergism

Mp – Mortality per cent of plant extract
Mv - Mortality per cent of virus
M v+p - Mortality per cent of virus and plant extracts

coefficient recorded in these treatments was 0.003, 0.023, 0.005 and 0.020 respectively, exhibiting lower order synergism.

4.4.3.2 Nuclear polyhedrosis virus and insecticides

The experiment conducted to evaluate the combined efficacy of NPV with insecticides revealed that NPV + insecticide mixtures significantly increased the per cent mortality of third instar larvae of *H. armigera* (Table 33). Cent per cent mortality was observed in NPV + spinosad combination and was significantly superior to other treatments. This was followed by NPV + triazophos treatment (90.0 per cent). The treatment combinations NPV +profenofos and NPV + carbosulfan recorded 86.6 and 83.3 per cent respectively. Spinosad alone recorded 80.0 per cent mortality.

The lowest LT_{50} was recorded in NPV + spinosad (0.7days). This was followed by NPV + profenofos (2.2 days), NPV+ triazophos (2.3 days) and NPV + carbosulfan (3.5 days). The time mortality responses in insecticide alone treatments ranged from 4.5 to 2.6 days. The highest LT_{50} was observed in NPV alone treatment (7.5 days).

The interaction effects are presented in Table 33a. Supplemental synergism was observed when the larvae were exposed to NPV and insecticides such as carbosulfan, profenofos, spinosad and triazophos treatments. The per cent combined mortality due to the above treatments recorded was 83.3, 86.6, 100.0 and 90.0 respectively, which were more than the algebraic sum of mortality caused individually by NPV and insecticides resulting in 57.9, 65.7, 72.7 and 66.6 per cent mortality respectively with synergistic coefficient of 0.020, 0.023, 0.030 and 0.026 exhibiting lower order synergism.

Table 32. Efficacy of nuclear polyhedrosis virus with essential oils against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Nuclear poly hedrosis virus	$1.5x10^{12}$ POB /ml	6.6	9.9	16.6	40.0	46.6	53.3	60.0	60.0	60.0^{c}	6.4
Citronella winterianus	5	13.3	26.6	43.3	46.6	50.0	50.0	53.3	53.3	53.3^{de}	5.5
Kaempferia galanga	5	20.0	30.0	36.6	43.3	46.6	50.0	50.0	50.0	50.0^{e}	6.2
Cymbopogon flexuosus	5	23.3	40.0	43.3	50.0	56.6	60.0	60.0	60.0	60.0^{c}	4.9
Cymbopogon martini	5	26.6	30.0	40.0	43.3	50.0	53.3	56.6	56.6	56.6^{d}	5.3
NPV + *C. winterianus*	$0.75x10^{12}$ POB /ml+ 2.5	30.0	36.6	40.0	56.6	60.0	63.3	63.3	63.3	63.3^{bc}	4.7
NPV + *K. galanga*	$0.75x10^{12}$ POB /ml+ 2.5	36.6	50.0	56.6	63.3	76.6	80.0	83.3	83.3	83.3^{a}	2.5
NPV+ *C. flexuosus*	$0.75x10^{12}$ POB /ml+ 2.5	30.0	46.6	53.3	56.6	60.0	63.3	63.3	66.6	66.6^{b}	3.0
NPV + *C. martini*	$0.75x10^{12}$ POB /ml+ 2.5	33.3	43.3	50.0	63.3	66.6	76.6	76.6	80.0	80.0^{a}	2.8

* Corrected for control mortality using Abbot's formula
Means followed by similar letters are not different statistically at 5%level by DMRT

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Table 32 a. Effect of the combination of nuclear polyhedrosis virus (NPV) and essential oils on *Helicoverpa armigera*

Essential oils	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Me	Mv	Mv+e		
Citronella winterianus	46.6 (27.8)	**60.0 (32.4)**	63.3	Supplemental synergism	0.003 (LOS)
Kaempferia galanga	50.0 (28.0)		83.3	Supplemental synergism	0.023 (LOS)
Cymbopogon flexuosus	60.0 (29.4)		66.6	Supplemental synergism	0.005 (LOS)
Cymbopogon martini	56.6 (28.8)		80.0	Supplemental synergism	0.020 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of virus and essential oils

LOS – Lower Order Synergism

Me – Mortality per cent of essential oil
Mv - Mortality per cent of virus
M v+e - Mortality per cent of virus and essential oil

4.5 IMPACT OF BIOCIDES (NPV AND *B. thuringiensis*) WITH PLANT EXTRACTS ON DIGESTIVE ENZYME ACTIVITY OF *H. armigera*

4.5.1 Nuclear polyhedrosis virus and plant extracts

4.5.1.1 Protease activity

The results of the protease activity in *H. armigera* after treatment with NPV and plant extract combinations are presented in Table 34. The enzyme activity recorded in plant extracts (*A. calamus*, *A. paniculata* and *V. negundo*) ranged from 13.5 to 14.9 x 10^{-3} µg $mg^{-1}min^{-1}$ at 24 h after treatment. The activity of protease enzyme was significantly decreased in NPV and plant extract combinations (10.2 to 11.3 x10^{-3} µg $mg^{-1}min^{-1}$). The treatment NPV alone also reduced the enzyme activity to 11.9 x 10^{-3} µg $mg^{-1}min^{-1}$ when compared to control insects (17.9 x 10^{-3} µg $mg^{-1}min^{-1}$).

After 48 h of treatment, a remarkably lesser activity of protease was noticed in all the treatments except control. The lowest enzyme activity of 9.0 x 10^{-3} µg mg^{-1} min^{-1} was observed in NPV and *V. negundo* treatment. This was followed by NPV + *A. calamus* treatment (10.7 x10^{-3} µg $mg^{-1}min^{-1}$) and NPV + *A. paniculata* (10.9 x 10^{-3} µg $mg^{-1}min^{-1}$). The enzyme activity observed in NPV alone treatment was 11.1 x 10^{-3} µg $mg^{-1}min^{-1}$. The highest enzyme activity was noticed in *A. calamus* treatment (13.6 x10^{-3} µg $mg^{-1}min^{-1}$) among the various treatments. But this treatment was significantly different from control (18.1x10^{-3} µg mg^{-1} min^{-1}).

4.5.1.2 α – amylase activity

Results are presented in Table 35. At 24 h after treatment, the lowest enzyme activity was observed in NPV+ *V. negundo* (15.2 x 10^{-3} µg mg^{-1} min^{-1}) followed by

Table 33. Efficacy of nuclear polyhedrosis virus with insecticides against third instar larvae of *Helicoverpa armigera*

Treatments	Dose (%)	Per cent mortality at the end of (Days)									
		1	2	3	4	5	6	7	8	Total*	LT_{50}
Nuclear poly hedrosis virus	$1.5x10^{12}$ POB /ml	0.0	3.3	16.6	16.6	33.3	40.0	46.6	56.6	56.6^{f}	7.5
Carbosulfan	0.05	30.0	36.6	43.3	50.0	53.3	56.6	60.0	60.0	60.0^{ef}	4.5
Profenofos	0.10	33.3	50.0	60.0	66.6	70.0	73.3	73.3	73.3	73.3^{d}	2.8
Spinosad	0.02	40.0	63.3	76.6	80.0	80.0	80.0	80.0	80.0	80.0^{c}	2.0
Triazophos	0.10	36.6	53.3	60.0	66.6	70.0	70.0	70.0	70.0	70.0^{d}	2.6
NPV + carbosulfan	$0.75x10^{12}$ POB /ml+ 0.025	30.0	40.0	46.6	56.6	63.3	73.3	80.0	83.3	83.3^{c}	3.5
NPV + profenofos	$0.75x10^{12}$ POB /ml+ 0.05	40.0	60.0	63.3	66.6	70.0	80.0	86.6	86.6	86.6^{bc}	2.2
NPV + spinosad	$0.75x10^{12}$ POB /ml+ 0.01	43.3	76.6	83.3	90.0	96.6	96.6	100.0	100.0	100.0^{a}	0.7
NPV + triazophos	$0.75x10^{12}$ POB /ml+ 0.05	36.6	56.6	66.6	70.0	73.3	80.0	86.6	90.0	90.0^{b}	2.3

*Corrected for control mortality using Abbot's formula

Means followed by similar letters are not different statistically at 5%level by DMRT

Table 33 a. Effect of the combination of nuclear polyhedrosis virus and insecticides on *Helicoverpa armigera*

Insecticide	Corrected percentage mortality			Effect	Synergistic co-efficient and effect
	Mi	Mv	Mv+i		
Carbosulfan	60.0 (28.0)	**56.6 (29.9)**	83.3	Supplemental synergism	0.020 (LOS)
Profenofos	73.3 (35.8)		86.6	Supplemental synergism	0.023 (LOS)
Spinosad	80.0 (42.8)		100	Supplemental synergism	0.030 (LOS)
Triazophos	70.0 (36.1)		90.0	Supplemental synergism	0.026 (LOS)

Figures in the parenthesis are theoretical mortality at half dose of virus and insecticides

LOS – Lower Order Synergism

Mi – Mortality per cent of insecticide
Mv - Mortality per cent of virus
M v+i - Mortality per cent of virus and insecticide

of treatment, the lowest enzyme activity of 11.8 x 10^{-3} µg $mg^{-1}min^{-1}$ was observed in *B. thuringiensis* + *V. negundo*. The other *B. thuringiensis* - plant extract combinations *viz.*, *B. thuringiensis* + *A. paniculata* and *B. thuringiensis*+ *A. calamus* recorded 12.8 and 14.6 x 10^{-3} µg $mg^{-1}min^{-1}$respectively. The highest enzyme activity was recorded in *A. paniculata* (18.7 x 10^{-3} µg $mg^{-1}min^{-1}$) and NPV alone (18.6 10^{-3} µg $mg^{-1}min^{-1}$). These treatments were on par with control (18.9 x 10^{-3} µg $mg^{-1}min^{-1}$).

The lowest enzyme activity of 11.1 x 10^{-3} µg $mg^{-1}min^{-1}$ was noticed in *B. thuringiensis* + *V. negundo* followed by *B. thuringiensis* + *A. paniculata* (12.2 x10^{-3} µg $mg^{-1}min^{-1}$) and *B. thuringiensis* +*A. calamus* (13.5 x 10^{-3} µg $mg^{-1}min^{-1}$) after 48 h of treatment. The highest enzyme activity was observed in control 18.9 x 10^{-3} µg $mg^{-1}min^{-1}$ among the treatments while in control the activity was significantly higher than other treatments. All the treatments were significantly different from control.

4.5.4 Lipase activity

The results are presented in Table 41. After 24 h of treatment, the plant extract, namely, *A. calamus, A. paniculata* and *V. negundo* recorded 6.6, 5.0 and 5.3 x 10^{-3} m eq mg^{-1} min^{-1}enzyme activity, respectively. The enzyme activity observed ranged from 3.0 to 4.6 x 10^{-3} m eq mg^{-1} min^{-1} in *B. thuringiensis* and plant extract combinations. When used alone, *B. thuringiensis* recorded 4.0 x 10^{-3} m eq mg^{-1} min^{-1} enzyme activity.

There was not much reduction in enzyme activity after 48 h of treatment. The lowest enzyme activity of 3.0 x 10^{-3} m eq $mg^{-1}min^{-1}$ was observed in *B. thuringiensis* + *V. negundo* and *B. thuringiensis* + *A. paniculata* 3.0 x 10^{-3} m eq $mg^{-1}min^{-1}$. The bacterial formulation recorded 3.6 x 10^{-3} m eq $mg^{-1}min^{-1}$ enzyme activity when used alone. The highest enzyme activity was observed in *A. calamus* 6.0 x 10^{-3} m eq min^{-1} and was significantly different from control (7.3 x 10^{-3} m eq mg^{-1} min^{-1}).

Table 34.Effect of nuclear polyhedrosis virus and plant extracts on protease enzyme activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity µg $mg^{-1}min^{-1}$ x 10^{-3}	
			24 HAT	48 HAT
1.	Nuclear polyhedrosis virus	$1.5x10^{12}$ POB/ha	11.9^{d}	11.1^{cd}
2.	*Acorus calamus*	5.0	13.7^{c}	13.0^{c}
3.	*Andrographis paniculata*	5.0	14.9^{b}	13.6^{b}
4.	*Vitex negundo*	5.0	13.5^{c}	11.2^{cd}
5.	NPV + *A. calamus*	$0.75x10^{12}$ + 2.5	10.7^{de}	9.5^{e}
6.	NPV + *A. paniculata*	$0.75x10^{12}$ + 2.5	11.3^{de}	10.7^{d}
7.	NPV + *V. negundo*	$0.75x10^{12}$ + 2.5	10.2^{e}	9.0^{e}
8.	Control	-	17.9^{a}	18.1^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

Table 35. Effect of nuclear polyhedrosis virus and plant extracts on α- amylase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity μg mg^{-1} min^{-1} x 10^{-3}	
			24 HAT	48 HAT
1.	Nuclear polyhedrosis virus	$1.5x10^{12}$ POB/ha	15.8^{d}	15.6^{de}
2.	*Acorus calamus*	5.0	16.7^{c}	16.5^{bc}
3.	*Andrographis paniculata*	5.0	17.6^{b}	17.4^{b}
4.	*Vitex negundo*	5.0	17.2^{bc}	16.4^{c}
5.	NPV + *A. calamus*	$0.75x10^{12}$ + 2.5	15.6^{de}	15.3^{de}
6.	NPV + *A. paniculata*	$0.75x10^{12}$ + 2.5	16.8^{bc}	16.5^{c}
7.	NPV + *V. negundo*	$0.75x10^{12}$ + 2.5	15.2^{e}	14.7^{e}
8.	Control	-	19.9^{a}	20.1^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

Table 36. Effect of nuclear polyhedrosis virus and plant extracts on β -amylase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity μg mg^{-1} min^{-1} x 10^{-3}	
			24 HAT	48 HAT
1.	Nuclear polyhedrosis virus	$1.5x10^{12}$ POB/ha	20.8^{b}	20.2^{b}
2.	*Acorus calamus*	5.0	19.7^{b}	18.6^{bc}
3.	*Andrographis paniculata*	5.0	21.9^{b}	21.4^{b}
4.	*Vitex negundo*	5.0	17.4^{c}	16.9^{cd}
5.	NPV + *A. calamus*	$0.75x10^{12}$ + 2.5	15.7^{de}	14.6^{e}
6.	NPV + *A. paniculata*	$0.75x10^{12}$ + 2.5	16.3^{d}	15.2^{de}
7.	NPV + *V. negundo*	$0.75x10^{12}$ + 2.5	13.5^{e}	12.4^{f}
8.	Control	-	25.3^{a}	25.3^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

After 48 h of treatment, the lowest enzyme activity of 8.4 $x10^{-3}$ µg mg^{-1} min^{-1} was observed in *B. thuringiensis* + *V. negundo* treatment. This was followed by *B. thuringiensis* + *A. calamus* treatment (9.0 $x10^{-3}$ µg mg^{-1} min^{-1}) and *B. thuringiensis* + *A. paniculata* (10.5 x 10^{-3} µg mg^{-1} min^{-1}). The highest enzyme activity was noticed in *A. paniculata* treatment (13.0 $x10^{-3}$µg mg^{-1} min^{-1}). But this treatment was significantly different from control (16.9 $x10^{-3}$µg mg^{-1} min^{-1}).

4.5.2.2 α – amylase activity

Results are presented in Table 39. After 24 h of treatment, the lowest enzyme activity was observed in *B. thuringiensis* + *A. paniculata* (12.1 x 10^{-3} µg $mg^{-1}min^{-1}$) followed by *B. thuringiensis* + *V. negundo* (13.0 $x10^{-3}$µg $mg^{-1}min^{-1}$) and *B. thuringiensis* + *A. calamus* (13.6 $x10^{-3}$µg $mg^{-1}min^{-1}$). The high α- amylase activity was recorded in *V. negundo* treatment 15.0 $x10^{-3}$ µg $mg^{-1}min^{-1}$, but was significantly lesser than control (17.5 $x10^{-3}$ µg mg^{-1} min^{-1}).

After 48 h of treatment, α- amylase activity was considerably decreased in all the treatments. α- amylase activity ranged from 14.5 to 13.7 x 10^{-3}µg $mg^{-1}min^{-1}$ in plant extracts (*A.calamus, A. paniculata* and *V. negundo*). However, *B. thuringiensis* in combination with plant extracts recorded 11.9 to 13.6 x 10^{-3}µg $mg^{-1}min^{-1}$. All the treatments observed for enzyme activity were significantly lesser than control (17.9 $x10^{-3}$ µg $mg^{-1}min^{-1}$).

4.5.3 β- amylase activity

The results of β- amylase activity in *H.armigera* after treatment with *B. thuringiensis* and plant extract combination are presented in the Table 40. After 24 h

Table 37.Effect of nuclear polyhedrosis virus and plant extracts on lipase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity meq/mg/min x 10^{-3}	
			24 HAT	48 HAT
1.	Nuclear polyhedrosis virus	$1.5x10^{12}$ POB/ha	7.3^{c}	7.0^{c}
2.	*Acorus calamus*	5.0	7.0^{d}	6.6^{d}
3.	*Andrographis paniculata*	5.0	7.6^{b}	7.3^{b}
4.	*Vitex negundo*	5.0	6.3^{e}	6.3^{e}
5.	NPV + *A. calamus*	$0.75x10^{12}$ + 2.5	5.0^{g}	4.6^{g}
6.	NPV + *A. paniculata*	$0.75x10^{12}$ + 2.5	5.6^{f}	5.0^{f}
7.	NPV + *V. negundo*	$0.75x10^{12}$ + 2.5	5.0^{g}	4.3^{h}
8.	Control	-	9.0^{a}	9.3^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

4.5.4 Lipase activity

The results are presented in Table 37. After 24 h of treatment, there was significant difference between the treatments. The plant extract, namely, *A. calamus, A. paniculata* and *V. negundo* recorded 8.0, 7.6 and 6.3 x 10^{-3} meq $mg^{-1}min^{-1}$enzyme activity respectively, when used alone. The enzyme activity observed ranged from 5.0 to 5.6 x 10^{-3} m eq $mg^{-1}min^{-1}$ in NPV and plant extract combinations. The nuclear polyhedrosis virus alone reduced the enzyme activity to 7.3 x 10^{-3} m eq $mg^{-1}min^{-1}$.

There was not much reduction in enzyme activity after 48 h of treatment. The lowest enzyme activity of 4.3 x 10^{-3} m eq $mg^{-1}min^{-1}$was observed in NPV + *A. calamus* and this was closely followed by NPV + *A. paniculata* (4.6 x 10^{-3} m eq $mg^{-1}min^{-1}$). The highest enzyme activity was observed in control (9.3 x 10^{-3} m eq $mg^{-1}min^{-1}$).

4.5.2 *B. thuringiensis* and plant extracts

4.5.2.1 Protease activity

The results of the protease activity in *H. armigera* after treatment with *B. thuringiensis* and plant extract combinations are presented in Table 38. The enzyme activity recorded in plant extracts (*A. calamus, A. paniculata* and *V. negundo*) ranged from 11.5 to 13.5 x 10^{-3} µg mg^{-1} min^{-1} after 24 h of treatment. The activity of protease enzyme was significantly decreased in *B. thuringiensis* and plant extract combinations (8.5 to 11.1 x10^{-3} µg mg^{-1} min^{-1}). The treatment *B. thuringiensis* alone also reduced the enzyme activity to 12.4 x 10^{-3} µg mg^{-1} min^{-1} when compared to control insects (16.4 x 10^{-3} µg mg^{-1} min^{-1}).

Table 38.Effect of *Bacillus thuringiensis* and plant extracts on protease activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity μg mg^{-1} min^{-1} x 10^{-3}	
			24 HAT	48 HAT
1.	*Bacillus thuringiensis* (Delfin)	0.2	12.4^{c}	11.5^{d}
2.	*Acorus calamus*	5.0	12.1^{c}	12.0^{c}
3.	*Andrographis paniculata*	5.0	13.5^{b}	13.0^{b}
4.	*Vitex negundo*	5.0	11.5^{d}	11.1^{d}
5.	*B.thuringiensis* + *A. calamus*	0.1+ 2.5	9.3^{f}	9.0^{f}
6.	*B. thuringiensis* + *A. paniculata*	0.1+ 2.5	11.1^{e}	10.5^{e}
7.	*B. thuringiensis* + *V. negundo*	0.1+ 2.5	8.5^{g}	8.4^{g}
8.	Control	-	16.4^{a}	16.9^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

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Table 39. Effect of *Bacillus thuringiensis* and plant extract on α- amylase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity μg $mg^{-1}min^{-1}$ x 10^{-3}	
			24 HAT	48 HAT
1.	*Bacillus thuringiensis* (Delfin)	0.2	14.0^{ab}	13.1^{b}
2.	*Acorus calamus*	5.0	14.7^{ab}	14.1^{ab}
3.	*Andrographis paniculata*	5.0	15.0^{ab}	14.5^{ab}
4.	*Vitex negundo*	5.0	13.9^{ab}	13.7^{b}
5.	*B.thuringiensis* + *A. calamus*	0.1+ 2.5	13.6^{ab}	13.6^{b}
6.	*B.thuringiensis* + *A. paniculata*	0.1+ 2.5	13.0^{ab}	12.8^{b}
7.	*B.thuringiensis* + *V. negundo*	0.1+ 2.5	12.1^{b}	11.9^{b}
8.	Control	-	17.5^{a}	17.9^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

NPV + *A. calamus* (15.6 x10^{-3} µg mg^{-1} min^{-1}) and NPV (15.8 x10^{-3} µg mg^{-1} min^{-1}). The high α- amylase activity was recorded in *A. paniculata* treatment treated larvae (17.6x10^{-3} µg mg^{-1} min^{-1}). However, this treatment was significantly lesser than control (19.9 x10^{-3}µg mg^{-1} min^{-1}).

A marginal reduction in enzyme activity was noticed after 48 h treatment. The lowest enzyme activity of 14.7x10^{-3} µg mg^{-1} min^{-1}mg min^{-1} was observed in NPV + *V. negundo*. This treatment was on par with NPV + *A. calamus* (15.3x10^{-3} µg mg^{-1} min^{-}) and NPV (16.6 x10^{-3}µg mg^{-1} min^{-1}). The treatment *A. paniculata* caused the high enzyme activity of 17.6 x 10^{-3} µg mg^{-1} min^{-1}. All the treatments observed for enzyme activity were significantly different from control (20.1x10^{-3}µg$mg^{-1}min^{-1}$).

4.5.3 β- amylase activity

The results of β- amylase activity in *H.armigera* after treatment with NPV and plant extracts combination are presented in Table 36. At 24 h after treatment, the lowest enzyme activity was observed in NPV +*V. negundo* (13.5 x 10^{-3} µg mg^{-1} min^{-1}). This was followed by NPV +*A. calamus* (15.7 x 10^{-3} µg mg^{-1} min^{-1}). The highest enzyme activity of 20.9 x 10^{-3} µg mg^{-1} $min^{-1}$was recorded in *A. paniculata* among the treated larvae.

A remarkable reduction in enzyme activity was observed after 48 h of treatment. The lowest enzyme activity of 12.4x 10^{-3} µg mg^{-1} min^{-1} was noticed in NPV + *V. negundo* followed by NPV + *A. paniculata* (14.6 x 10^{-3} µg mg^{-1} min^{-}) and NPV + *A. calamus* (15.2 x 10^{-3} µg mg^{-1} min^{-1}) The highest enzyme activity was observed in NPV (20.2x 10^{-3} µg mg^{-1} min^{-1}). There was no change in enzyme activity in control after 48 h of treatment.

Table 40. Effect of *Bacillus thuringiensis* and plant extracts on β -amylase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity μg mg^{-1} min^{-1} x 10^{-3}	
			24HAT	48HAT
1.	*Bacillus thuringiensis* (Delfin)	0.2	18.6^{bc}	18.3^{b}
2.	*Acorus calamus*	5.0	17.4^{c}	16.3^{c}
3.	*Andrographis paniculata*	5.0	19.7^{b}	19.1^{b}
4.	*Vitex negundo*	5.0	16.9^{c}	15.7^{c}
5.	*B.thuringiensis* + *A. calamus*	0.1+ 2.5	14.6^{d}	13.5^{d}
6.	*B.thuringiensis* + *A. paniculata*	0.1+ 2.5	12.8^{e}	12.2^{e}
7.	*B.thuringiensis* + *V. negundo*	0.1+ 2.5	11.8^{e}	11.1^{e}
8.	Control	-	20.4^{a}	20.9^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

Table 41.Effect of *Bacillus thuringiensis* and plant extracts on lipase activity of *Helicoverpa armigera*

Sl.no	Treatments	Dose (%)	Enzyme Activity meq mg^{-1} min^{-1} x 10^{-3}	
			24HAT	48HAT
1.	*Bacillus thuringiensis* (Delfin)	0.2	$4.0b^{cd}$	3.6^{be}
2.	*Acorus calamus*	5.0	6.6^{ab}	6.0^{b}
3.	*Andrographis paniculata*	5.0	5.0^{abc}	4.6^{cd}
4.	*Vitex negundo*	5.0	5.3^{bc}	5.3^{bc}
5.	*B.thuringiensis* + *A. calamus*	0.1+ 2.5	4.6^{cd}	4.0^{de}
6.	*B.thuringiensis* + *A. paniculata*	0.1+ 2.5	3.3^{de}	3.0^{e}
7.	*B.thuringiensis* + *V. negundo*	0.1+ 2.5	2.6^{e}	2.0^{f}
8.	Control	-	7.3^{a}	7.3^{a}

Means followed by similar letters are not significantly different at P= (0.05) by DMRT

4.6. FIELD EFFICACY OF PROMISING BIOCIDES AND ITS COMBINATIONS ON *H.armigera*

4.6.1. Per cent mortality

The results pertaining to the field experiments are presented in Table 42. In the first spray, the highest per cent mortality was observed in Delfin + spinosad (82.6 per cent) followed by *N. rileyi* + spinosad (72.20 per cent) and spinosad alone (69.0 per cent). The lowest per cent mortality of 23.80 was recorded in NPV alone treatment.

In the second spray, the highest per cent mortality was observed in Delfin + spinosad (81.53 per cent) followed by *N. rileyi* + spinosad (80.23 per cent) and NPV + spinosad (76.13 per cent). The lowest per cent of mortality of 16.67 was recorded in *V. negundo* and was on par with *C. martinii* alone treatment (22.58 per cent).

4.6.2. Effect on fruit borer infestation

The data on the per cent fruits infested by *H. armigera* are presented in Table 43. The analysis of variance on the per cent of borer infestation indicated that the treatments were significantly different. The treatments Delfin + spinosad and spinosad alone were significantly superior and recorded 15.92 and 19.60 per cent infestation on fruit number basis. The highest damage of 47.70 per cent was recorded in control.

Table 42.Field evaluation of promising biocides and its combinations against tomato fruit borer *Helicoverpa armigera*

Treatments	Dose	Corrected per cent mortality	
		First Spray	Second spray
Bacillus thuringiensis (Delfin)	0.2 Per cent	38.07 (6.202)	30.96 (5.606)
Nuclear Polyhedrosis virus	$1.5x10^{12}$ POB/ha	23.80 (4.236)	30.92 (5.602)
Nomuraea rileyi	$2x10^{8}$ spores/ml	26.86 (5.489)	33.33 (5.813)
Vitex negundo	5.0 per cent	28.73 (5.406)	16.67 (4.143)
Cymbopogon martinii	5.0 per cent	24.99 (5.003)	22.58 (4.776)
Spinosad	0.02 per cent	69.09 (7.896)	71.42 (8.480)
Delfin+ *V.negundo*	0.1+2.5per cent	41.66 (6.472)	61.85 (7.892)
Delfin + *C.martini*	0.1+2.5per cent	46.41 (6.846)	42.85 (6.584)
Delfin + Spinosad	0.1+0.01 per cent	82.60 (9.112)	81.53 (9.055)
NPV + *V.negundo*	0.75×10^{12} + 2.5 per cent	54.68 (7.339)	53.54 (7.348)
NPV + *C.martinii*	0.75×10^{12} + 2.5 per cent	41.66 (6.471)	50.00 (7.106)
NPV + Spinosad	0.75×10^{12} +0.01per cent	61.85 (7.892)	80.23 (8.980)
N. rileyi + *V. negundo*	1×10^{8}spores/ml +2.5per cent	37.50 (6.201)	46.33 (6.839)
N. rileyi + Spinosad	1×10^{8}spores/ml +0.01per cent	72.20 (8.584)	76.13 (8.748)
CD 5%		1.29	1.11

Figures in parenthesis are $\sqrt{x+0.5}$-transformed values

Table 43. Effect of promising biocides and its combinations against tomato fruit borer *Helicoverpa armigera* infestation

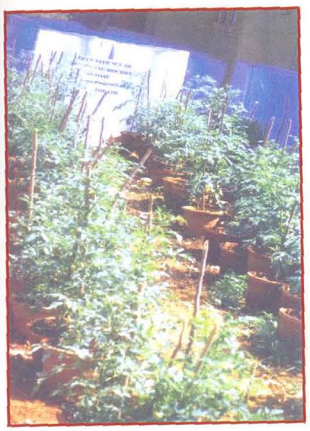
Treatments	Dose	Per cent borer infestation	
		Fruit No	Fruit weight (kg)
Bacillus thuringiensis (Delfin)	0.2 Per cent	30.93 (5.544)	30.47 (5.605)
Nuclear Polyhedrosis virus	$1.5x10^{12}$ POB/ha	44.30 (6.802)	40.43 (6.490)
Nomuraea rileyi	$2x10^{8}$ spores/ml	34.73 (5.756)	32.46 (5.935)
Vitex negundo	5.0 per cent	42.60 (6.508)	39.30 (6.346)
Cymbopogon martinii	5.0 per cent	39.40 (6.364)	36.07 (6.115)
Spinosad	0.02 per cent	19.60 (4.566)	20.40 (4.896)
Delfin+ *V.negundo*	0.1+2.5per cent	41.60 (6.470)	38.60 (6.233)
Delfin + *C.martinii*	0.1+2.5per cent	37.10 (6.134)	32.07 (5.862)
Delfin + Spinosad	0.1+0.01 per cent	15.92 (3.998)	17.67 (4.345)
NPV + *V.negundo*	0.75×10^{12} + 2.5 per cent	43.25 (6.632)	36.03 (6.111)
NPV + *C.martinii*	0.75×10^{12} + 2.5 per cent	34.00 (5.938)	33.60 (5.921)
NPV + Spinosad	0.75×10^{12} +0.01per cent	20.70 (4.865)	28.50 (5.398)
N. rileyi + *V. negundo*	1×10^{8}spores/ml +2.5per cent	30.90 (5.606)	30.08 (5.584)
N. rileyi + Spinosad	1×10^{8}spores/ml +0.01per cent	20.30 (4.886)	25.82 (5.001)
Control	-	47.70 (6.895)	44.90 (6.816)
CD 5%		0.92	0.69

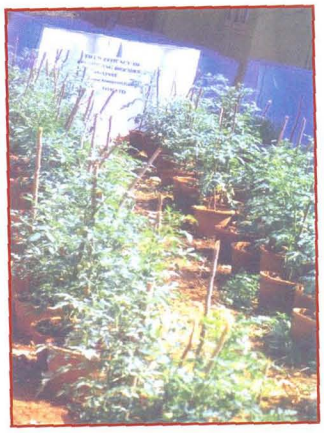
Figures in the parenthesis are $\sqrt{x+0.5}$ transformed values

Plate 6. Field Experiment

Insect cage

Experimental Plot





4.6.3 Effect of promising biocide on yield parameters

4.6.2.1 Total yield

The total yield obtained in different treatments is presented in Table 44. The treatment *C. martini* recorded the highest mean yield of 2.195 kg per treatment followed closely by Delfin + *V. negundo* and NPV+ *V. negundo* which recorded 2.085 and 2.047 kg per treatment, respectively. Control recorded the lowest yield of 1.380 kg per treatment.

4.6.2.2 Marketable yield

The results are presented in Table 44. The treatment Delfin + spinosad recorded the highest marketable fruit yield of 1.515 kg per treatment followed by spinosad alone (1.485 kg per treatment) and *N. rileyi* + spinosad (1.480 kg per treatment). The control recorded the lowest marketable fruit yield of 0.810 kg per treatment.

4.6.2.3 Economics of Biocides combinations

The benefit: cost ratio of different treatment was calculated and furnished in Table 45. The highest benefit: cost ratio of 6.26:1 was recorded in *B.thuringiensis* + spinosad. The treatment combinations, *B.thuringiensis* + *C.martini* and NPV + spinosad recorded a high benefit: cost ratio of 5.42:1 and 5.42: 1. The lowest BCR was recorded in *B.thuringiensis* alone treatment (1.06: 1).

Table 44. Effect of promising biocides and its combinations on tomato fruit yield

Treatments	Dose (%)	Total yield		Marketable yield	
		Fruit No.	Fruit weight (kg)	Fruit No.	Fruit weight (kg)
Bacillus thuringiensis (Delfin)	0.2 Per cent	67.00	1.470	46.33	0.960
Nuclear Polyhedrosis virus	$1.5x10^{12}$ POB/ha	87.33	1.700	48.66	1.013
Nomuraea rileyi	$2x10^{8}$ spores/ml	65.33	1.450	42.67	0.980
Vitex negundo	5.0 per cent	94.00	1.935	54.00	1.175
Cymbopogon martinii	5.0 per cent	108.33	2.195	65.67	1.405
Spinosad	0.02 per cent	96.67	1.790	76.67	1.485
Delfin+ *V. negundo*	0.1+2.5per cent	112.33	2.085	65.67	1.280
Delfin + *C. martinii*	0.1+2.5per cent	102.66	1.965	64.64	1.435
Delfin + Spinosad	0.1+0.01 per cent	96.33	1.840	81.00	1.515
NPV + *V.negundo*	0.75×10^{12} + 2.5 per cent	111.00	2.047	63.00	1.310
NPV + *C. martinii*	0.75×10^{12} + 2.5 per cent	103.00	2.025	68.00	1.345
NPV + Spinosad	0.75×10^{12} +0.01per cent	95.32	1.860	76.67	1.462
N.rileyi + *V. negundo*	$1x\ 10^{8}$spores /ml+2.5per cent	89.67	1.895	62.00	1.325
N.rileyi + spinosad	$1\ x10^{8}$spores/ml +0.01per cent	98.67	1.995	78.67	1.480
Control		64.67	1.380	33.67	0.810
CD 5%		NS	NS	NS	NS

Table 45. Economics of promising biocides and its combinations

Treatments	Dose (%)	Value of increased yield over control Rs. 6.0 per kg	Cost of Labour and insecticide used (Rs.)	Benefit cost ratio
Bacillus thuringiensis (Delfin)	0.2 Per cent	8333	4044	1.06:1
Nuclear Polyhedrosis virus	$1.5x10^{12}$ POB/ha	11277	4544	1.48:1
Nomuraea rileyi	$2x10^{8}$ spores/ml	9444	4376	1.16:1
Vitex negundo	5.0 per cent	20277	4464	3.54:1
Cymbopogon martinii	5.0 per cent	33054	6744	3.90:1
Spinosad	0.02 per cent	37498	6744	4.56:1
Delfin+ *V. negundo*	0.1+2.5per cent	26110	4254	5.14:1
Delfin + *C. martinii*	0.1+2.5per cent	34721	5394	5.42:1
Delfin + Spinosad	0.1+0.01 per cent	39165	5394	6.26:1
NPV + *V.negundo*	0.75×10^{12} + 2.5 per cent	27777	4504	5.17:1
NPV + *C. martinii*	0.75×10^{12} + 2.5 per cent	29721	5644	4.27:1
NPV + Spinosad	0.75×10^{12} +0.01per cent	36221	5644	5.42:1
N.rileyi + *V. negundo*	1×10^{8}spores/ml +2.5per cent	28610	4736	5.04:1
N.rileyi + spinosad	1×10^{8}spores/ml +0.01per cent	37221	5876	5.33:1

Discussion

5. DISCUSSION

Tomato is the one of the important vegetable crops grown all over the world. But the crop is highly vulnerable to fruit borer, *Helicoverpa armigera*. It is a major ubiquitous impediment to production and productivity of tomato. Insecticides have been in use to combat this insect menace. However, their indiscriminate use has resulted in adverse effects like resistance and resurgence to pests, contamination of food materials, adverse effects on animal and human health and environmental pollution.

The repeated failure of unilateral approach of using chemical pesticides, and the increasing concern for environmental safety and global demand for pesticide residue free food necessitated the use of effective, economically viable, eco-friendly and biodegradable pest control materials with greater selectivity. The microbial pesticides such as nuclear polyhedrosis virus, *Bacillus thuringiensis* and entomopathogenic fungi in combination with botanicals and new insecticide molecules evoked a great deal of interest owing to their unique advantages over the conventional synthetic insecticides. Therefore the present study was carried out for testing the joint action potentials of bio pesticides with botanicals and new molecules. The experimental results obtained in the study are briefly discussed hereunder drawing the various inferences.

5.1 NATURAL ENEMIES OF *H.armigera* IN VEGETABLE ECO SYSTEM

Not much information on the incidence and damage of this pest on vegetable crops in Kerala is available. The seasonal abundance of the larvae in the vegetable ecosystem is also not available. The availability and proper exploitation of native natural enemies will reduce the pesticide pressure on the pest. For efficient

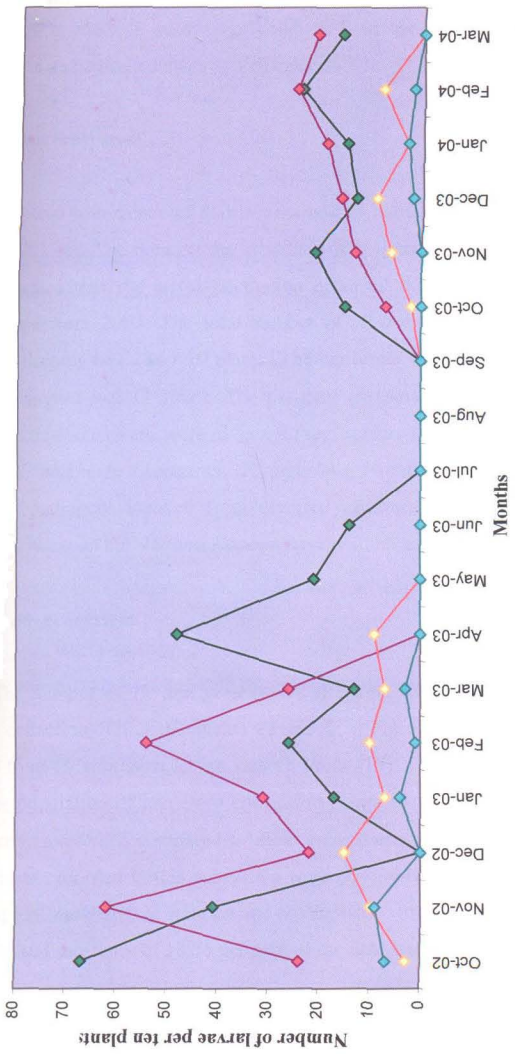


Fig.1. Seasonal fluctuation of *Helicoverpa armigera* in Vegetable ecosystem

management of any pest, knowledge of its interaction with its host plant is necessary. Keeping this in view, a study was conducted on the seasonal occurrence of *H. armigera* and its natural enemies at Vellanikkara.

5.1.1 Tomato ecosystem

Seasonal occurrence of *H.armigera* and its natural enemies were observed in tomato ecosystem. The data on the incidence of *H. armigera* (Table 1. ; Fig. 1.) on tomato revealed that the larval population occurred throughout the survey period except July – Sept 2003. The total number of *H. armigera* larvae recorded in 18 monthly collection was 388 / 10 plants. The important tachinid parasitoid recovered from *H. armigera* was *C. illota.* The per cent parasitism was 6.18. The predators recorded in this ecosystem were *C. carnea* and spiders (*O. sunandae, O. shweta, C. citricola, N. mukherji, Lycosa* sp., *P. lugerbris, P. paykulli, Theridion* sp. and *C. feae*). The pathogens isolated from diseased cadavers of *H. armigera* were the fungus – *N. rileyi* and protozoa - *Nosema* sp.

5.1.2 Bhendi ecosystem

The number of *H. armigera* recorded during the survey period was in 18 monthly collection 320 / 10 plants (Table 2. ; Fig. 1.). The tachinid parasitoid emerged from *H. armigera* larvae was *C. illota* (17) . The per cent parasitism was 5.30. The population of predatory coccinellid *C. sexmaculata* was found to be more in this ecosystem when compared to other ecosystems. The per cent predators was 5.31. Spiders recorded in this ecosystem were *O. sunandae, O. shweta, C. citricola, Lycosa* sp., *N. mukherji, N. nautica* and *N. elliptica.* Protozoan infection was severe in the field and mortality of 13.75 per cent of the field larval populations.

5.1.3 Bittergourd ecosystem

Bittergourd ecosystem recorded 89 numbers of larvae from10 plants during the survey period (Table 3. ; Fig.1.). The parasitoid recorded in this ecosystem was *A. taragamae*. The incidence of this parasitoid was rare. The per cent parasitism was 2.24. Only few grubs of *C. sexmaculata* were observed in this ecosystem. The per cent predators was 5.61. There was no incidence of disease infection in *H. armigera* larvae.

5.3.4 Cowpea ecosystem

The population of *H. armigera* (33/10 plants) was found to be low in this ecosystem (Table 4. ; Fig. 1.) The only natural enemy recorded in this ecosystem was the coccinellid *C. sexmaculata.*

The graph (Fig. 1.) shows the importance of seasonality. The period between April to October is remarkably pest free. It is safe for us to cultivate tomato or bhendi during this season. During rainy season we can go for protected cropping with plastic cover. The results indicate a definite preference of *H. armigera* larvae for tomato. Cowpea is the least preferred crop. That may be due to interspecific competition with other pod borers and very high population of natural enemies especially coccinellids. The normal feeding pattern of tomato fruit borer *H. armigera* is by putting its head inside and abdomen outside, but interestingly the observation here is that the larva was fully inside the fruit from third instar onwards. This is a very interesting ecological adaptation made by the pest.

Cowpea and bittergourd ecosystems do not have many spiders when compared to bhendi and tomato ecosystems. The population of *H. armigera* was

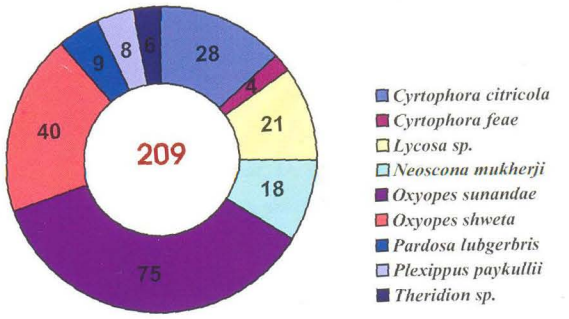


Fig.2. Abundance of predatory spiders in tomato ecosystem during Oct 2002- Mar 2004

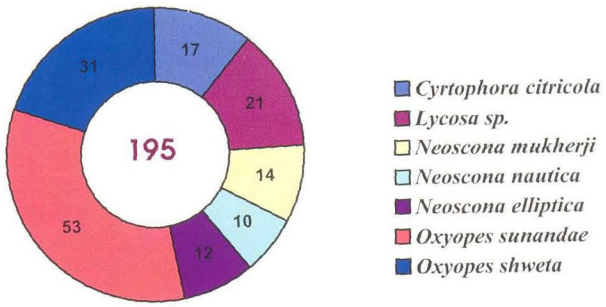


Fig.3. Abundance of predatory spiders in bhendi ecosystem during Oct 2002 - Mar 2004

found to be more in tomato ecosystem in spite of high spider population. This poses a very important question. What do the spiders do?. Do they predate *H.armigera* or do they feed the natural enemies? (Fig. 2. and 3.). Dhulia and Yadav (1991) reported that spiders *O. ratnae*, *Clubiona* sp. and *Thomisus* sp. predated on larvae of *Anomis flava* and *H. armigera* in cotton ecosystem. The spiders found to be predating on *H. armigera* in pigeon pea ecosystem were *O. ratnae*, *O. shweta*, *Neoscona* sp. and *P. paykulli* (Borah and Dutta, 2003). A further work should be based on host and prey preference of spiders and their role in the control of *H. armigera* and other pests under simulated natural environment in the laboratory.

Among the four ecosystems, tomato recorded the highest number of *H.armigera* larvae followed by bhendi, bittergourd and cowpea (Fig 4.). In nature, selection of plants for oviposition appears to be dictated by chemical receptors, the female being attracted by chemical cues emitted by plants (Lozina, 1946).

Figure 5 indicates the importance of predators in the cowpea ecosystem. The total number of predators was found to be more than the number of *H.armigera*. As cowpea has a balanced ecosystem and supports large numbers of natural enemies that discourage the *Helicoverpa* population, it will be prudent to cultivate cowpea or bittergourd around tomato crop in inter cropping systems and not to go for bhendi. The least amount of natural enemies was noticed in tomato ecosystem. This indicates the non-preference or unfavourable situation for natural enemies in tomato crop.

The species of natural enemies recorded on *H. armigera* during the survey period were *Carcelia illota, Apanteles taragamae, Chrysoperla carnea, Nosema* sp. and *Nomuraea rileyi.*

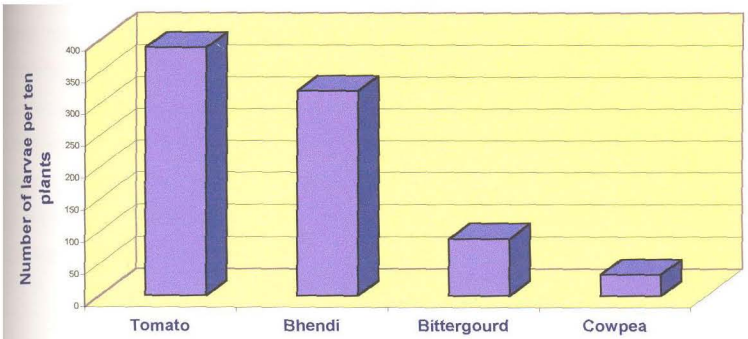


Fig. 4. Population of *Helicoverpa armigera* in vegetable ecosystem

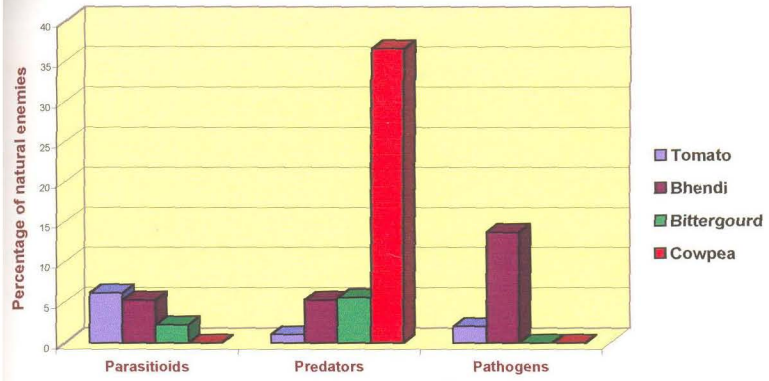


Fig. 5. Occurrence of natural enemies of *Helicoverpa armigera* in vegetable ecosystem

Previous workers have also reported various natural enemies; *viz*; *C. illota* on pupal mortality of *H. armigera* (Koshiya and Patel, 1987), larval parasitoids *C.chloridae* and *C.illota* on *H.armigera* (Singh *et al.*, 1982), *Trichogramma* sp., *C.chloridae, C.illota* and *Hexameris* sp. on tomato (Krishnamoorthy and Mani, 1990) and the tachinids, *C.illota* and *Exorista xanthopsis* providing about 24.54 per cent parasitism on sun flower (Patel and Talati, 1987). The wasps *Delta* sp. and chrysopid, *Chrysoperla* sp. have been observed to be important predators of *H.armigera* (Manjunath *et al.*, 1990).

The entomopathogenic fungus *Nomuraea rileyi* is an important natural control agent of many lepidoptera through out the world (Fuxa, 1984) and is frequently observed in soybean ecosystem causing natural epizootics of at least six major caterpillar pests (Puttler *et al.*, 1976). In India, though the occurrence of *N. rileyi* under natural condition has been reported from *H. armigera* on tomato (Gopalakrishnan and Narayanan, 1988), the occurrence of this fungus in Kerala has not been reported earlier.

Hence this is the first report of this fungus on *H.armigera* from Kerala. Similar observations on the occurrence of *N. rileyi* on *H.armigera* was reported by Gopalakrishnan and Narayanan (1989) on *Cajanus cajan, Phaseolus vulgaris* and tomato, Hugar and Hegde (1996) on sorghum, Singh (1999) on cotton and chickpea and Manjula *et al.* (2003) on cotton, chilli, tomato, red gram, black gram and groundnut.

It can be envisaged that the crop played a vital role in the activity and behaviour of natural enemies, as the natural enemies were recorded throughout the year in varying levels except for a few months and this depended on the availability

of the suitable host crop for the pest. The availability and proper exploitation of native natural enemies will reduce the pesticide pressure on the pest.

Our control strategies should include methods to conserve or enhance existing natural enemies as naturally occurring beneficial arthropods can maintain pest populations at sub economic levels. In order to find out the efficacy of naturally occurring beneficial arthropods, appropriate sampling techniques, their behaviour, seasonal abundance and influence of environment and host plants are to be determined.

5.2 SCREENING OF BOTANICALS AGAINST THIRD INSTAR LARVAE OF *H. armigera*

Integration of the use of botanicals with entomopathogens is a novel approach. Since both the components are ecologically non disruptive and safe to higher animals and man, they can be used in IPM without the fear of undesirable side effects.

In the present investigation, three plant species *viz.*, *Acorus calamus*, *Andrographis paniculata* and *Vitex negundo* and four essential oils *Citronella winterianus, Cymbopogon flexuosus, Kaempferia galanga* and *Cymbopogon martini* were screened at different concentrations (one, two and half and five per cent) for their ability to improve the mortality in *H.armigera* due to NPV, *B. thuringiensis* and entomopathogenic fungi.

Fig. 6. Efficacy of aqueous plant extracts against *Helicoverpa armigera*

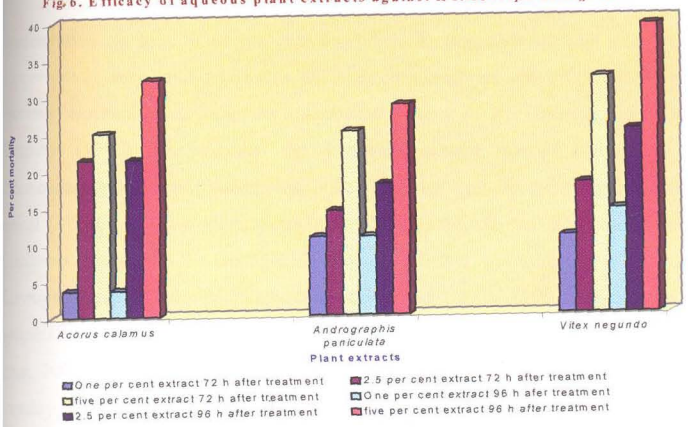
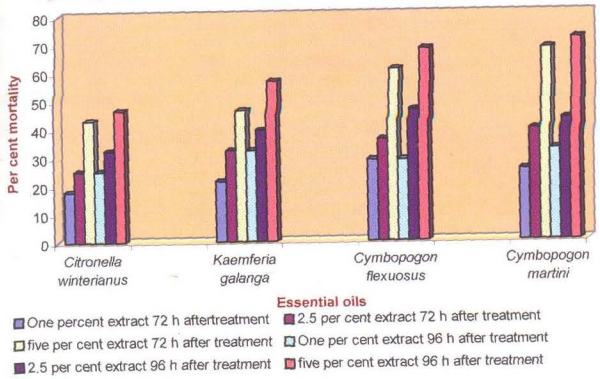


Fig.7. Efficacy of essential oils (emulsions) against *Helicoverpa armigera*



5.2.1 *A. calamus* (Vayambu)

The rhizome extracts of *A. calamus* were found to be toxic at five per cent concentration causing 32.14 per cent mortality. At two and half and one per cent concentration, they caused less than 20.0 per cent mortality only (Table 8. ; Fig. 6.). Previous workers have reported insecticidal action of *A. calamus*. Behera and Satapathy (1996) observed that the *A.calamus* extract induced highest per cent abnormalities due to morphogenetic effect both by leaf dip (38.60 per cent) and topical (37.30 per cent) applications in treated *S. litura* larvae. Rhizome extract of *A.calamus* caused 57.77 and 72.22 per cent larval mortality of *S.litura* and *H.armigera* respectively (Venkadasubramanian and David, 1999). On *S.litura* and *Lipaphis erysimi*, the extract caused more than 30 per cent mortality (Desai and Desai, 2000). At 0.4 per cent, *A. calamus* extract gave 63.3 per cent accumulated mortality of *P. xylostella*, within 48 h (Jiyavorranant *et al.*, 2003).

5.2.2 *A. paniculata* (Kiriyathu)

At five per cent concentration, *A. paniculata* recorded the maximum mortality of 28.56 per cent (Table 8. ; Fig. 6.). Other concentrations produced less than 20.0 per cent mortality. The main constituent of *A.paniculata* has been isolated and established as andrographolide (Moktadar and Guha-sincar, 1939 and Chakravarthi and Chakravarthi, 1952).

Insecticidal activity of *A. paniculata* extracts has been reported against several pests. A *paniculata* extracts had higher antifeedant activities on third instar larvae of *S.litura* (Gunasekaran and Chelliah, 1985). In laboratory studies, extracts of *A. paniculata* acted as an antifeedant to *P. xylostella*, and as an oviposition deterrent to *C. chinensis* (Hermawan *et al.*, 1993; Hermawan *et al.*, 1994; Hermawan *et al.*,

1998). Ethyl acetate fraction was found to be possessing highest ovipositional deterrency against *S. obliqua* and the methanol fraction of *A. paniculata* extracts had the highest growth inhibitory activity on larval and adult stages of *S. obliqua* according to Tripathi *et al.* (1999).

5.2.3 *V. negundo* (Karinotchi)

Among the plant extracts tested, *V. negundo* recorded the highest mortality of 39.27 percentages at five per cent strength (Table 8. ; Fig. 6.). Bai and Kandasamy (1985) reported that acetone extracts of *V.negundo* exhibited 100 per cent mortality on *S.litura* at 0.05 per cent level. The essential oil extracted from dried leaves of *V.negundo* was found to contain terpenes, cinole1, sabinene and sesquiterpenes (Itikawa and Yamastia, 1940 and Manalo, 1982). Viteralones and phenolic glucosides have been isolated from *V.rotundifolia* (Tada and Yasuda, 1984 and Kouno *et al.*, 1988). Sahayaraj (1998) reported the morphogenetic effects of *V.negundo* extracts on *S.litura* larvae.

The aqueous extracts are not very highly effective and they cause only about 30 to 40 per cent mortality. The plants are easily available and can be recommended for the management of vegetable and fruit crop pests. Even though the extract cannot act as insecticide on its own, there is a potential to mix it with microbial pesticides. The farmers at farm level can directly adopt this technology without any infrastructural facilities and at a low cost.

5.2.4 *C. winterianus* (Citronella oil)

Among the three concentrations tested for their insecticidal action against third instar larvae of *H. armigera*, the highest mortality per cent 46.42 was recorded

at five per cent concentration (Table 9. ; Fig. 7.). Previous workers have also reported insecticidal action of *C. winterianus*. Dale and Saradamma (1981) tested eight essential oils of plant origin (citronella, palmarosa, geranium, eucalyptus, wintergreen, patchouli, citriodora and camphor oils) against third instar larvae of *Pericallia ricini* at concentrations of two and half, five and ten per cent on castor leaves. All the oils had some antifeedant properties. Rao *et al.* (2000) evaluated *C. winterianus* oil at 0.5 per cent against second instar larvae of *H.armigera*. Significant antifeedant activity was recorded compared to untreated control. *C. winterianus* oil at 0.5 per cent strength exhibited moderate ovipositional deterrency and ovicidal action. At three and five per cent concentration it had higher leaf protection (65.37 and 85.05 per cent) and an LC_{50} value of 370 ppm on the third instar larvae of *S.litura* (Suresh, 2002).

5.2.5 *K. galanga* (Kacholam oil)

The kacholam oil (*K. galanga)* caused more than 50.0 per cent mortality of *H. armigera* larvae at five per cent concentration (Table 9. ; Fig.7.). Other concentrations caused less than 40.0 per cent mortality only. Rhizomes of *Curcuma xanthorrhiza, C. zeodoaria, K. galanga* and *K. pandurata* were analysed for insecticidal constituents (Pandji *et al.* 1993). They reported that sesquiterpenoids, xanthorrhizol and furanodienone have pronounced toxicity against neonate larvae of *S. littoralis*. Suresh (2002) reported the ovicidal action of kacholam oil at 0.1 and 0.5 per cent strengths (43.32 and 79.60 per cent respectively). Kacholam oil induced very high larval starvation on *S.litura* (62 to 82 per cent) at three and five per cent concentrations respectively. It greatly affected the assimilation of ingested and digested food and induced pupal and adult malformations when applied on the third instar larvae of *S.litura*.

5.2.6 *C. flexuosus* (Lemongrass Oil)

The highest mortality per cent 67.85 per cent was observed at five per cent concentration after 96 h of treatment (Table 9. ; Fig. 7.). Rajapakse and Jayasena (1991) observed that treatment with lemongrass oil reduced the damage caused by *S.litura* on peanut. Rao *et al.* (2000) reported that *C. flexuosus* oil at 0.5 per cent caused significant reduction in larval weight and adult emergence of *H.armigera.* Suresh (2002) reported the ovipositional deterrency and ovicidal action of *C. flexuosus* at 0.5 per cent. He also observed higher adult malformation of *S.litura* at one per cent concentration.

5.2.7 *C. martinii* (Palmarosa oil)

Among the four essential oils tested for their insecticidal action, *C. martinii* recorded the highest mortality per cent 71.42 at five per cent concentration (Table 9. ; Fig. 7.). Citronella oil and palmarosa oil gave total protection to groundnut pods by inhibiting oviposition by the bruchid for six months with an efficacy equal to that of malathion dust (Kumari *et al.*, 1998). Venkadasubramanian and David (1999) reported that the palmarosa oil at one per cent caused 91 and 100 per cent mortality of third instar larvae of *H. armigera* and *S. litura* respectively. *C.martini* at 0.5 per cent resulted in significant reduction in larval weight of *H.armigera* and adult emergence (Rao *et al.*, 2000). The palmarosa oil exhibited highest insecticidal toxicity with LC_{50} value of 178 ppm against *S.litura* larvae (Suresh, 2002).

All the essential oils are causing more than 50 per cent mortality except citronella oil. Palmarosa oil at five per cent concentration recorded the highest per cent mortality. Hence it was selected for further field studies.

5.3 COMPATIBILITY OF BIOCIDES AND PESTICIDES

Compatibility with chemical insecticides and botanicals is important in the effective usage of entomopathogens in pest management systems. It is essential that pathogens are not inactivated by chemicals in tank mixes of sprays or by deposits of chemicals on the plant. Enhanced effectiveness through joint action of pathogens and chemical pesticides, especially insecticides, is particularly interesting because the reduced amount of chemical insecticide required for crop protection would reduce chemical contamination of the field habitat and permit maximum impact of predaceous and parasitic arthropods on pest species.

The information on the sensitivity of different isolates of fungi and bacteria to insecticides and botanicals was required for interaction studies. In the present study, the insecticides and botanicals at the recommended doses for *H.armigera* management were evaluated against entomopathogenic fungi *viz.*, *N. rileyi*, *B. bassiana* and *M. anisopliae* and bacteria *B. thuringiensis*.

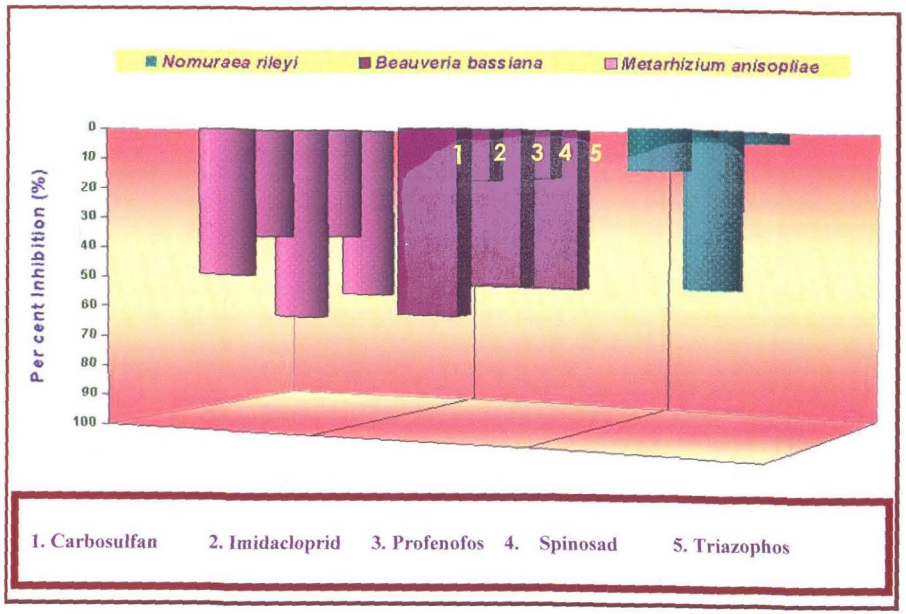
5.3.1 *Nomuraea rileyi*

5.3.1.1 N. rileyi and insecticides

Among the insecticides profenofos brought about the maximum inhibition of fungal growth. The per cent inhibition brought about by spinosad, imidacloprid, carbosulfan and triazophos was below 20.0 per cent. Among these spinosad recorded the least per cent inhibition of 1.2 (Table 10. ; Fig. 8.).

Ignoffo (1981) studied the compatibility of 44 chemical pesticides with *N.rileyi*. The three most inhibiting insecticides were monocrotophos, phenthoate and

Fig.8. Per cent inhibition of growth of entomopathogenic fungi by insecticides



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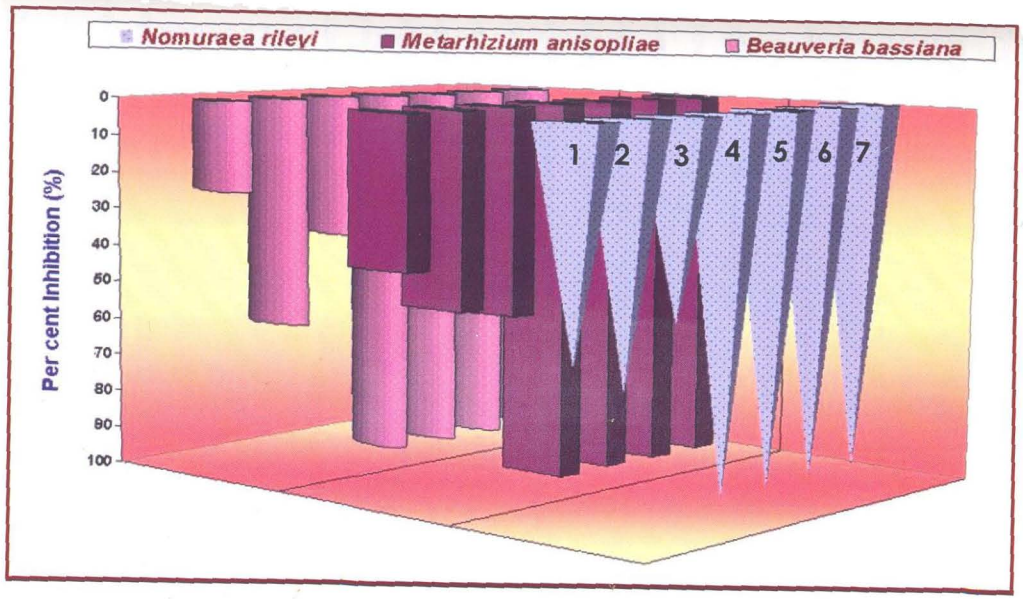
methyl parathion. The insecticides compatible with *N. rileyi* in *in vitro* tests were pyroxychlor, acephate, carbaryl, carbofuran, DBCP, DDT, dimilin, endrin, methoxychlor, bentazone, chlorbromuron, chloroxuron, dalapon-Na, metribuzin, naptalam and trifluran.

Monocrotophos, phosphamidon and dimethoate were safe to *N. rileyi* at all concentrations tested. Quinalphos (0.025), carbaryl (0.025), endosulfan (0.035) and fenvalerate (0.005) were safe at low concentrations and they were highly detrimental to the fungus at higher concentrations (0.075, 0.150, 0.106 and 0.015) respectively (Gopalakrishnan and Mohan, 2000).

5.3.1.2 N. rileyi and botanicals

In this study, it was found that under laboratory condition the essential oils (*C. winterianus, C. flexuosus, K. galanga* and *C. martinii*) were least compatible with *N. rileyi*. The botanicals were not as deleterious as the essential oils. Among them, the descending order in terms of rate of inhibition was *A. calamus, A. paniculata* and *V. negundo* (Table 11. ; Fig. 9.).

Devi and Prasad (1996) in their compatibility test of seed kernel extracts from *A. indica, Melia azedarach* and *Pongamia pinnata,* whole plant extract from *Tephrosia purpurea, Parthenium hysterophorus* and *Cleome viscosa* and vegetable oils from sunflower, safflower, groundnut, rapeseed, sesame, coconut and cotton seed with the entomogenous fungi *N. rileyi*. They reported that none of the oils were detrimental to the fungus. Devi *et al.* (2002) reported that neem did not inhibit the growth and sporulation of *N. rileyi.*



1. *Acorus calamus*
2. *Andrographis paniculata*
3. *Vitex negundo*
4. *Citronella winterianus*
5. *Kaempferia galanga*
6. *Cymbopogon flexuosus*
7. *Cymbopogon martinii*

5.3.2 *Beauveria bassiana*

5.3.2.1 B. bassiana and insecticides

Among the insecticides, spinosad and profenofos recorded less than 20.0 per cent inhibition of growth of *B. bassiana*. The other insecticides tested inhibited the growth of *B. bassiana*. The per cent inhibition was more than 50.0 per cent (Table 12. ; Fig. 8.). Aguda *et al.* (1984) evaluated the effect of insecticides on the germination of *B. bassiana* spores. The insecticides *viz.*, monocrotophos, BPMC, carbosulfan and azinphos-ethyl inhibited spore germination of *B. bassiana*. The BPH resurgence causing insecticides deltamethrin and methyl parathion also greatly reduced spore germination. Moino and Alves (1998) evaluated the effect of imidacloprid and fipronil on *B.bassiana*. Imidacloprid was less toxic to the fungi than fipronil. *In vitro* fungitoxic effect of the neonicotinoid insecticides acetamiprid, imidacloprid and thiamethoxam to *B.bassiana* showed that use of insecticides in the recommended formulations had no negative effect on conidia germination, conidia production and vegetative growth of *B.bassiana* (Neves *et al.*, 2001). Xu *et al.* (2002) reported that imidacloprid 10 WP, Yashiling 22 WP (a mixture of imidacloprid and buprofezin), methomyl 20 EC, triazophos 20 EC and fipronil 5 FF exhibited high compatibility with the fungus.

5.3.2.2 *B. bassiana and botanicals*

The essential oils *viz.*, *C.winterianus, C.flexuosus, K.galanga* and *C.martinii* completely inhibited the growth of fungus. The plant extracts namely, *A. calamus*, *A. paniculata* and *V. negundo* caused more than 50.0 per cent inhibition (Table 13.; Fig. 9.). Devaprasad *et al.* (1989) studied the effect of certain botanicals *viz.*, *O. sanctum*,

A. sativum, A. calamus, T. terrestris as well as neem seed kernel extract and neem oil on the conidial germination of *B.bassiana*. They have also reported neem oil and neem seed kernel extract were deleterious to the spore germination *B. bassiana*.

5.3.3 *Metarhizium anisopliae*

5.3.3.1 M. anisopliae and insecticides

All the insecticides included in the study inhibited the growth of *M. anisopliae*. Inhibition in the descending order was with spinosad, imidacloprid, carbosulfan, triazophos and profenofos (Table 14. ; Fig. 8.) Aguda *et al.* (1984) reported that the insecticides *viz.*, monocrotophos, BPMC, carbosulfan and azinphos ethyl inhibited spore germination of *M.anisopliae*. Moino and Alves (1998) found that imidacloprid was less toxic to the fungi *M.anisopliae* than fipronil. The neonicotinoid insecticides acetamiprid, imidacloprid and thiamethoxam (Neves *et al.*, 2001) monocrotophos, chlorpyrifos and azadirachtin (Gupta *et al.*, 2002) were well tolerated by the fungus *M.anisopliae*.

5.3.3.1 M.anisopliae and botanicals

The essential oils completely inhibited the growth of *M. anisopliae*. The plant extracts also exhibited significantly greater inhibition (Table 15. ; Fig. 9.). Neem oil at five per cent concentration inhibited germination and sporulation of *M.anisopliae* (Aguda and Rombach, 1986). Nicotine sulphate, and Repellin inhibited the growth of *M.anisopliae*. But Neemark did not inhibit the growth (Vyas *et al.*, 1992).

In the present investigation, the green muscardine fungus *M. anisopliae* was more sensitive to insecticides when compared to *B. bassiana* and *N. rileyi*. The

fungus *N. rileyi* was compatible with all the insecticides except profenofos. The insecticide profenofos was incompatible with all the three fungi as its per cent inhibition was more than 50.0.

All the three fungi exhibited complete inhibition of growth when exposed to essential oils. Among the three fungi, the white muscardine fungus, *B. bassiana* was more sensitive to plant extracts. Among the plant extracts, *A. calamus* caused more than 50.0 per cent inhibition followed by *A. paniculata*.

5.3.4 *Bacillus thuringiensis*

5.3.4.1 B. thuringiensis and insecticides

The insecticides namely, carbosulfan, imidacloprid, profenofos, spinosad and triazophos were compatible with the commercial formulation of *B. thuringiensis* (Halt, Delfin and Dipel). Most insecticides were found compatible with *B. thuringiensis,* having little or no effect on spore germination or cell multiplication. Low concentrations of carbamates (carbaryl and carbofuran) and organophosphates (diazinon, malathion and phorate) did not affect the bacterial growth, whereas others, especially the chlorinated hydrocarbons (DDT, aldrin and heptachlor) inhibited the growth (Sutter *et al.*, 1971).

5.3.4.2 B. thuringiensis and botanicals

The study clearly indicated that the botanicals *viz.*, *A. calamus, A. paniculata, V. negundo, C. winterianus, C. flexuosus, K. galanga* and *C. martini* were safe and compatible with commercial formulation of *B. thuringiensis*. The neem preparations Neemazal–F and Neemazal–T/S and their active neem ingredient Neemazal pulvar,

were screened *in vitro* for the presence of antibacterial activity. Neemazal –F and a one per cent Neemazal pulvar solution (3000 ppm azadirachtin) inhibited the growth of *B. cereus, B. mycoides, B. thuringiensis* and *B. subtilis* (Coventry *et al.*, 1997).

The present study on compatibility has indicated that the botanicals and insecticides did not play much effect on *B. thuringiensis*. The essential oils completely inhibited the growth of three entomopathogenic fungi. So the essential oils were not included for the interaction studies involving fungi. The plant extracts also reduced the growth of fungi to some extent. Because of this, half doses of all the plant extracts and insecticides were used in the interaction studies. All the essential oils and plant extracts were compatible with *B. thuringiensis*, so they were included in interaction studies with *B.thuringiensis*. However their doses were reduced to half.

5.4 INTERACTION STUDIES ON BIOCIDES AND INSECTICIDES

Compatibility of microbial pesticides with chemical pesticides and botanicals is of special concern because of increasing interest in using them together in IPM. Chemical pesticides and microbial agents used in combination are reported to be effective in field due to the synergistic effect. Such combinations may also help to reduce the mean lethal time (LT_{50}) of microbial agents. The integration of chemical insecticides with biological one is recognised as an effective means to reduce the quantity of insecticide used in field which would in turn reduce environmental pollution, resistance development and harmful effects on non target organism.

Though considerable information has been gathered on the use of microbial agents (virus, bacteria and fungi) combined with insecticide, the studies on new molecules are limited. Hence investigation was taken up to gather the information on the possibility of combining microbial pesticides with new molecules and botanicals.

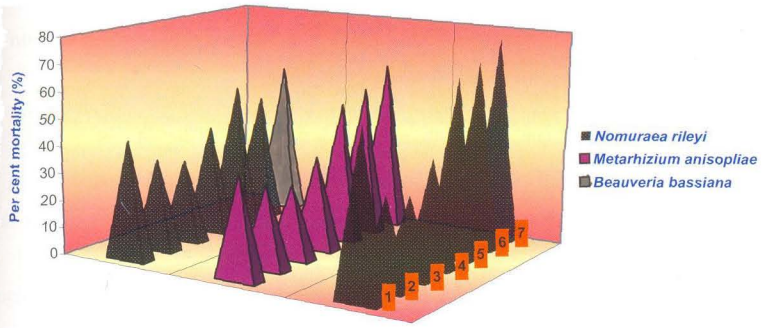


Fig.10. Effect of entomopathogenic fungi and plant extracts against third instar larvae of *Helicoverpa armigera*

1.Fungi alone 2. *Acorus calamus* 3. *Andrographis paniculata*
4.*Vitex negundo* 5. Fungi + *A. calamus* 6. Fungi + *A. paniculata* 7. Fungi + *V. negundo*

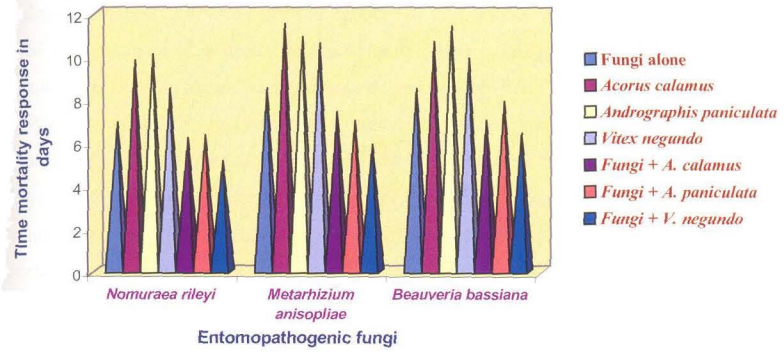


Fig. 11. Effect of entomopathogenic fungi and plant extracts on time mortality response (LT 50) against *Helicoverpa armigera*

5.4.1 Entomopathogenic fungi with plant extracts and insecticides

5.4.1.1 Entomopathogenic fungi and plant extracts

In the present investigation, *N. rileyi* +*V. negundo, M. anisopliae* + *V. negundo* and *B. bassiana* + *V. negundo* recorded the maximum mortality per cent (Table 16, 18, 20. ; Fig. 10.). The plant extracts when combined with entomopathogenic fungi *viz., N. rileyi, B. bassiana* and *M. anisopliae* showed an increase in the per cent mortality of *H. armigera* larvae. It is probable that the aqueous extracts of botanicals might have predisposed the larvae for infection and thereby enhanced the efficacy of the fungi. There was also a reduction in mean lethal time (LT_{50}) when both entomopathogenic fungi and plant extracts were combined (Fig.11.).

Among the three fungi, *N. rileyi* was found to be more virulent than *B. bassiana* and *M. anisopliae* against third instar larvae of *H. armigera.* Botanicals are known to inhibit certain vital enzymes resulting in the disruption of digestive physiology of insects and cause general weakness. The results of the present study revealed the existence of supplemental synergism between entomopathogenic fungi and plant extracts of *A. calamus, A. paniculata* and *V. negundo* (Table 16a, 18a and 20a). Patil *et al.* (2003) reported that combinations of *N. rileyi* with botanicals performed better than individual treatments. They had reported that botanicals with *N. rileyi*, NSKE (five per cent) + *N. rileyi* ($2x10^{11}$ conidia /l) and *V. negundo* (five per cent) + *N. rileyi* ($2x10^{11}$ conidia/l) proved to be as effective as recommended insecticides in reducing larval incidence of *S. litura* and defoliation and recorded higher yield of ground nut.

The results of the present study give very good support for a strategy to encourage the use of combination of botanicals and fungi. The fungi when used alone caused about 60 per cent mortality. The other plant extracts caused only about 30 to 40 per cent mortality. When these were combined at half their doses, about 60 to 70 per cent mortality was achieved. More importantly, *V. negundo* combination reduced the LT_{50} substantially. Under field conditions, advancing the mortality even by few hours is going to reduce the damage to foliage/fruit in geometric proportion.

The supplementary synergism that is being exhibited when entomopathogenic fungi and plant extracts were combined is a result, which can be directly passed on to the field. Further study is required to find out various combinations involving plant extracts and microbial agents. The commercial formulation has to be developed considering their stability in storage for longer duration. These products can be used in farming situations where the insecticides cannot be sprayed, or in organic farming and in export oriented cultivation.

5.4.1.2 Entomopathogenic fungi and insecticides.

From the present findings, it is evident that there is a possibility of positive interaction between the insecticides and entomopathogenic fungi for enhanced bio efficacy against *H. armigera*. Hiromori and Nishigaki (1998) suggested that the joint action between *M. anisopliae* and synthetic insecticides resulted from easy penetration of this fungus under conditions of distribution or decline of the larval defence system caused by the existence of a small quantity of insecticides.

Among the different combinations of entomopathogenic fungi with insecticide *viz.*, *N. rileyi*+ spinosad, *B. bassiana*+ spinosad and *M. anisopliae* + spinosad recorded the highest mortality per cent (Table 17,19,21. ; Fig. 12.). A

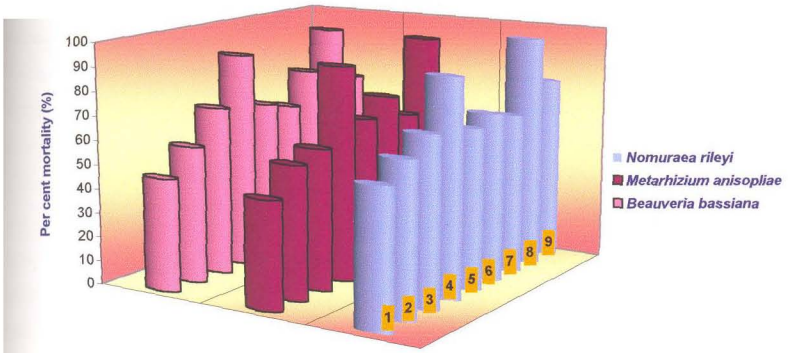


Fig 12. Effect of entomopathogenic fungi and insecticides against third instar larvae of *Helicoverpa armigera*

1.Fungi alone	2.Carbosulfan	3. Profenofos	4. Spinosad	5. Triazophos
6.Fungi + carbosulfan	7. Fungi + profenofos	8. Fungi + spinosad	9. Fungi + triazophos	

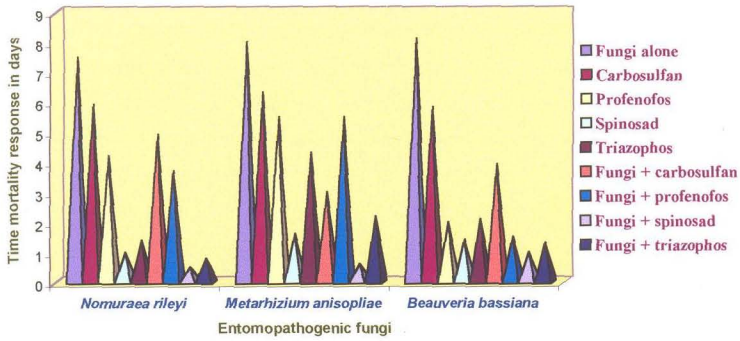


Fig 13.Effect of entomopathogenic fungi and insecticides on time mortality response (LT50) against *Helicoverpa armigera*

drastic reduction in LT_{50} value was noticed in combination treatments (Fig.13.). The decrease in LT_{50} over that of individual treatment can be attributed to synergistic action of the insecticide. Effects produced by the combined activity of entomopathogenic fungi and insecticides could be categorised as sub additive synergism and supplemental synergism (Table 17a, 19a and 20a). The supplemental synergism was observed when the larvae were treated with entomopathogenic fungi and insecticides, *viz.*, profenofos, spinosad and triazophos. When the third instar larvae were treated with entomopathogenic fungi and carbosulfan, it resulted in sub additive synergism.

The interaction between *B. bassiana* and mineral oil was evaluated by Batistafilho *et al.* (1995) in order to control the banana plant borer, *Cosmopolites sordidus* (Gem). These investigators observed an additive effect of the combination, which caused about 98 per cent adult insect mortality compared to 70 per cent caused by the fungus alone and 33 per cent by mineral oil alone.

Neves *et al.* (2002) reported the synergistic interaction between the entomopathogenic fungi, *B.bassiana* and *M.anisopliae* and commercial insecticides *viz.*, DDT and triflumuron and its positive implications in integrated pest management. The application of fungus *N.rileyi* at $1.6x10^8$ spores /ml along with endosulfan (0.035 per cent) gave good control of *H. armigera*, *S. litura* and *Trichoplusia ni* on cabbage. The treatment combination increased the yield also (Gopalakrishnan and Mohan, 2003).

These results have a direct implication for the field. The fungal preparation of *N.rileyi*, *B. bassiana* and *M.anisopliae* are effective about 50 per cent only. Insecticides are effective in bringing in mortality of to the tune of about 70 per cent roughly except spinosad, which is highly effective. Being a new product, it has not

shown any resistance to insects in India. But resistance has been reported from outside the country, (Moulton *et al.*, 1999; Toshio and Scott, 2003) and it may only a matter of time before resistance is developed. But possibility of resistance to a combination is remote because of two different modes of action. The present results have given a good combination involving fungi and new product like spinosad. This is going to reduce the cost of combined application of the insecticide and fungi. *B.bassiana* and *M.anisopliae* are available as commercial products and hence it can be used along with spinosad. Moreover the spectacular reduction in LT_{50} has direct benefit to farmers. The Fungi on its own, take eight days for 50 per cent mortality but the combination does it in half a day so that damage to the fruits/leaves will be minimal.

5.4.2 Commercial formulations of *Bacillus thuringiensis* with botanicals and insecticides

5.4.2.1 B. thuringiensis and plant extracts

The results obtained from the present study indicated that Delfin recorded the highest mortality per cent in combination with *V.negundo*. Among the bacterial formulations + plant extract combinations, Delfin + *V. negundo* recorded the highest mortality per cent with the entire three combination product (Table 22,23,24. ; Fig. 14.). There was a reduction in LT_{50} when larvae were treated with bacterial formulation and plant extracts combination (Fig. 15.). A study on effect of joint action of bacterial formulations and plant extracts showed supplemental synergism in all combinations (Table 22a, 23a and 24a).

There are no reports available on combinations involving *B.thuringiensis* and plant extracts namely, *A.calamus, A.paniculata* and *V.negundo*. Hellpap (1984)

Fig.14. Effect of commercial formulations of *Bacillus thuringiensis* and plant extracts against third instar larvae of *Helicoverpa armigera*

1.*Bacillus thuringiensis* alone 2.*Acorus calamus* 3.*Andrographis paniculata* 4. *Vitex negundo*
5. *B. thuringiensis* + *A. calamus* 6. *B. thuringiensis* + *A. paniculata* 7. *B. thuringiensis* + *V.negundo*

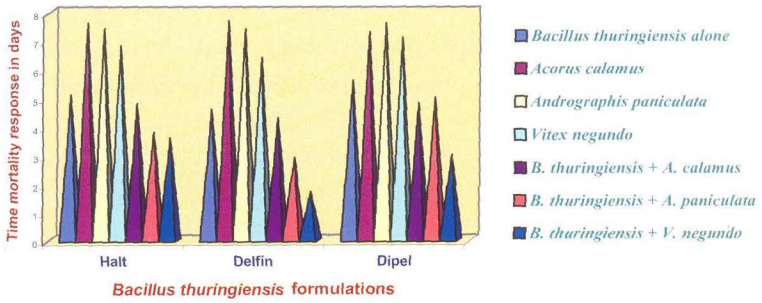


Fig.15. Effect of *Bacillus thuringiensis* and plant extracts on time mortality response (LT50) against *Helicoverpa armigera*

reported that a combination of the methanolic extract of neem seed kernel with *B.thuringiensis* increased the mortality of *S. frugiperda* larvae and reduced considerably the LT_{50} and LT_{100}. Hellpap and Zebitz (1986) found that the combination of neem seed extracts and *B.thuringiensis* had an additive effect in the control of *S. frugiperda* and *Aedes toigoi*. Justin *et al.* (1987) found that when *B.thuringiensis* was combined with neem seed kernel extract (five per cent), LC_{50} value was reduced by 1.9 times. Similarly, the combination of *B.thuringiensis* with *Catharanthus roseus* (three per cent) also resulted in a reduced LC_{50}.

Salama and Sharaby (1988) observed a marked increase in the potency of *B.thuringiensis* endotoxin preparation against *S. littoralis,* when combined with plant products like orange and pomegranate peel. Combination of neem products Neemazal-F one percent (1.26ml/l) and Neemazal –F 5 per cent (0.36ml/l) with *B.thuringiensis* products Delfin (0.25g/l) and Bio-asp (0.23g/l) at sub lethal concentrations resulted in increased mortality from 50 to 70 percent of *P.xylostella* (Jeyarani, 1995). Murugan *et al.* (1998) studied the combined effect of *B.thuringiensis* subsp. *kurstaki* (Dipel) and certain botanical insecticides on *H.armigera*. Mortality was significantly increased by pongamia oil with *B.thuringiensis*.

The results with commercial formulations of *B.thuringiensis* + plant extracts also gave the indications of enhanced synergism. There is also a drastic reduction in lethal time when *B.thuringiensis* and plant extracts are combined. Plant extracts take long time (six to seven days) for establishing the theoretical half mortality. The time mortality response was reduced from six to seven days to three to four days in combinations. The plant *V.negundo* was identified as the most important plant extract that can be combined with any of the commercial formulations. The plant extracts, which contain a number of compounds, may represent an additional stress

on insect system, which allows enhanced pathogen performance. The advantage of adding these plant extracts to the bacterial formulations is the potential to decrease the dosage of both the extract and the bacteria. The use of plant extracts, as an additive to the *B. thuringiensis* may be a promising approach because larval feeding and subsequent defoliation would be reduced greatly without interference with bacterial activity. The results may vary under field conditions depending upon the crop situation.

5.4.2.2 B. thuringiensis and essential oils

The studies made on the toxicity of commercial formulations of *B. thuringiensis* and essential oils to third instar larvae of *H. armigera* indicated that *C. martinii* recorded the highest mortality per cent followed by *C. flexuous, K. galanga* and *C. winterianus* (Table 25. ; Fig. 16.). The combination Halt + *C. martinii* recorded the highest mortality per cent followed by Halt + *C. flexuosus*. All the Halt + essential oil combinations produced supplemental synergism (Table 25a.).

Delfin when combined with essential oil showed an increase in the per cent mortality of the larvae and highest mortality was recorded in Delfin + *C. martinii* followed by *C. winterianus* (Table 26. ; Fig. 16.). Effects produced by the combined activity of Delfin and essential oil could be categorised as sub additive and supplemental synergism. Sub additive synergism was observed when the larvae were treated with Delfin + *C. flexuosus*. All the other Delfin+ essential oil combinations produced supplemental synergism (Table 26a.).

In the case of Dipel +essential oil combinations, Dipel + *C. martinii* recorded the maximum mortality and was closely followed by Dipel + *K. galanga* (Table 27 ;

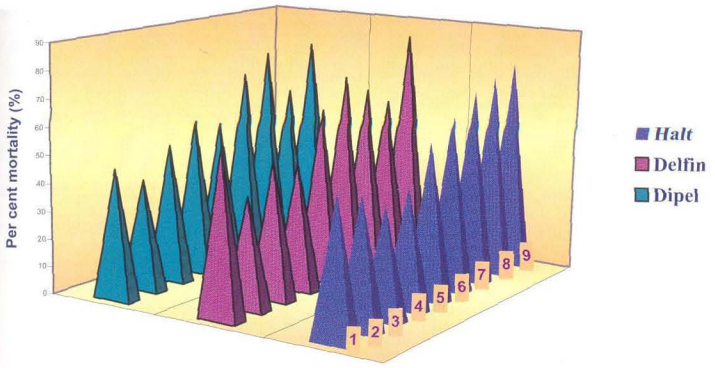


Fig. 16. Effect of commercial formulations of *Bacillus thuringiensis* and essential oils against third instar larvae of *Helicoverpa armigera*

1. *Bacillus thuringiensis* alone	2. *Citronella winterianus*	3. *Kaempferia galanga*
4. *Cymbopogon flexuous*	5. *Cymbopogon martinii*	6. *B. thuringiensis + C.winterianus*
7. *B.thuringiensis + K. galanga*	7. *B. thuringiensis+ C. flexuous*	8. *B. thuringiensis + C. martinii*

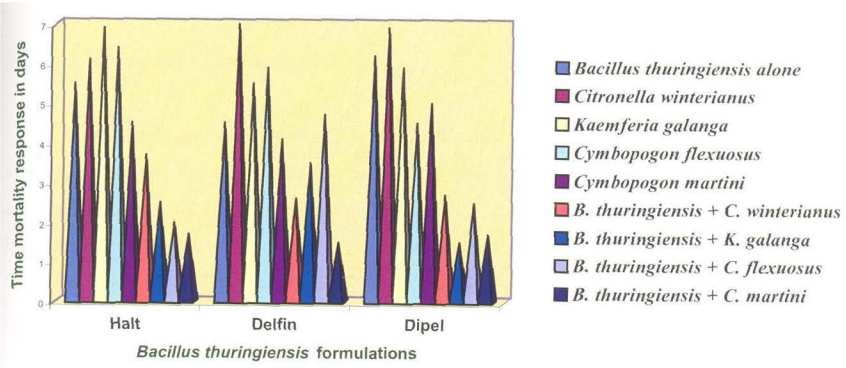


Fig.17. Effect of commercial formulations of *Bacillus thuringiensis* and essential oil on time mortality response (LT50) against *Helicoverpa armigea*

Fig.16.). The combination Dipel + *C. flexuosus* produced sub additive synergism. All the other combinations produced supplemental synergism (Table 27a.).

The study also revealed a drastic reduction in dose time mortality response (LT_{50}) in all the bacterial formulation (Halt, Delfin and Dipel) and essential oil combinations (Fig. 17.). The above results are in consonance with the findings of Venkadasubramaniam and David (1999) who reported the toxic effects of *B. thuringiensis* products and palmarosa oil against *S.litura* and *H. armigera*.

The essential oils are commercially available and more effective than the plant extracts. The farmer can go for any of the commercial formulations, as they are equally effective. The result indicated *C.martinii* (Palmarosa oil) as the best oil amongst the essential oils tested. But spectacular results were obtained with all the other oils (*C.winterianus*, *K.galanga* and *C.flexuosus*). The modes of action of essential oils are not known. Generally they are antifeedant and repellent in their action so that the insect is weakened due to starvation. This gives a better possibility for delta endotoxin of *B.thuringiensis* to act faster and the inherent resistance mechanism is reduced, thus paving the way for better pest control.

5.4.2.3 B. thuringiensis and insecticides

The results of the above study revealed the existence of supplemental synergism between bacterial formulations (Halt, Delfin and Dipel) and insecticides, *viz*., carbosulfan, profenofos, spinosad and triazophos (Table 28, 29, 30. ; Fig. 18.). Regarding the mortality per cent, bacterial formulation and spinosad recorded the maximum mortality. The mean lethal time could be decreased drastically in combinations when compared to individual treatments (Fig.19.).

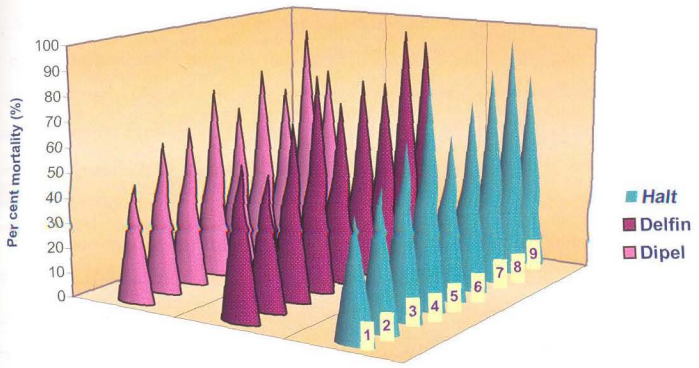


Fig.18. Effect of commercial formulations of *Bacillus thuringiensis* and insecticides against third instar larvae of *Helicoverpa armigera*

1. *Bacillus thuringiensis* alone 2. Carbosulfan 3. Profenofos 4. Spinosad
5. Triazophos 6. *B. thuringiensis* + carbosulfan 7. *B. thuringiensis* + profenofos
8. *B. thuringiensis* + spinosad 9. *B. thuringiensis* + triazophos

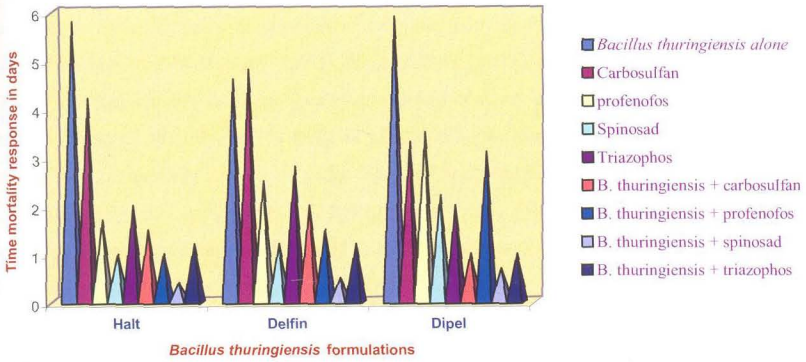


Fig.19. Effect of commercial formulations of *Bacillus thuringiensis* and insecticides on time mortality response (LT50) against *Helicoverpa armigera*

The possible mechanism for synergism might be due to the physiological stress of the insecticide treatment that might have debilitated and predisposed the insect to microbial infection. Sub lethal doses of insecticides caused leakage of the gut, since intestinal bacteria might enter the haemocoel within 24 h as in the case of *B. thuringiensis* and DDT (Benz, 1971).

Combinations of insecticides, quinalphos (310 ppm) and acephate (302ppm) with *B.thuringiensis* products *viz.*, Delfin (0.25g/l) and Bioasp (0.23g / l) at sublethal concentrations resulted in increased mortality from 50 to 70 per cent of *P. xylostella* (Jeyarani, 1995). Dipel (0.05 per cent) + chlorpyriphos (0.025 per cent) has also been proved superior and significantly effective by reducing 71.86 per cent population over control (Obulapathi *et al.*, 2000). Khaliqus and Ahmed (2001) reported the compatibility and synergism of *B. thuringiensis* and lambda-cyhalothrin against *H.armigera.*

Like fungi, insecticides in combination with *B.thuringiensis* too gave a fruitful result. *B.thuringiensis* formulations are effective causing mortality up to 60 per cent. Spinosad produced very good effect in this case also. The superior efficacy of spinosad in reducing larval population and tomato fruit damage caused by *H. armigera* was reported by Sathish (2003), Shobanadevi (2003) and Suganyakanna (2003). The combinations involving spinosad is almost cent per cent effective. This combination reduced the LT_{50} to 0.4 days. A treated larva would not cross a single day after the combined application of *B.thuringiensis* and insecticide. There is also an added advantage that the cadaver will function as a source for natural inoculum. These combinations produced immediate and also long-term control.

5.4.3 Nuclear polyhedrosis virus with botanicals and insecticides

5.4.3.1 Nuclear polyhedrosis virus and plant extracts

The results of the present study revealed the existence of supplemental synergism between nuclear polyhedrosis virus and plant extracts (Table 31a.). The combination, NPV + *V. negundo* recorded the highest per cent mortality (Table 31.; Fig. 20.) This is in conformity with earlier reports with other plant products. Nicotine sulphate and pyrethrum were found to produce additive to potentiation effect on the NPV in *S. litura* larva (Choudhari and Ramakrishnan, 1983). Devaprasad *et al.* (1989) had also found that the addition of methanol fractions of *O. sanctum* and *A. calamus* at 0.01per cent increased the efficacy of NPV against third instar larvae of *H. armigera* and *S. litura.*

Rabindra *et al.* (1994) found that aqueous leaf extracts of *T. patula* (ten per cent) and *Calotropis gigantea* (ten per cent) when fed to apparently healthy *H.armigera* larvae, expressed latent NPV infections resulting in mortality ranging from 36 to 50 per cent. The NPV–Neem bitter, crude sugar combination recorded the shortest LT_{50}. The enhanced action was seen even at a lower dose of neem bitter (0.025 per cent) with NPV ($1x10^5$POB / ml) and crude sugar (one per cent). The larval weight and growth rate were significantly reduced in the NPV-neem combinations (Rabindra *et al.,* 1997). Neem seed kernel extract at two and half per cent enhanced the activity of NPV at 1 x 10^2 PIB /ml against *H.armigera* on cotton leaves suppressing overall consumption, fecundity and survival (Murugan *et al.*, 1998; Murugan and Jeyabalan, 1998). Here, our results also have recorded higher efficiency at lower doses in combinations involving NPV and plant extracts.

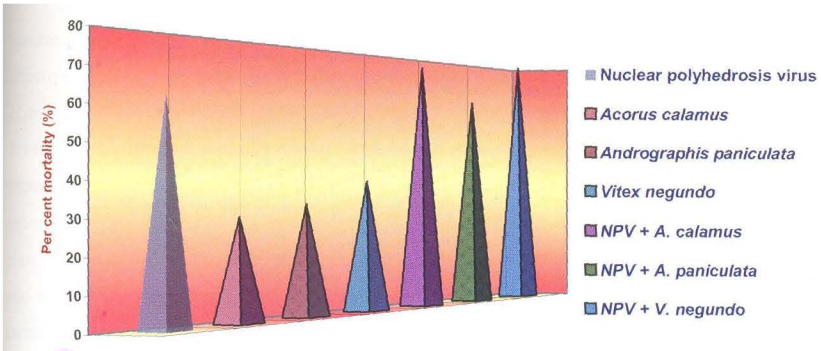


Fig. 20.Effect of nuclear polyhedrosis virus and plant extracts against third instar larvae of *Helicoverpa armigera*

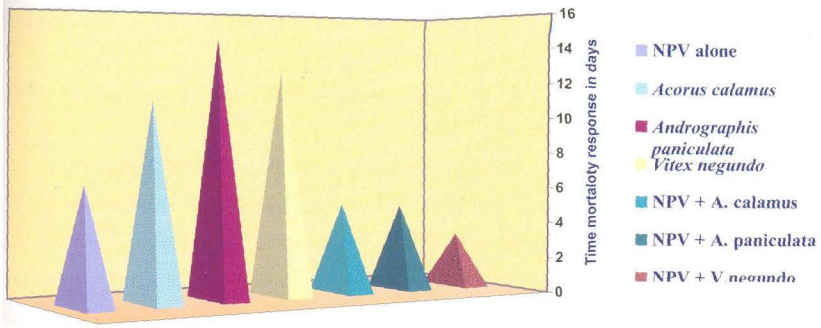


Fig. 21. Effect of nuclear polyhedrosis virus and plant extracts on time mortality response (LT50) *Helicoverpa armigera*

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In the present study, the commercial formulation of NPV by itself was not spectacular in its results. By using the half dose of plant product and NPV, the effectiveness could be increased. Addition of plant extracts had additive effect with *H.armigera* nuclear polyhedrosis virus and did not have any adverse effect upon the development of lethal virus infections. The larvae exposed to both virus and plant extracts died faster than larvae exposed to virus alone. Reduction in LT_{50} was reported by Muthiah (1988) when neem products were mixed with *Ha*NPV. Use of plant extracts as adjuvants to *Ha*NPV is a promising approach because larval feeding and subsequent damage to fruits would be reduced greatly without interference with larval activity. Here also *V.negundo* proved to be effective against *H.armigera* (Fig. 21.). Compounds of *V. negundo* like terpenes, cinole, sabinene and sesquiterpenes (Manalo,1982) might have weakened and predisposed the larvae of *H. armigera* to the action of nuclear polyhedrosis virus. Simple addition of the plant extracts along with commercially available products will produce a very good field control. Overall effectiveness (mortality and LT_{50}) plays a major role in the management of *H.armigera.* When used in the field, it can either activate latent virus infections in field populations of pest insects or increase the efficacy of field applied virus.

5.4.3.2 Nuclear polyhedrosis virus and essential oils

Among the NPV + essential oil combinations, NPV + *K. galanga* recorded the highest mortality per cent followed by NPV + *C. martini*, NPV + *C. flexuosus* and NPV + *C. winterianus* (Table 32. ; Fig. 22.). The results of the above study revealed the existence of supplemental synergism between NPV and essential oils, *viz., C. martinii, K. galanga, C. flexuosus* and *C. winterianus* (Table 32 a.). The dose mortality response (LT_{50}) was also reduced in combined treatments.

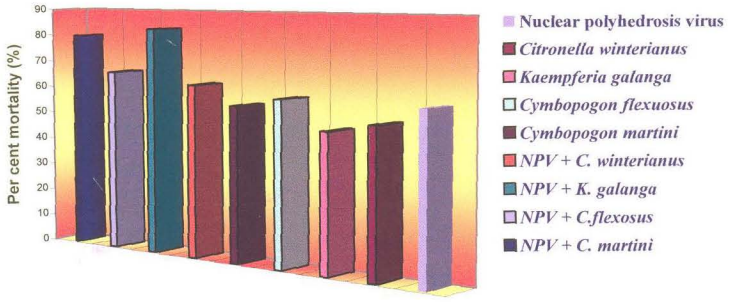


Fig. 22.Effect of nuclear polyhedrosis virus and essential oils against third instar larvae of *Helicoverpa armigera*

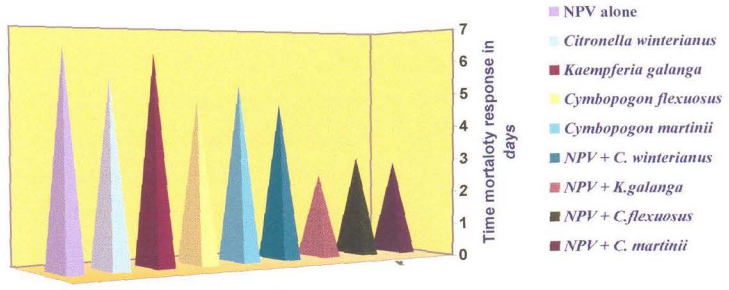


Fig. 23. Effect of nuclear polyhedrosis virus and essential oil on time mortality response (LT50) *Helicoverpa armigera*

The combination involving essential oil also enhanced the effectiveness in bringing high mortality and reduced the LT_{50} to as much as 6.5 to 4.2 days (Fig. 23.). There are no previous reports involving NPV and essential oil.

5.4.3.3 Nuclear polyhedrosis virus and insecticides

In the present study, combined use of *Ha*NPV and insecticides effected higher mortality than NPV/insecticide applied individually (Table 33. ; Fig. 24. and Fig. 25.). NPV + Spinosad combination recorded the highest mortality per cent. This might be due to the synergistic interaction between the NPV and chemical insecticides.

A lot of combination studies have been carried out earlier both in the laboratory and under field conditions. Laboratory studies conducted earlier revealed that *Ha*NPV enhanced the action of insecticides like endosulfan and cypermethrin (Rajasekhar *et al.*, 1996), cyhalothrin, deltamethrin, methomyl, phoxim and acephate (Song *et al.*, 2000).

Combination of *Ha*NPV with insecticides like endosulfan gave the maximum control of the pest as well as higher yield in chickpea (Sathiah, 1987), pigeonpea (Bijjur *et al.*, 1994), sunflower (Bijjur *et al.*, 1994; Balikai *et al.*, 1998; Balikai and Sattigi, 2000) and tomato (Gopal and Senguttuvan, 1997; Pokharkar and Chaudhary, 1999; Ganguli and Dubey, 1998; Sivaprakasam, 1998; Satpathy *et al.*, 1999; Satpathy and Rai, 2000). Gopal and Senguttuvan (1997) reported that NSKE 3 per cent + endosulfan 0.035 per cent + *Ha*NPV 250 LE/ha applied three times at 45, 55 and 65 days after planting gave the highest larval mortality and Pokharkar and Chaudhary (1997) observed that *Ha*NPV 250 LE/ha with half the dose of pyrethroids

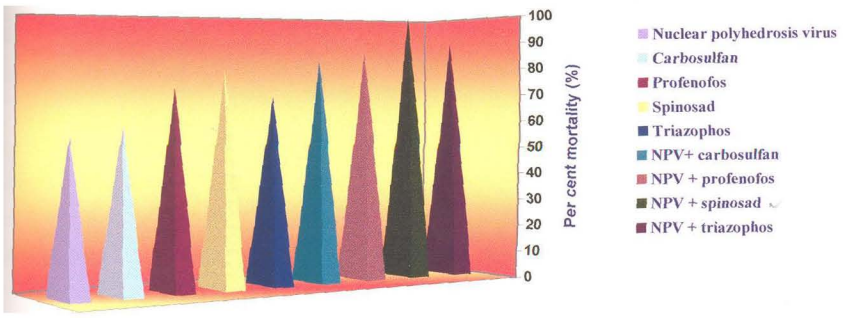


Fig. 24.Effect of nuclear polyhedrosis virus and insecticides on the third instar larvae of *Helicoverpa armigera*

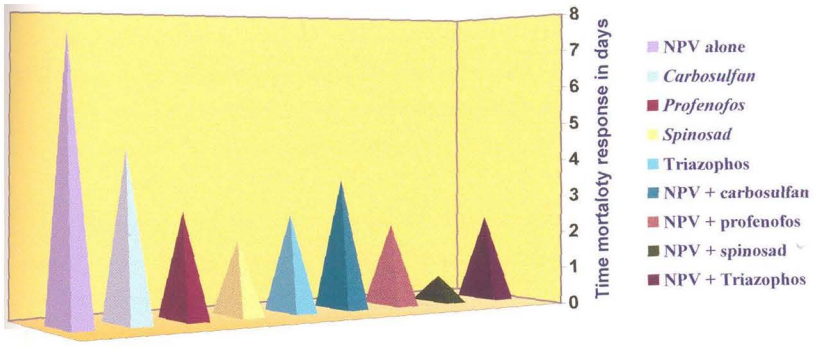


Fig. 25. Effect of nuclear polyhedrosis virus and insecticides on time mortality response (LT50) *Helicoverpa armigera*

like cypermethrin, fenvalerate, deltamethrin, carbaryl and endosulfan controlled the tomato fruit borer *H. armigera* effectively.

In all the combinations tested in the present study, the enhanced activity observed must be due to sub acute infection of polyhedrosis virus influencing the insecticide susceptibility as reported in case of cabbage looper *T. ni* (Girardeau and Mitchell, 1968), *H. armigera* and *S. litura* (Rabindra and Jayaraj, 1990).

This suggestion is supported by the findings of Pokharkar *et al.* (1999) who reported that sequential application of endosulfan 0.07 per cent followed by two sprays of *Ha*NPV 250 LE/ha greatly reduced larval population. Pokharkar and Chaudhary (1999) suggested evening application of *Ha*NPV 250 LE/ha + endosulfan 0.035 per cent or jaggery one per cent against tomato fruit borer.

Combinations of insecticides with alternative mortality agents such as virus, bacteria and fungi serve to reduce selection pressure for resistance by lowering the dose and perhaps the number of applications of insecticides. Such combinations also introduce multiple mortality factors, so that individuals with genes for insecticide resistance may still fall prey to microbial agents.

Based upon the lab results the best combinations for field experiment were selected. The following are the combinations, which were screened for field evaluation *Bacillus thuringiensis,* Nuclear polyhdrosis virus, *Nomuraea rileyi*, *Vitex negundo*, *Cymbopogon martinii*, spinosad, *B.thuringiensis* + *V.negundo*, *B.thuringiensis* + *C .martinii*, *B.thuringiensis* +spinosad, NPV + *V.negundo*, NPV + *C. martinii*, NPV + spinosad, *N.rileyi* + *V.negundo* and *N.rileyi*+ spinosad.

5.5 EFFECT OF BIOCIDES AND PLANT EXTRACTS ON DIGESTIVE ENZYME ACTIVITY

5.5.1 Nuclear polyhedrosis virus and plant extracts

In the present study, the combined treatment of NPV and plant extracts significantly reduced the digestive enzyme activity in the midgut of *H. armigera* (Table 34,35,36 and 37. ; Fig. 26.). The lowest protease, α – amylase and β – amylase activity was observed in NPV + *V. negundo* treatment. The combined application of NPV + *A. calamus* caused lowest lipase activity. The NPV alone treatment did not affect the β – amylase activity when compared to control. There was no significant difference in α – amylase activity between treatments except control. The NPV alone treatment significantly reduced the protease activity.

In third instar larvae, these combinations probably inhibited digestive enzymes indirectly by acting on a physiological system affecting digestive activity or secretion. The extracts were ingested by the larvae along with the food. The reduction in enzyme activities can be attributed to the direct action of the extract on the enzyme-secreting walls of the midgut cells, rupturing the tissues that can induce subsequent disruption in enzyme secretion.

The reduction of enzyme activity is in conformity with the earlier studies. The activity of amylase, invertase and protease in the *Bm*NPV infected larva increased initially but as the disease progressed, it decreased significantly (Gururaj *et al.*, 1999). Kumar and Murugan (1999) reported that the combined treatment of nuclear polyhedrosis virus and azadirachtin significantly reduced the digestive enzyme activity in the midgut of *H.armigera*. The protease activity was reduced to $1x10^{-4}$ mg/ min, amylase activity to 3.1 $x10^{-4}$ mg/ min and lipase activity to 0.4 $x10^{-4}$

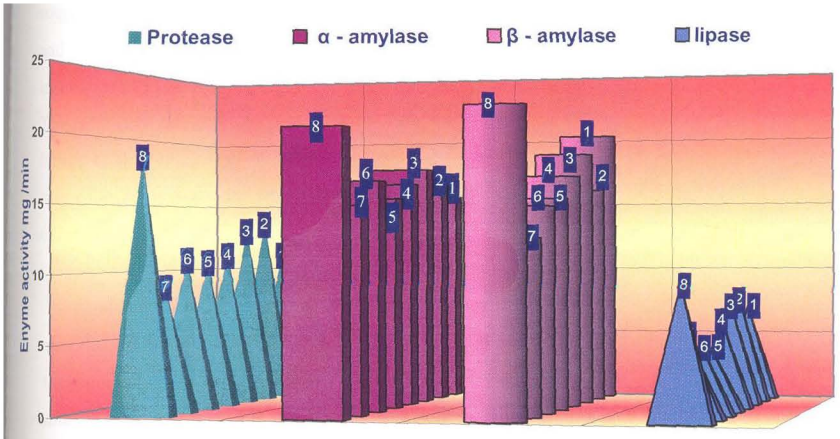
mg/ min at $1x10^4$ PIB /ml NPV +2.0 azadirachtin treatment. Lavanya and Chitra (2002) reported that sub lethal concentration of *Annona* seed extracts reduced the amylase and invertase activities in the midgut of the fifth instar larvae of *S. litura.*

5.5.2 *B. thuringiensis* and plant extracts

The present investigation on digestive enzyme activity indicated that *B. thuringiensis* and plant extract combinations significantly reduced the enzyme activity in *H. armigera* (Table 38,39,40 and 41. ; Fig. 27.). The lowest protease and β – amylase activity was observed in *B. thuringiensis* + *V. negundo* treatment. The *B. thuringiensis* and *A. paniculata* alone treatment did not affect the β- amylase activity as it was on par with control. The treatment combination *B. thuringiensis* + *A. paniculata* produced the lowest α – amylase activity. There was no significant difference in α- amylase activity among the treatments, but was significantly less than control. A drastic reduction in lipase activity was noticed in *B. thuringiensis* + plant extracts (*A. calamus* and *A. paniculata*) and *B. thuringiensis* alone when compared to control.

It has been reported that *B. thuringiensis* caused swelling of the gut musculature and it led to the disruption of the gut physiology (Endo and Uwo, 1980). Generally pathogenic effect of gram positive, spore forming and crystalliferous bacteria, *B. thuringiensis* is determined by the activity of the spore to pass through the gut wall, which is lined by the peritrophic membrane.

El-Ghar *et al.* (1995) studied the effect of abamectin and *B. thuringiensis* on digestive enzymes of *S.littoralis.* In enzyme assay, abamectin caused a remarkable decrease in invertase, amylase and trehalase activities by 81, 76 and 54 per cent respectively compared to those recorded in the control larvae. Sub lethal dosages of



1. NPV 2. *Acorus calamus* 3. *Andrographis paniculata* 4. *Vitex negundo*
5. NPV + *A. calamus* 6. NPV+ *A. paniculata* 7. NPV + *V. negundo* 8. Control

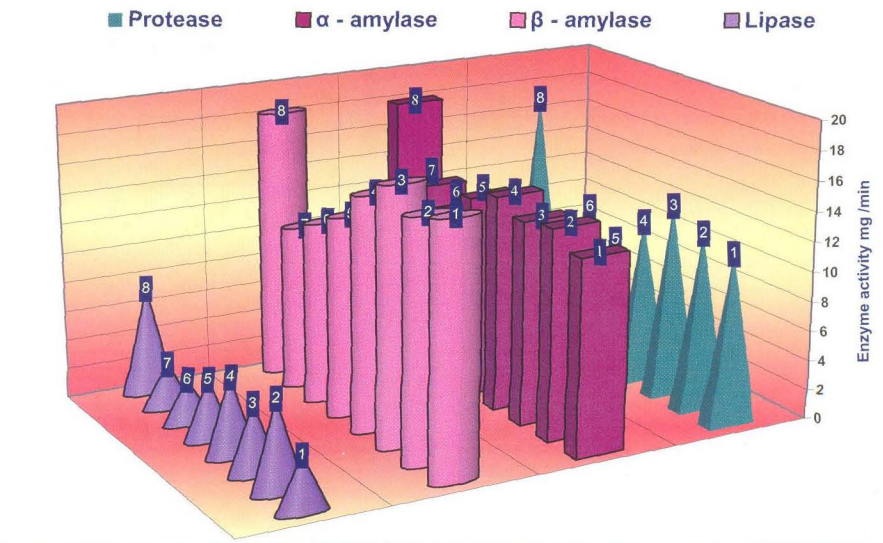
Fig.26. Effect of Nuclear polyhedrosis and plant extracts on digestive enzyme activity of *Helicoverpa armigera*

the toxin crystal of *B. thuringiensis* subsp. *galleriae* reduced the proteinase, trypsin and amylase in the gut of larvae of *Galleria mellonella* (Shen and Qian, 1995). The profiles of digestive enzymes were decreased by *B. thuringiensis* subsp. *kurstaki* treatment. The inhibition of digestive enzymes in the midgut of *H.armigera* suggested that *B. thuringiensis* and plant extracts greatly affect the gut homeostasis and suppress the feeding physiology (Murugan *et al.*, 1998). *C. roseus* alkaloids inhibited the activity of digestive enzymes of *Euproctis fraterna* (Sundari, 1998). The activities of the midgut digestive enzymes of the third, fourth and fifth instar larvae after feeding on *B.t.* transgenic cotton were lower than that of the control. The rate of enzyme activities decreased significantly as the larval development advanced (Zhou *et al.*, 2001).

Results of the present study thus indicate that the plant extracts reduce the enzymatic function of the gut, to cause reduced feeding, indigestion and blockages in the gut. This may damage the peritrophic membrane and allow the spore entry by *B. thuringiensis* or infection of NPV. These predisposing causes enhanced effectiveness of both *B.thuringiensis* and NPV.

5.6 FIELD EFFICACY OF PROMISING BIOCIDES ON TOMATO FRUIT BORER *H. armigera*

Based on the laboratory investigations, field efficacy on pot culture was conducted during December 2003 to April 2004. In contrast to the conventional experiments, here we have given an assured level of larval population to each plant by artificial inoculation. The presence was confirmed based on feeding. On the second day evening, spraying was done and the mortality was found out every day up to pupation. The results of the pot culture experiment on the management of *H. armigera* on tomato are discussed (Table 42. ; Fig. 28.)



1. *Bacillus thuringiensis* 2. *B. t + V. negundo* 3. *B. t + A. paniculata* 4. *B.t + A. calamus*
5. *Vitex negundo* 6. *Andrographis paniculata* 7. *Acorus calamus* 8. Control

Fig. 27. Effect of *Bacillus thuringiensis* and plant extracts on digestive enzyme activity of *Helicoverpa armigera*

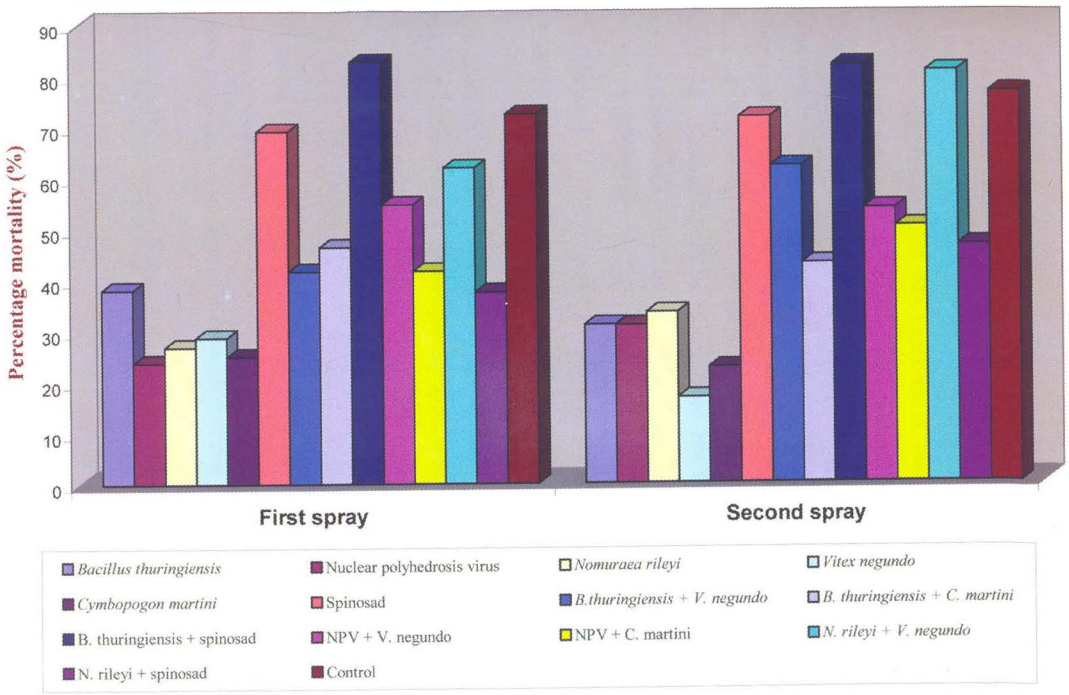
5.6.1 Per cent mortality

The results of the experiment indicated that the per cent mortality was found to be more in biocide (*B. thuringiensis*, NPV and *N. rileyi*) and insecticide combinations when compared to the individual treatments. All the combination treatments produced more than 40 per cent mortality whereas the individual treatments could cause only 16.6 to 38.07 per cent mortality. The plant extract (*V. negundo*) and essential oil (*C. martinii*) alone recorded low mortality per cent.

The inefficiency of botanicals in the field may be due to the instability of the active principles concerned. Probably, the alkaloids, terpenoids and other substances were quickly degraded and hence the negative results. Use of suitable adjuvant and antioxidants to preserve the activity of these botanicals might be helpful.

In the present investigation, under field condition, Spinosad alone and its combinations with other microbial pesticides showed higher insecticidal activity towards *H. armigera*. This high level of larval mortality might be due to high insecticidal action of Spinosad as contact and stomach poison. In the toxicity tests under laboratory condition, Spinosad was found to be highly toxic to all instars of *H. armigera* followed by *Ha*NPV+ spinosad + *B. thuringiensis*, spinosad + *B.thuringiensis* recorded cent per cent mortality at 72 h. Spinosad kills through activation of acetylcholine nervous system through nicotinic receptors. Continuous activation of motor neurons causes the insect to die of exhaustion. There may be some effects on the Gama Amino Butyric Acid (GABA) and the nervous system (Salgado *et al.*, 1998; Thompson and Hitchins, 1999). The present findings are comparable with the findings of Patil *et al.* (1999); Dandale *et al.* (2001) and Vadodaria *et al.* (2001).

Fig.28. Effect of promising combinations on mortality of *Helicoverpa armigera* in tomato



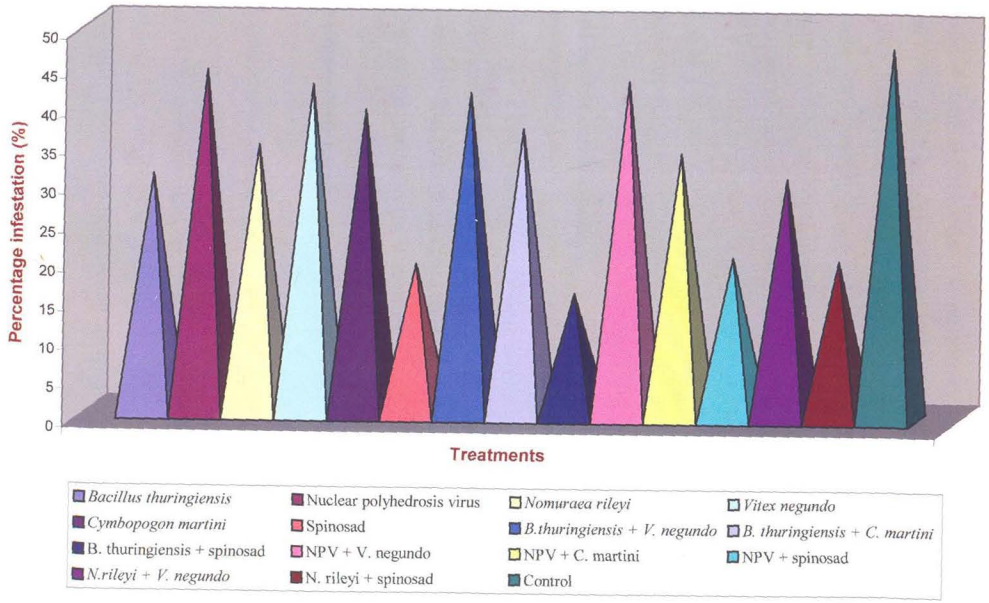
5.6.2 Borer infestation

The results on fruit borer infestation indicated that all the treatments caused more than 35 per cent infestation except spinosad alone and its combinations with biocides on fruit number basis. The treatments *B. thuringiensis* + spinosad and spinosad alone were found better than other treatments since they recorded only 15.92 and 19.60 per cent of infestation on fruit number basis (Table 43. ; Fig. 29.). On fruit weight basis, the treatment *B. thuringiensis* + spinosad was significantly superior among the rest of the treatments and registered 17.67 per cent infestation. Efficacy of *B. thuringiensis* + acephate combination was earlier reported by Dibyantro and Siswajo (1988) on tomato crop. The highest yield of tomato was obtained in plots treated with *B. thuringiensis* 0.05 per cent + acephate 0.05 per cent.

Mathur *et al.* (1996) conducted field trials in Rajasthan and reported that *B. thuringiensis* var. *kurstaki* in combination with methomyl can protect the tomato crop from *H.armigera*. Mehta *et al.* (2000) evaluated the effect of deltamethrin alone and in combination with *B.thuringiensis* against tomato fruit borer *H.armigera*. A mixture of Deltamethrin + *B.thuringiensis* application revealed a fruit damage of 5.58 per cent. The mean fruit damage was also lowest in this combination.

Athira (2003) conducted a field experiment to assess the combined efficacy of *B.thuringiensis* var. *kurstaki* (Delfin, Spicturin) and insecticides (spinosad, Indoxacarb, Quinalphos and Thiocarb) against *P.xylostella*. Delfin + spinosad and Delfin + Indoxacarb were superior in reducing the larval population of *P.xylostella*.

Fig.29.Effect of promising combinations on fruit borer infestation in tomato



5.6.3 Marketable fruit yield

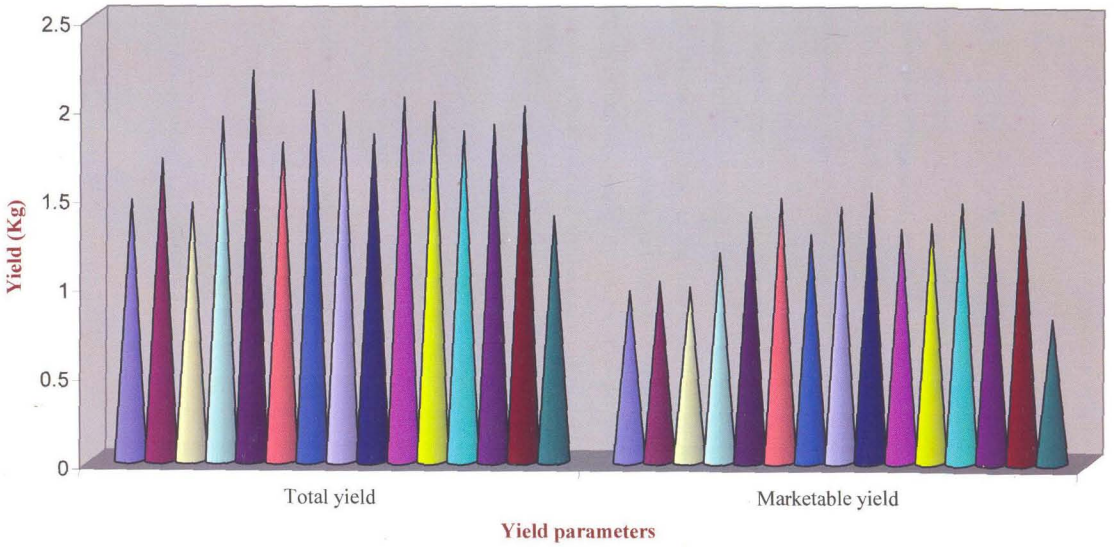
Analysis of data on the marketable yield (on number and weight basis) revealed that Delfin + spinosad recorded the marketable yield of 1.515 kg per treatment followed by spinosad alone (1.485 kg) and *N. rileyi* + spinosad (1.480 kg) (Table 44. ; Fig. 30.). Singh *et al.* (1999) evaluated Dipel 8L(one litre / ha), Delfin WG (1 Kg /ha) and NPV (250LE /ha) in combination with endosulfan for their effectiveness against *H.armigera* on chickpea in the field in Bihar. The results indicated that when bio pesticides were used in combination with endosulfan, they were more effective resulting in relatively low average pod damage of 4.21 (Delfin), 5.65 (Dipel) and 6.65 per cent (NPV) resulting in 49.7, 47.2 and 46.7 per cent increase in yield respectively.

5.6.4 Economics of biocide combinations

The increased in the yield and higher net profit resulting from the pest control programme are the main consideration in recommending an insecticide. In the present study, the benefit: cost ratio (6.26:1) as well as the marketable yield (6527 kg fruits ha^{-1}) were the highest among the treatments evaluated (Table 45.). The higher benefit: cost ratio obtained in the above treatment might be due to the effectiveness of these treatments against fruit borer.

In our experiments, we used a rate of 75 g a.i / ha when spinosad was used alone. In the combinations, the dose was halved to 37.5g a.i / ha, much less than the dose used by any of the previous workers. The insecticidal activity was not just conserved, but enhanced in many of the combinations. What is more significant is that the results have been reconfirmed by a second spray given one month after the first spray (Fig. 28.). The results of the second spray also did not deviate from the

Fig.30. Effect of promising combinations on tomato fruit yield



first spray significantly. The present study, hence would recommend large-scale field studies involving NPV + spinosad, *B. thuringiensis* + spinosad and *N. rileyi* + spinosad.

Exclusive dependence on chemical pesticides will not provide sustained solutions to pest problems. Hence, safer and effective alternatives such as application of mixtures of botanicals with bio pesticides, and or chemical pesticides are desirable for an eco-friendly interdisciplinary approach to fruit borer management.

The recommendation is also immediately field worthy. This recommendation is being given as an immediate tool of insecticide resistance management against *H. armigera*, which has caused losses to the tune of millions of rupees to the farmers cultivating cotton, chickpea, tomato and bhendi. This technology is considered as ecofriendly and economically viable in nature. The present findings will be of immense help to the ordinary farmer and take him out of debt traps and offer him a better future.

Future line of work proposed

⇒ Ascertain the role spiders in the vegetable ecosystem and evaluating the impact on natural enemies

⇒ Standardisation, mass multiplication and characterisation of *Nomuraea rileyi* Vellanikkara isolate.

⇒ Methods to enhance the efficacy of *N. rileyi* and its pathogenicity to other lepidopteran pests.

⇒ Field efficacy involving new molecules in combination with microbial agents (Virus, Bacteria and entomopathogenic fungi).

⇒ Find out the effectiveness of sequential application of biocides and insecticides

SUMMARY

6. SUMMARY

Investigations were conducted at the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, during 2002-2004 to study the "Synergistic interaction of biocides and insecticides for the management of tomato fruit borer, *Helicoverpa armigera*". The salient findings of the present study are summarized hereunder.

- During the survey period, the population of *H. armigera* was found to be more during October to March period. There was no incidence of pest from July to September. The period from April to June recorded lower population levels of *H. armigera*.

- Among the four vegetable ecosystems observed for occurrence of *H. armigera* and its natural enemies, tomato recorded the maximum (388) number of *H. armigera* larvae during the survey period. This was followed by bhendi ecosystem (320). The bittergourd and cowpea ecosystems recorded 89 and 33 number of larvae per ten plants in 18 monthly observations throughout the survey period.

- Regarding the percentage of parasitism, the highest parasitism was observed in tomato (6.18 per cent). The maximum percentage of predation was noticed in cowpea ecosystem (36.60 per cent). Bhendi ecosystem recorded 13.75 per cent diseased cadavers.

- The species of natural enemies recorded on *H. armigera* during the survey period were *C. illota, A. taragamae, C. carnea, Nosema* sp. and *N. rileyi.* All

these natural enemies are being reported for the first time on *H. armigera* in Kerala.

- The spiders recorded in the tomato ecosystem were *O. sunandae, O. shweta, C. citricola, N. mukherji, Lycosa* sp. *P. lugerbris, P. paykulli, Theridion* sp. and *C. feae.* In bhendi ecosystem, seven species of spiders were recorded.

- The plant aqueous extracts (*A. calamus, A. paniculata* and *V. negundo*) were not very highly effective on their own. They caused only about 30 to 40 per cent mortality at five per cent concentration.

- All the essential oils viz., *C. winterianus, C. flexuosus, K. galanga* and *C.martini* provided more than 50 per cent mortality except citronella oil (*C.winterianus*). Palmarosa oil (*C. martinii*) at five per cent concentration recorded the highest mortality of 71.42 per cent.

- In the compatibility study, the green muscardine fungus *M. anisopliae* was more sensitive to insecticides than *B. bassiana* and *N. rileyi.* The fungus *N. rileyi* was compatible with all the insecticides except profenofos. The insecticide profenofos was incompatible with all the three fungi as its percentage inhibition was more than 50.0 per cent.

- All the three fungi exhibited complete inhibition of growth when exposed to essential oils. Among the three fungi, the white muscardine fungus *B. bassiana* was more sensitive to plant extracts. Among the plant extracts, *A. calamus* caused more than 50.0 per cent inhibition followed by *A. paniculata.*

- All the commercial formulations of *Bacillus thuringiensis* were compatible with plant extracts, essential oils and insecticides, and hence they were included in interaction studies with *B.thuringiensis.*

- In the laboratory bioassay of entomopathogenic fungi and plant extract combinations, *N. rileyi* +*V. negundo, M. anisopliae* + *V. negundo* and *B. bassiana* + *V. negundo* recorded the maximum mortality percentage. The fungi when used alone caused about 60 per cent mortality. The other plant extracts caused only about 30 to 40 per cent mortality. When they were combined, at half their doses, they could produce about 60 to 70 per cent mortality.

- In the case of entomopathogenic fungi and insecticide combinations, *N. rileyi*+ spinosad, *B. bassiana*+ spinosad and *M. anisopliae* + spinosad recorded the highest mortality percentage. A drastic reduction in LT_{50} value was noticed in the combination treatments.

- Among the bacterial formulations + plant extract combination, Delfin + *V. negundo* recorded the highest mortality percentage among the entire three combination products. Plant extracts take long time (six to seven days) for establishing the theoretical half mortality. The time mortality response was reduced from six to seven days to three to four days in combinations.

- The combination Halt + *C. martini* recorded the highest mortality percentage followed by Halt + *C. flexuosus*. All the Halt + essential oil combinations produced supplemental synergism. Delfin when combined with essential oil showed an increase in the percentage mortality of the larvae and highest mortality was recorded with Delfin + *C. martini* followed by *C. winterianus.*

In the case of Dipel +essential oil combinations, Dipel + *C. martini* recorded the maximum mortality and was closely followed by Dipel + *K. galanga.*

- The treatment combinations Halt + spinosad, Delfin + spinosad and Dipel + spinosad recorded the maximum mortality percentage. The mean lethal time could be decreased drastically in combinations when compared to individual treatments.

- The combination NPV + *V. negundo* recorded the highest mortality percentage in NPV + plant extract combinations. Among the NPV + essential oil combinations, NPV + *K. galanga* recorded the highest mortality percentage followed by NPV + *C. martini*, NPV + *C. flexuosus* and NPV + *C. winterianus.* The combination involving essential oil also enhance the effectiveness in bringing high mortality and reduce the LT_{50} to as much as 6.5 to 4.2 days. NPV + spinosad combination recorded the highest mortality percentage.

- In digestive enzyme activity, the lowest protease, α – amylase and β – amylase activity was observed in larvae treated with NPV + *V. negundo* treatment. The combined application of NPV + *A. calamus* caused lowest lipase activity. The NPV alone treatment did not affect the β – amylase activity when compared to control. There was no significant difference in α – amylase activity between treatments except control. The NPV alone treatment significantly reduced the protease activity.

- The lowest protease and β – amylase activity was also observed in *B. thuringiensis* + *V. negundo* treatment. The *B. thuringiensis* and *A. paniculata* when fed alone did not affect the β- amylase activity, while the treatment

combination of these two produced lowest α – amylase activity. A drastic reduction in lipase activity was noticed in *B. thuringiensis* + plant extracts (*A. calamus* and *A. paniculata*) and *B. thuringiensis* alone treatment when compared to control.

- In the field experiments, *Bacillus thuringiensis* (Delfin) + spinosad recorded the highest larval mortality with lowest borer infestation. Delfin + Spinosad recorded high marketable yield of 1.515 kg treatment followed by spinosad alone (1.485 kg) and *N. rileyi* + spinosad (1.480 kg).

- The results of the present study indicate that the percentage mortality was more in biocide (*B. thuringiensis*, NPV and *N. rileyi*) and insecticides combinations when compared to the individual treatments. The study has identified eight such combinations, which are immediately field worthy. The combinations are *B. thuringiensis* + *V. negundo*, *B. thuringiensis* + *C. martinii*, *B.thuringiensis* +spinosad, NPV + *V.negundo*, NPV + *C. martinii*, NPV + spinosad, *N. rileyi* + *V. negundo* and *N. rileyi* + spinosad. This recommendation can be given as an immediate tool for insecticide resistance management against *H. armigera.*

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* Originals not seen.

SYNERGISTIC INTERACTION OF BIOCIDES AND INSECTICIDES ON TOMATO FRUIT BORER *Helicoverpa armigera* (Hubner)

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ABSTRACT OF THE THESIS

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ABSTRACT

The tomato fruit borer *Helicoverpa armigera* (Hubner) is a serious pest of several cultivated crops and has attained global importance. *H. armigera* displays formidable biological profiles based on multihost feeding, strong flying ability and genetic versatility, and consequently it resists any synthetic insecticide used to control it world wide. This pest is out of control precisely because of overuse of synthetic insecticides, which has led to development of resistance and destruction of natural enemy complex. Hence the present study was carried out to investigate the efficacy and interaction of different plant extracts, microbial pesticides and synthetic chemicals, alone and in combination against *H. armigera* to reduce the cost, to avoid the after effects of commonly used insecticides and to consider its fitness in different management options.

The population of *H. armigera* was found to be more during October to March period. There was no incidence of pest from July to September period. Among the four ecosystems, tomato recorded the highest number of larvae followed by bhendi, bittergourd and cowpea. The species of natural enemies recorded on *H. armigera* during the survey period were *Carcelia illota, Apanteles taragamae, Chrysoperla carnea, Nosema* sp. and *Nomuraea rileyi.* All these natural enemies are being reported for the first time on *H. armigera* in Kerala.

The plant aqueous extracts (*Acorus calamus* Linn. , *Andrographis paniculata* Wall. and *Vitex negundo* Linn.) on their own, are not very highly effective, as they cause only about 30 to 40 per cent mortality at five per cent concentration. All the essential oils (*Citronella winterianus* Jowitt., *Cymbopogon flexuosus* Steud., *Kaempferia galanga* Linn. and *Cymbopogon martinii* Roxb.) are causing more than

50 per cent mortality except citronella oil (*C.winterianus*). Palmarosa oil (*C. martinii*) at five per cent concentration recorded the highest percentage of mortality.

In the compatibility study, all the three-entomopathogenic fungi (*Nomuraea rileyi, Metarhizium anisopliae and Beauveria bassiana*) were found to be incompatible with essential oils and compatible with plant extracts and insecticides. But all the commercial formulations of *Bacillus thuringiensis* were compatible with plant extracts, essential oils and insecticides.

The bioassay of entomopathogens with botanicals and insecticides under laboratory conditions produced the following results

In the entomopathogenic fungi and plant extracts combinations, *N. rileyi* + *V. negundo* (76.6 per cent) *M. anisopliae* + *V. negundo* (63.3 per cent) and *B. bassiana* + *V. negundo* (56.6 per cent) recorded the maximum mortality.

The treatments *N. rileyi*+ spinosad (96.6 per cent), *B. bassiana*+ spinosad and *M. anisopliae* + spinosad (93.3 per cent) recorded the highest mortality in entomopathogen + insecticide combinations. A drastic reduction in LT_{50} value was noticed in combination treatments.

Among the bacterial formulations + plant extract combination, Delfin + *V. negundo* (83.3 per cent) recorded the highest mortality percentage among the entire three (Halt, Delfin and Dipel) combination products. The time mortality response was reduced from six to seven days to three to four days in combinations.

In the case of bacterial formulations + essential oil, the maximum mortality was recorded in *B. thuringiensis* (Halt, Delfin and Dipel)+ *C. martinii* followed by *B. thuringiensis* + *K. galanga.*

The treatment combinations Halt + spinosad, Delfin + spinosad and Dipel + spinosad recorded the maximum mortality percentage. The mean lethal time could be decreased drastically in combinations when compared to individual treatments.

The combination NPV + *V. negundo* recorded the highest mortality percentage in NPV + plant extract combinations. Among the NPV + essential oil combinations, NPV + *K. galanga* recorded the highest mortality percentage followed by NPV + *C. martinii*, NPV + *C. flexuosus* and NPV + *C. winterianus.* NPV + spinosad combination recorded the highest mortality percentage in NPV + insecticide combinations.

The combined treatment of NPV and *B. thuringiensis* with plant extracts significantly reduced the digestive enzyme (protease, α - amylase, β - amylase and lipase) activity in the midgut of *H. armigera.*

The results of the field experiment indicate that the percentage mortality was more in biocide (*B. thuringiensis*, NPV and *N. rileyi*) and insecticides combinations when compared to the individual treatments. The study has identified eight such combinations, which are immediately field worthy. This technology is considered as eco- friendly and economically viable in nature. The present findings will be of immense help to the ordinary farmer and take him out of debt traps and offer him a better future.