MANAGEMENT OF

HELICOVERPA ARMIGERA(HUBNER)

USING MICRO ORGANISMS AND PLANT PRODUCTS

Dissertation submitted

to

MONTESSORI MAHILA KALASALA

VIJAYAWADA.

in

partial fulfillment of the requirement for the award

of Degree of Master of Science in Microbiology

By

Y.V.N.Sushma

Department of Microbiology

Nagarjuna University

Nagarjunanagar,

A.P., India.

Work done at

International Crops Research Institute for the Semi-Arid Tropics

(ICRISAT), Patancheru, Andhra Pradesh 502 324

TO WHOMSOEVER IT MAY CONCERN

It is certified that **Ms. Y.V.N. Sushma** an Apprentice at ICRISAT, Patancheru, India has satisfactorily completed the assigned experiments as part of the project titled **"Management of *Helicoverpa armigera* (Hubner) using Microorganisms and Plant Products "** at ICRISAT in about three months. Much of the work was of high quality. We have no objection on submitting this work as part of her MSc degree thesis.

Dr. G.V.Ranga Rao
Research Guide
Special Project Scientist
IPM Project
ICRISAT

Dr. O. P. Rupela
Research Guide
Senior Scientist
Natural Resources Management
Program
ICRISAT

Dated: February 2000

ACKNOWLEDGEMENT

It is a great pleasure and satisfactory to present this copy of my project work entitled "Biological Control of Helicoverpa armigera (Hubner) using Micro-organisms and plant products - Done at ICRISAT".

I sincerely enpress by heart felt gratitude to Dr.Smt. V.Koteswarammagaru, M.A.B.Ed.,Ph.D., Principal of Montessori Mahila Kalasala, Vijayawada for constant moral support, encouragement during the course of my study. I also thank DR. C.V. RAMACHNDRA RAO, Ph.D., Dr. MADHAVA RAO, Ph.D., Mr. KISHORE, M.Tech., and Mr. DHANRAJ, M.Tech., for their valuable suggestions.

Working at ICRISAT was a dream come true which was made possible by DR. C.L.L.GOWDA, Co-ordinator cereals and Legumes Asia Network (CLAN) and incharge of TAFP and Programme Director of NRMP for giving me an opportunity to work at ICRISAT.

I was fortunate that I got an opportunity to work under the guidance of two eminent Scientists, DR. G.V.RANGA RAO (Special Project Scientist IPM Programme), DR.O.PRUPELA, Senior Sc ientist (NRMP) ICRISAT. I sincerely thank both of them for patient counselling, invaluable guidance and constant motivation which helped me a lot in the sucessful completion of the project.

I am thankful to MR. RAMESHWAR RAO and MR. S. GOPALA KRISHNAN, Scientific Officer's ICRISAT, for their encouragement and valuable suggestions during the project.

I sincerely express my gratitude to all Lab-Technians without whose help this project work would not be complete.

(Y.V.N. SUSHMA)

D E C L A R A T I O N

I, hereby declare that, the dissertation or part has not been previously submitted by me/anyone for a degree of any University.

Y.V.N. Sushma

(Y.V.N. SUSHMA)

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Introduction

Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) is one of the most serious insect pests of several crops in the world. It is distributed from the Cape Verde Islands in Atlantic Ocean through Africa, Asia and Australia to the south Pacific islands and from southern Europe to New Zealand (Reed and Pawar 1982).

H. Armigera has a wide host range and in India it is known to attack at least 181 plant species from 45 families (Manjunath, 1989), including major crops such as cotton (*Gossypium spp*), Sorghum (*Sorghum bicolor Linn.*), Tomato (*Lycopersicon esculentum Mill*), Pigeonpea (*Cajanus Cajan (Linn). mill*), and Chickpea *(Cicer arietinum Linn*). Annual loss due to *H.armigera* in Pigeonpea and chickpea has recently been estimated to exceed US$ 600 million (ICRISAT 1992).

Application of 15- 20 insecticidal sprays in cotton and 4-5 sprays in pigeon pea and chickpea on a calendar basis was common practice followed by farmers to combat this insect. Continuous and indiscriminate use of chemicals led to the development of resistance to most classes of chemical insecticides (Organochlorines, Phosphates, Carbamates and Pyrethroids). Resistance to synthetic pyrethroids in *H.armigera* is threatening the cotton and legumes production in South Asia . (Armes, 1992). Total dependence on chemical pesticides for long time also led to several crop failures in the past. To avoid crop loss due to insect pest and manage them in an ecologically sound way, the present day research focuses on alternative control methods and led to the concept of Integrated Pest Management (IPM). IPM is the containment of a pest below economically damaging level using a combination of different, feasible control measures. Biological control plays an important role in developing IPM strategies. The possibility for biological control of Helicoverpa through conservation and augmentation of natural enemies, classical biological control and use of microbial insecticide has been reviewed by king and Coleman (1989). The IPM relies on host plant

resistance, biological control, cultural control and use of biorational pesticides with minimal use of chemical pesticides.

Among the various biocontrol agents, parasites, predators and microbial agents are in the forefront of plant protection. In recent years the use of microorganisms in plant protection gained considerable momentum. Among the various microbial organisms bacteria, fungi and viruses are the known examples. The microbial insecticides account for only 1% of the total insecticide market in India.

Bacteria:

Although 100 bacterial species have been identified as insect pathogens, only certain *Bacillus* species have attained commercial success. Of these, *Bacillus thuringiensis* is the most widely used microbial control agent. In recent years, there has been increased interest in using bacteria as an insecticide and several new products have been identified. Most bacterial products are effective only through ingestion. Bacteria produce delta endotoxins in the form of crystals, which are toxic to insects. Each bacterial strain shows specific activity, being effective against a limited spectrum of insects. The Entomopathogenic bacteria are nontoxic to humans and other nontarget organism.

Bacillus popillae and *Serratia* are the other notable strains in insect pest management in different parts of the world.

Fungi:

Fungi hold a special place with regard to insect control. Over 500 fungi are known to be associated with insects of which some have the potential biological control properties. Five organisms that are currently been considered for commercial development are *Beauveria bassiana, Metarhizium anisopliae*, *Verticillum lecanii, and Paecilomyces fumosa – rosea, Hirsutella thompsonii* with several others in early stage of testing as potential microbial pesticides.

Of the various entomopathogens considered for insect control, fungi are the only ones, which do not require ingestion by the insects. Most fungi invade insect by penetrating the exoskeleton. This gives them activity that is more analogous to contact insecticides. The successful fungal infection on insects is highly dependent on the environmental conditions.

Botanicals:

This group contain biologically active products such as plant derived products. These products are usually safe for human -beings and for environment. For this reason as well as the increasing problems of pest resistance to chemical pesticides, interest in botanical insecticides has increased rapidly in recent years.

Among the numerous ingredients of plants studied during the last 20 years, extracts and the compounds from the neem tree *Azadirachta indica* has attracted special interest of entomologists.

The present study aims at finding effective, eco-friendly alternatives to chemical pesticides against *H. armigera,* with the following objectives:

1. To study the effect of potential bacteria and fungi on *Helicoverpa* larvae.

2. To study the effect of potential plant products on *Helicoverpa larvae.*

3. Extraction, Identification and storage of entomopathogens on *Helicoverpa larvae* collected from the farmer's field.

Review of Literature

Helicoverpa and Insecticides

Economic losses of agriculture owing to pests are huge. It is estimated that in the world about 15% of these are due to insect attacks on the crop, (Bravo and Quintero, 1993). In 1987, failure to control the pest within the cotton crops of coastal A.P state led to an estimated loss of income US$150 million, around 15% of the annual state income (Kishore, 1990). The residual effects produced by insecticides, environmental pollution, toxicity and induced resistance in insects provoked the use of microorganisms as an alternative means of control (Lacey and Harper, 1986).

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a polyphagous pest of economic interest through out the world. It has been found on over 181 plant species, both cultivated and wild through out India (Manjunath et al., 1985). It is known to cause significant damage to several crops grown in south India .The management of *H.armigera* is difficult and in many crops relies heavily on the use of chemical insecticides (King, 1992). High dependency on chemicals over two decades has led to high level of resistance in *H.armigera* to major groups of insecticides - Organochlorines, Phosphates, Carbamates and Pyrethroids. (Armes, 1992).

The most important mechanisms of insecticide resistance in pests are thought to be due to:

- Detoxification of the insecticide by mixed function oxidases (mfo resistance) (Gunning, 1991).

- Reduced permeability of insect cuticle to pyrethroids (Gunning 1991).
- The sprayed insecticide metabolized by esterases (Phokela and Mehrotra, 1989).

To sustain the use of synthetic organic pesticides, the present day protection rely upon Integrated Pest Management.

Microbial Insecticides

Insect pathology dates back to Aristotle with his description of the disease of Honeybee. Entomopathogens cause a regular and tremendous mortality of many pests in many parts of the world and in fact, constitute an extremely important natural control factors (Steinhaus 1949). Scientific work on this line was started with the discovery of *Beauveria bassiana* from the silk worm in1834 by Agostino Bassi. Metchnioff (1887) described *Metarhizium anisopliae* and tested preparation for the control of wheat cochaker *Anisoplia austriaca*

Among the various organisms which are being studied for biocontrol potential, bacteria belonging to the genus *Bacillus*, within which the most important is *Bacillus thurigiensis*.

B. thurigiensis has been successfully exploited as a microbial pesticide against a wide range of insect pests (Rushtapakornachari and Vattanatangam, 1986). *B. thurigiensis* is a gram positive *Bacillus* which produce crystalline proteinic inclusion during the sporulating process. The spores or inclusion bodies of this species are toxic to insects. The proteins of the crystal are called delta-endotoxins (Aronson, 1991) or parasporal crystal (Tanafda, 1993). The need to develop safe pesticides has accelerated the

search for new strains of *B.thuringiensis* with different modes of action (Bernhard and Utz, 1993).

The infective unit of entomogenous fungi is conidium. At favourable temperature and in presence of sufficient moisture, conidia germinate, generally on the cuticle and form a germ tube. The conidia penetrates the cuticle directly, as in *Verticillum lecanii* (Hughes and Gillespie, 1985) and *Beauveria bassiana* (U.Hilber and A.T.Gillespie, unpublished observation), or forms an appressorium which produces a penetrate peg e.g. *Metarhizium anisopliae* (Zaccharuk, 1970 a, b, c). Penetration of the insect cuticle by germinating spores has long been thought to result from a combination of enzymatic degradation of the cuticle and mechanical pressure. Strains of *B.bassiana, M.anisopliae, paecilomyces spp.* and *V.lecanii* produced large quantities of proteases and chitinases in liquid culture (St.Leger, 1986). Production of protease, lipase and chitinase on insect cuticle has also been demonstrated with *M.anisopliae* by enzyme specific straining (St.Leger, 1986).

Once through the cuticle, the fungus must overcome the host defence system before it can enter the haemolymph and spread throughout the insect. In some fungi, toxins may inactivate the host defence system (Robert, 1981). A crude partially purified extract from a *M.anisopliae* culture filterate containing the cyclic dipsipeptides, destruxins, inhibited prophenol oxidase production by insect haemocytes, suggesting that dextrins may suppress insect immune responses (Huxham, 1986). Once in the haemolymph, growth morphology changes and the fungus assumes a 'yeast- like' budding .The fungus spreads through the haemocoel, killing the insect in 3-14 days after

spore application. Probably death eventually results from a combination of mechanical damage, nutrient exhaustion and toxicosis. After death, provided water availability is high, the fungus emerges outwards through the cuticle and sporulates on the cadaver, providing inoculum for other insects. If conditions are unfavorable, the fungus remains inside the insect, where it can survive for several months, eventually producing spores when favorable conditions return (Wilding 1973).

Epizootics

Fungi often cause spectacular epizootics with large number of dead insects showing visible fungal outgrowth. More often, insects are found covered with white mycelium and it is necessary to use Koch's postulates to determine if the fungus is growing saprophytically or a true pathogen. The potential of the fungus to cause epizootics can be estimated in terms of secondary mortality including infectivity bioassays, measurement of extend and mode of sporulation and information obtained from field trails (Hall 1982). A fungus that produces toxin is less likely to cause epizootics due to rapid kill of target insects. This can affect the possibility of long term control of the target pest, as the toxemic potential of a fungus may decrease its epizootic potential (Ferron 1981). In India natural occurrence of *N.rileyi* as an epizootic was first reported by Singh and Gangrade (1975) and observed on *H.armigera* by Gopalkrishnan and Narayanan 1989. Of all the meteorological variable that influence an epizootic, none is more critical for sporulation, germination and invasion of the host than high humidity (90% RH) or moisture (2-6h as dew) (Getzin, 1961). Wind is probably one of the most important means of dislodging and dispersing spores (Garcia and Ignoffo1977).

Common Fungi used in Insect Management

Entomopathogenic fungi are receiving renewed interest as biocontrol agents in instances where chemical pesticides present a risk to human health. Of all the entomogenous fungi, *Beauveria bassiana* is most extensively studied. *Beauveria bassiana* occurs most commonly than the *Metarhizium spp.* It also has a wide host range in the tropics (Vanninen, 1995).

Metarhzium anisopliae occurs as two forms differing in conidial size (Tulloch,1976). *M.anisopliae* is used in Brazil to control *Mahanarva posticata* on sugarcane, which gave sufficient reduction of pest and increased sugarcane production (Ferron 1981). *M.anisopliae* is also being developed for control of the pasture cockchafer, *Aphodius tasmaniae*, in Australia by Coles and Pinnock,(1984) where fungus was persistent resulted in 80% mortality with reduced cost . *M.anisopliae* is also effective against termite *Nasutitermes exitiosus* in the laboratory(Hanel,1982).

Nomuraea rileyi is primarily found parasitising Lepidoptera, although there are occasional reports from Coleoptera (Ignoffo 1981). *N.rileyi* was described over 100 years ago, but was not used for biological control until 1955 (Chambarlin and Dutky,1958).

Biorational pesticides

The negative environmental effects of pesticides have evoked worldwide concern to minimize their usage and to develop and utilize less hazardous pest control methods (Luna and House, 1990). In addition to this concern there is a need to develop pest control methods that are economically feasible to the developing countries and require minimal technical knowledge on the part of the farmers (Zelazyn, 1985). One

approach towards this is to study the readily available, safe, effective and less expensive pest control options that are of plant origin. Interest has now focused on biorational insecticides which are based on natural products or synthesized analogues of naturally occurring biochemicals (Bentz and Neal ,1995). Studies on insect growth regulators
(Biddinger and Hull 1995), neem (extract from the neem seed) was studied by A.Juss; Spollen and Isman 1996, an extract from *Nicotiana gossei* domain was studied by Bentz and Neal 1995.

Extensive study was carried out on neem *Azradirachta indica* plant. It possesses insect repellant and antifeedant properties (Saxena 1983,87). Farmers in the Indian subcontinent have used neem seed derivatives for centuries to protect agriculture crops from insect's attacks (Saxena and Khan 1985). Azardirachtin the primary component of neem seed show selective activity against many pest species (Jacobson, 1986). Unlike insecticides, neem products do not kill the insects instantly but incapacitate them (Saxena,1984). Neem is relatively non toxic to humans beings (Sardesh Pande, 1976) and has no carcinogenic effects in rats fed with 25% neem seed cake protein for nine months. The biorational insecticides usually are less toxic to natural enemies than conventional insecticides . Specific programs require research into potential compatibility among biorational insecticides (Biddinger and Hull 1995). Using chemicals to enhance efficacy of entomopathogenic fungi has been tested using organo phosphates, carbamates and organo chlorines such as DDT (Anderson 1989) . In these studies no consistent interactions were observed with insecticides. The insecticides and herbicides tested with *B.bassiana* , *M.anisopliae* and *N.rileyi*

their action was not inhibited (Gardner and storey,1985). Synergistic effects between chemicals and entomopathogenic fungi was already obtained by using *B.bassiana* preparation with 0.005ppm abamectin when applied to control the Colorado potato beetle (Anderson 1989). Studies conducted by Vimala Devi (1997) indicate that the fungus sprayed in combination with 1% seed kernal extracts of neem, melia and pongamia facilitated higher initial mortality due to mycosis at 6^{th} day when compared to fungus alone. Studies conducted by Rabindra and Rajasekharan in 1997 showed that combined action of NPV and neem against *Spotoptera litura* showed significant mortality.

Materials and Methods

Micro-organisms and plant material

Seventeen bacterial strains and 3 fungal strains were obtained from the microbial germplasm collection at ICRISAT and were used in this study. The bacterial strains were named as biocontrol bacteria (BCBs) because of their potential in control of diseases (Rupela and Gopalakrishnan 1999, Sriveni 1999). The fungal cultures *Metarhizium anisopliae, Beauveria spp.,and Nomuraea rileyi* were obtained from different sources. *M.ansiopliae* from microbial collection of ICRISAT, *Beauveria sps* from Department of Botany, Andhra University Vishakapatam and *N.rileyi* from Directorate of Oil Seeds Research Rajendranagar Hyderabad. A total of five experiments were set up. These were:

1. Evaluation of pathogenicity of some bacteria and fungal strains on *Helicoverpa* larvae.
2. Effect of selected BCBs in combination with neem on *Helicoverpa* larvae.
3. Effect of some Botanicals on *Helicoverpa* larvae.
4. Effect of green chili fruit paste and leaf creeper on *Helicoverpa* larvae.
5. Isolation, identification and preservation of pathogens from naturally killed Helicoverpa larvae.

Each treatment in an experiment had three replications.

Collection of *Helicoverpa armigera* larvae

Third instar larvae were collected in cell wells from unsprayed fields at ICRISAT, Patancheru location. Each cell well has 12 compartments or sections. One larva was placed per section. Initial weight of the larvae was

recorded using four digit electronic balance, after about 2hrs of starvation before setting the experiment.

Preparation of the food for larvae

The larvae were fed on soaked chickpea seeds. The seeds of cultivar ICCC 37 (kranti) were soaked for 3 to 4 hrs in water. After draining, the dripping water was removed with a sterilized muslin cloth and then placed in a Laminar flow until surface was free from moisture. Two seeds were given per larvae and food was changed at alternate days.

Inoculum preparation for bacteria

Sterilized 30 ml of potato dextrose broth (PDB) was taken in the 250ml conical flasks and inoculated with a given strain from its mother culture, in a Laminar flow. The inoculated flasks were then incubated for overnight on a shaker at 25°C.

Inoculum preparation for fungal cultures

Half strength potato dextrose agar (½ PDA) plates were prepared, and a given culture was inoculated at the center of the plate and incubated at 28°C until well grown. A circle was marked at the back of the plate with well-grown fungus. The fungal growth including the spores were scraped from that marked area and suspended in sterilized 5ml water in a test tube. It was blended thoroughly, using a test tube blender, (Tissumizer Mark II, Tekmark company, Japan) to break mycelium into small bits.

Preparation of plant extracts

Water extracts of plant parts of different species used in the study were neem (Azardirachta indica) seed kernel, leaves and fruit of Datura *(Datura stromonium)* leaves of Calotropis *(Calotropis gygantia)*, fruits of green chillies (*Capsicum annuam*) , fruit pulp of Nuxvomica, and leaves of a creeper (yet to be identified) at ICRISAT. A given material was ground in pistle mortar. 5% and 10% suspension in water was prepared and kept for overnight. The contents were filtered using a clean muslin cloth before applying to the larvae.

Application of microbial cultures and plant extracts

Treatments were applied to the larvae in a Laminar flow to minimize contaminants. In case of bacterial strains the 0.1ml (per larvae) of the bacterial cultures was dropped on chickpea seeds using a micropippete. And in the case of fungal strains, 0.1ml of the Inoculum was dropped on the body surface of the larvae using a micropippete. For the control treatment, 0.1ml of sterile water was added to chickpea seeds. Inoculum was added only once during the experiment. The inoculated larvae were then kept in laboratory conditions for 10days for recording observations. In case of plant extracts, 0.1ml was dropped on the seeds and on the body surface of the larvae using a micropipete. For the control treatment also 0.1ml sterile water was sprayed on both the body surface of the larvae and on seeds. Wherever a mixture of more than one strain of bacteria or a combination of bacteria and neem extract was tried (e.g. experiment no 2), 0.1 ml of each of the materials was applied to the seeds and larvae with a micropipette.

Statistical analysis: The data was subjected to analysis of variance to find out the difference between treatments using Randomized block division

Observations

Mortality was recorded after every 24hrs for 10 days. The 10day period of the observation on a given larva was taken as 100% life span. The number of days a larva survived out of the total 100% (10 days) was calculated as life span percentage. Dead larvae were removed at the first observation to prevent contamination of other larvae in the tray. And after every two days fresh food was added to prevent the larvae from starvation. Initial weight of each larva or a group of larvae was observed in all the three experiments except for the first experiment. In three of the four experiments the weight of the larvae at death was also taken. The difference between the final and the initial weight was calculated as weight gained or lost by the larvae.

Isolation of potential pathogens from naturally killed larvae

Naturally killed 16 *Helicoverpa* larvae were brought from unsprayed cotton fields in Guntur on 5 Dec 1999.The main aim of the experiment was to isolate identify and store potential pathogens from naturally killed larvae. A small piece of dead larva was taken aseptically from each of the 16 larvae and placed on different plates containing half-strength potato dextrose agar (PDA). After inoculation, these plates were incubated at 28°C. After 24 hours microbial colonies were picked for further purification. At least three serial transfers were made for all the isolates. Finally, all these were transferred on slopes having ¼ PDA in duplicates.

Short Term Preservation: Purified bacterial cultures were grown on ½ potato dextrose agar slopes and also in ½ potato dextrose broth and incubated at 28°C for overnight in the incubator. After the growth was apparent in the cultural tubes, 1ml of glycerol (sterilized separately) was added into each tube and sealed with parafilm. These slopes were stored at about 4°C in the refrigerator. The purified fungal cultures were then transferred on ½ potato dextrose agar slants. The inoculated slopes were incubated at 28°C. After the fungal cultures were grown on potato dextrose agar slants, 1 ml of sterile paraffin oil was poured in the slants and stored in refrigerator at 4°C. And for fungal cultures that are sporulating, preservation was done by sand culture method.

Sand Culture Preservation: Very fine dried sand was taken on a screw cap tube and autoclaved for 15 lb. pressure for one hour. Fungal cultures that were sporulated were taken and 1ml of sterilized distilled water was added. With the inoculation wire, the culture was scraped so that the spores get suspended in water. This inoculum (fungal spores in water) was added to the pre-sterilized dry sand test tubes. The screw caps were kept loose to allow drying of water at 25-28°C and then dry tubes were stored at 4°C.

Some of the purified cultures were then identified solely on the cultural characteristics, using standard microscopic procedures at ICRISAT pathology unit.

Results

Studies conducted at ICRISAT, Patancheru location, to develop Eco-friendly, Bio-Intensive Pest Management Strategies against the key pod borer, *Helicoverpa armigera* , using the Microbial and Bio-rational, products, reveled the following results.

Pathogenicity of some bacterial and fungal strains on H.armigera larvae

The experiment to evaluate the efficacy of 17 bio-control bacteria (BCB's) and three well known fungal pathogens on the 3 rd instar *H.armigera* indicated following results:

Table 1. Pathogencity of some bacterial[1] and Fungal Strains on 3rd instar *Helicoverpa* larvae.

Treatment	Mortality[2] %
BCBs85	40.7
BCBs114	58.3
ARC-B-2[3]	43.9
BCBs117a	58.3
BCBs98	34.3
BCBs117b	52.8
BCBs75	44.4
BCBs135	44.4
BCBs97	3.3
BCBs122	52.8
BCBs69	47.2
BCBs106	50.0
BCBs111	71.7
BCBs136	48.7
BCBs91	47.2
BCBs103	41.7
BCBs74	36.1
Metarhizium anisophile	94.4
Beauveria spp (BbH)	72.2
Nomuraea rileyi	94.4
Control	36.4
Mean	53.4
SE±	8.03
CV%	26

1. Bacteria used in the study were isolated from Natural sources such as compost.
2. Mortality (%) = No of larvae died in 10 days / No. of larvae X 100
3. ARC-B-2= Bacteria isolated from dead Hairy caterpillars.

It is evident from the data in table 1 that, among the potential bacterial strains screened, BCB 111, BCB 117(a), BCB 114, BCB 117(b), & BCB 122 [72, 58, 58,53, and 53% respectively over control.

Among the other BCB 's, BCB 106 (50%), BCB 136 (49%), BCB 91 (47%) and BCB69 (47%) have shown higher mortality over control. Others were not of much significance.

All the Fungal strains [*Metarhizium spp., Nomureae spp., Bevaria spp.*] have caused high mortality. Among them, *Metarhizium anisophae & Nomuraea rileyi* (94 %) and *Beauveria* spp. (72%) were effective over most of the BCB's. The symptoms caused from fungal (M.anisopliae, *N.rileyi*) infection on *H.armigera* can be observed in fig 1 , 2 and 3.

Interaction of bio-control bacteria and neem on Helicoverpa armigera larvae:

The effect of some bio-control bacteria alone and in combination with Neem were tested in this experiment and the results were given in Table 2

Among the potential bacterial strains screened, BCB 114, BCB 111, BCB 117(a) & BCB 117(b) were pathogenic to *H.armigera* larvae (as evident from Table I), and these BCB's were tested in combination with neem and among themselves.

The results from Table 2 remitted that, the potential combination of the bacterial strains among themselves and the interaction of neem 10 % with each strain has shown a decrease in weight gain when compared to single treatment of each.
Neem in combination with all four bacterial strains, neem + 117(b) [0.46 mg], neem +117(a) [7.5g], neem + 114 [11.3g] and neem + 111 [13.2g] have shown considerable decrease in weight over control [151.7mg].

The combination, 117 (a) + 117(b) [91.8mg) was effective than the other combinations BCB111+114 [157.3mg], in causing deleterious effects on larval growth and development.

All the four independent BCB treatments, BCB 117 [131.6 mg], BCB 111 [150.6 mg], BCB 117(b) [159.1 mg] and BCB 114[208.1 mg] were on par with control.

Table 2 Interaction of biocontrol bacteria[1] and neem on 3rd instar *Helicoverpa* larvae

Treatment	Initial wt Of larvae (mg)	Weight gained by larvae until death (mg)	Life span[1] (%)
Control	10.6	151.7	76.2
Neem10%	9.5	7.4	59.0
Neem+117a	11.7	7.5	62.7
Neem+117b	13.0	4.6	64.1
Neem +111	12.7	13.2	64.7
Neem+114	14.3	11.3	69.0
BCB117a+117b	8.9	91.8	58.1
BCB111+114	9.2	157.3	60.0
BCB117a	14.0	131.6	65.0
BCB117b	13.8	159.1	71.9
BCB111	18.3	150.6	72.1
BCB114	7.1	208.1	75.1
Mean	11.9	91.2	66.5
SE±	3.650	16.30	16.27
CV%	48	107	40

1. Life span %=The 10 day period of the observation on a given larvae was taken as 100%. The number of days it survived out of the total 100% (10 days) was calculated as "Life span (%)"

All these combinations, except Neem + 117 (b) [4.6mg] were ineffective when compared to Neem at 10% concentration [7.4mg], in decreasing the larval weight and life span.

Though there was substantial differences in the life spans of the larvae, with combined treatments over control and the independent treatments, the differences were not statistically significant.

The Bacterial combinations, 117(a) + 117(b) [58%] and 111 + 114 [60%] were more effective than the combination with Neem in decreasing the life span.

Among all the treatments and in combinations of each, Neem at 10% concentration was superior in decreasing the life span from 76% (control) to 59%.
Neem alone and in different combinations had profound effect on the larval growth when compared to other BCB strains alone or in different combinations.

Effect of some Botanicals on Helicoverpa armigera [3rd Instar]:

The studies organized on the Bio-intensive control of H.armigera larvae with four
Botanicals, at 5% and 10% concentrations indicated
The following results [Table 3]

Table 3. Effect of some Botanicals on *Helicoverpa* larvae (3rd instar)

Treatment	Initial weight of larvae (mg)	Weight gained by larvae until Death (mg)	Life span [1] %
Control	7.2	213.6	81.1
Neem 5%	6.1	4.2	71.0
Neem 10%	7.3	1.6	60.3
Calotropis 5%	7.6	177.2	81.2
Calotropis 10%	7.8	122.7	73.2
Datura 5%	8.5	117.0	55.8
Datura 10%	8.9	130.4	67.0
Nuxvomica 5%	11.7	72.5	53.6
Nuxvomica 10%	8.2	71.5	53.9
Mean	8.1	101.2	66.3
SE ±	3.90	77.51	18.14
CV (%)	81	124	44

1. Life span %=The 10 day period of the observation on a given larvae was taken as 100%. The number of days it survived out of the total 100% (10 days) was calculated as "Life span (%)

As seen from the data in table 3, Neem [4.2, 1.6mg], Calotropis [177.2, 122.7mg], Datura [117.0, 130.4mg] & Nuxvomica [72.5, 71.5mg] have shown a decrease in weight gained by the larvae, over Control [213.6mg].

Among the treatments, all the plant extracts tested had marginal effect on the life span. However the effects of Neem 10% decreased the larval weight from 213.6 mg to 1.6 mg. the least effective was Calotropis 5% [117.2 mg]. In retarding the growth the plant products were effective in order of neem, Nuxvomica, Datura and least was Calotropis.

The other treatments, Neem [71%, 60%], Calotropis [81%, 76%] and Datura [56%, 67%] at 5% and 10% concentrations respectively, were not much effective when compared to Nuxvomica treatment [54%, 60%] at 5% and 10% concentrations respectively. Incase of Life span the plant products were effective in order of Nuxvomica (more effective) then Datura, Neem and least was Calotropis.

Effect Of Chili Fruit Paste And Leaf Extract Of A Creeper On The Growth And Survival Of H.armigera :

The efficiency of the Chili Fruit Paste and leaf Extract of a creeper, on the growth and survival of *H.armigera* were tested and the results were presented in Table 4

Table 4 . Effect of Chili fruit paste and leaf extract of a creeper[1] on growth and survival of 3rd *Helicoverpa* larvae[2].

Treatment	Initial weight of the larvae (mg)	Weight gained by larvae until death (mg)	Life span[3]
Control	73.0	139.0	69.6
Green Chilli5%	67.0	149.0	68.5
Green Chilli10%	72.0	186.0	68.6
Leaf[1] 10%	85.0	218.0	77.3
Mean	74.0	173.0	71.0
SE ±	26.41	8.52	22.33
CV(%)	46	72	45

1. The creeper had an unpleasant smell. Its botanical identification is pending.
2. Third-instars larvae were used in the studies.
3. Life span %=The 10 day period of the observation on a given larvae was taken as 100%. The number of days it survived out of the total 100% (10 days) was calculated as "Life span %

As seen in the Table 4, the chili fruit paste (149.0, 186.0 mg) and leaf extract of the creeper fed larvae did not show any decrease in the larval weight (218.0 mg), when compared to Control (139.0 mg) and there was no effect of these treatments on the larval life span. (Table 4).

Isolation, Identification and Preservation of microbial cultures

Eighteen fungal and two bacterial cultures were isolated from the dead larvae. Out of these only six fungal cultures were identified based upon on their morphological characters. The following six cultures which are isolated and identified and preserved were *Penicillum expansum, Aspergillus niger, Fusarium oxysporum, Alternaria tennuissima, Culavaria lunata* and *Nomureae rileyi* . Their identification characters were as follows:

Penicillum expansum : The colony of *Penicillum expansum* are dull green colour 30-40 mm diameter, radially sulcate, moderately deep to very deep, with surface typically coremial in one or more annular bands, mycelium white. Conidiophores borne from surface or subsurface hyphae, singly, in fascicles or in definite coremia, stripes 200-500um long, with smooth walls, bearing terminal penicilli, typically terverticillate; ramiborne singly, 15-25um long; metulae12-15um long. Phialides closely packed, ampulliform to almost cylindroidal ,8-11um long with short collula conidia ellipsoidal ,3.0-3.5um long, smooth walled, borne long, densely packed, irregular chains. Importance: *Penicillum expansum* is a broad-spectrum pathogen. Isolation from saprophytic habitats have been much less frequent.

Aspergillus niger: *Aspergillus niger* is recognized by the production of compact, brownish black or carbon black, spherical or columnar spore heads. Conidiophores are smooth hyaline or faintly brownish near the apex. Apices are spherical .75um in diameter. Two series of conidia bearing cells are produced. Supporting cells are of varying length and sometimes septate. Phialides are more uniform in length, usually 7-10um x 2-3um. Conidia are typically spherical at maturity often very rough or spiny, very

dark in color or with conspicuous longtitudinal striations. *Aspergillus niger* is world wide in distribution and produces a large number of air disseminated spores.

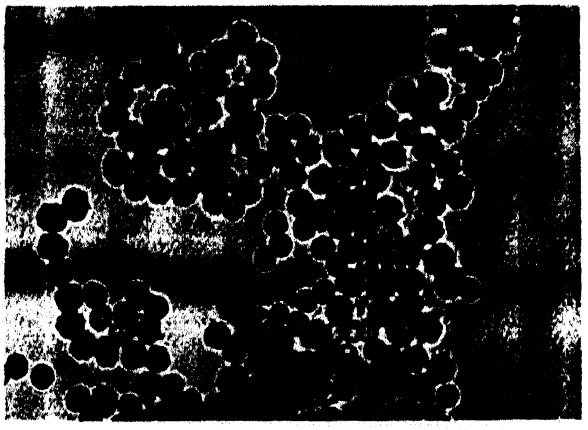


Fig 1. Conidia of *Aspergillus niger*.

Fusarium oxysporum : *Fusarium oxysporum* can be recognized by observing the culture where growth is rapid and white aerial mycelium is cream to orange in color. Microconidia are abundant, generally single celled, oval to kidney shaped and produces only false head. Macro conidia are abundant, only slightly sickle shaped, thin walled and delicate with an attenuated apical cell and a foot shaped basal cell. Conidiophores may be branched or unbranched monophialides. Chlamdospores are present.

Alternaria tennuissima: The culture is golden brown or black growth on the surface. Conidiophores are solitary or in groups, simple or branched, straight or flexuous, more or less cylindrical, septate, pale or mid pale brown, smooth, with one or several conidial scars, up to 115um long and 4um thick. Conidia are solitary or in short chains, straight or curved, obclavate or ellipsoidal tapering gradually to the beak which is up to half the length of the conidium, which is usually shorter, sometimes tapered to a point but more frequently swollen at the apex

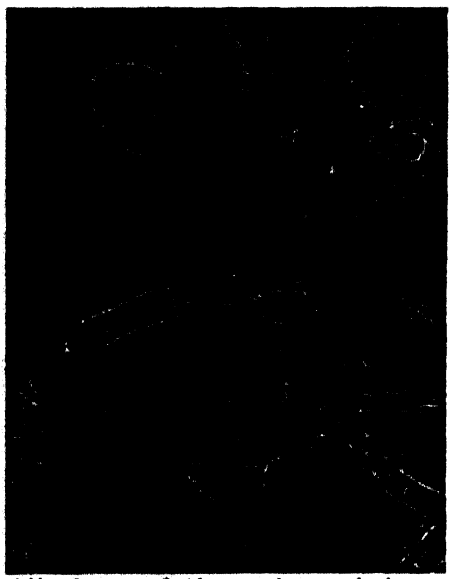


Fig 2. Conidia and Conidiophores of *Alternaria tenuissima.*

Nomuraea rileyi : Preliminary identification of *N.rileyi* is the appearance of white mycelial growth initially then changes to malachite green colour. Vegetative hyphae, 2-3um in diameter are smooth, septate and hyaline to slightly pigmented. Conidiaphores are seen bearing dense whorls of branches and phialides conidiogenous cells are short necked. Conidia are broadly ellipsoidal to cylindrical with size 3.5-4.5 x 2-3.1um and pale green.

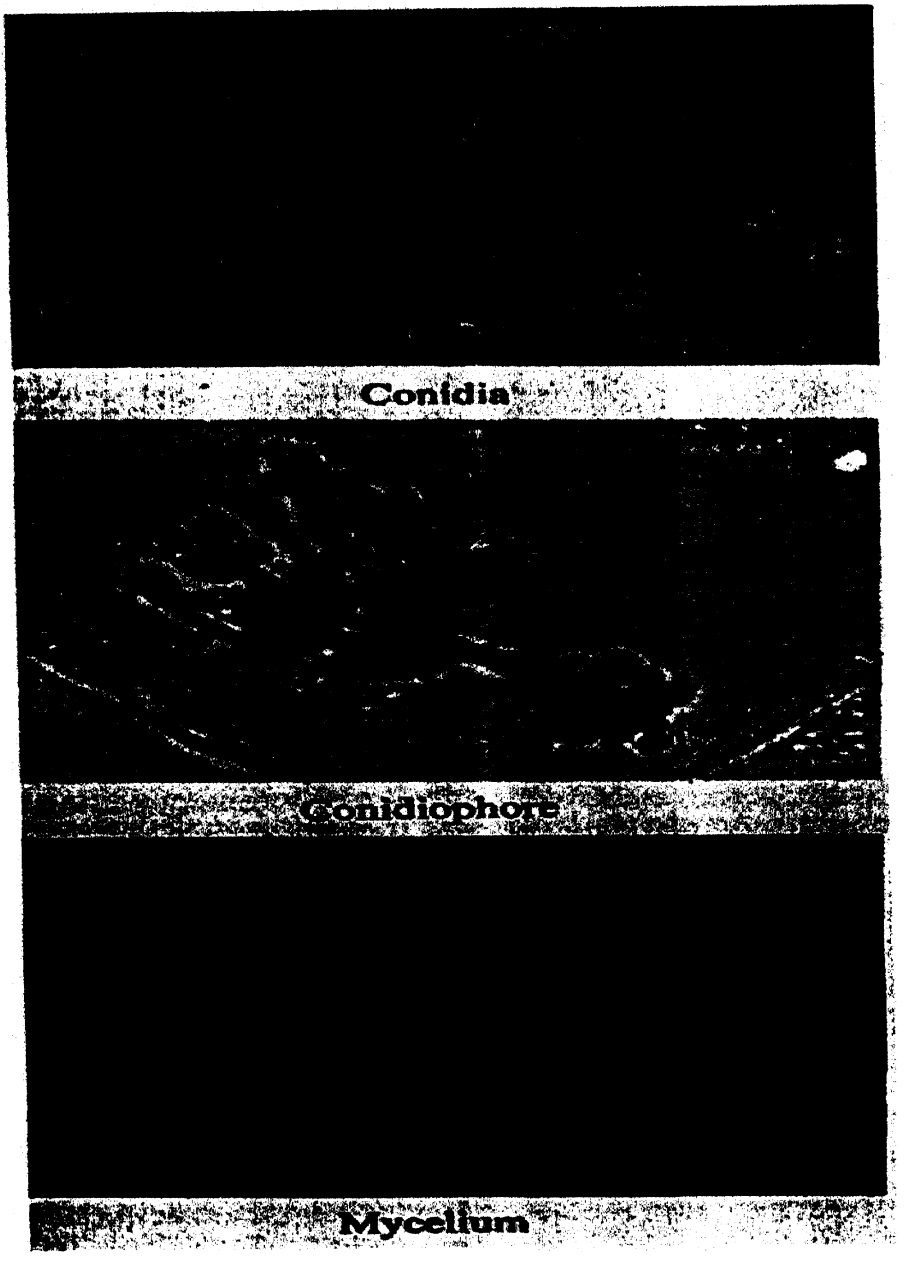
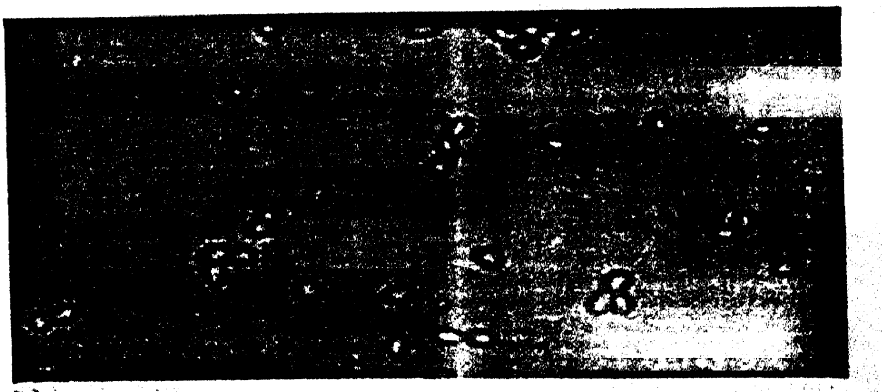


Fig 3. Nomuraca rileyi.



Conidia

Conidiophore

Mycelium

Fig 3. Nomuraea rileyi.

Plate 1 **Comparision Of** Metarhizium anisophiae **Treated Larvae (Right) With Control**

Plate 2 **A Close View Of** Metarhizium anisophiae **Treated Larvae** (Right) With The Control(Left)

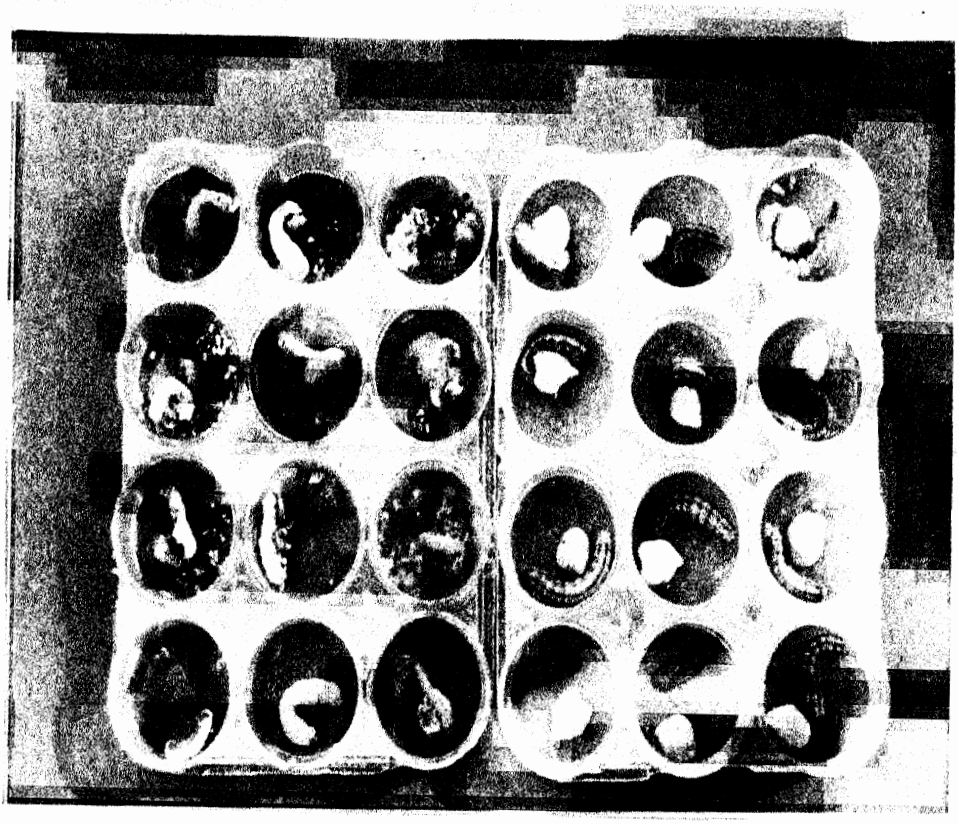


Plate 3 Pathogenecity Of Nomurea rileyi(Left) Over Control(Right)

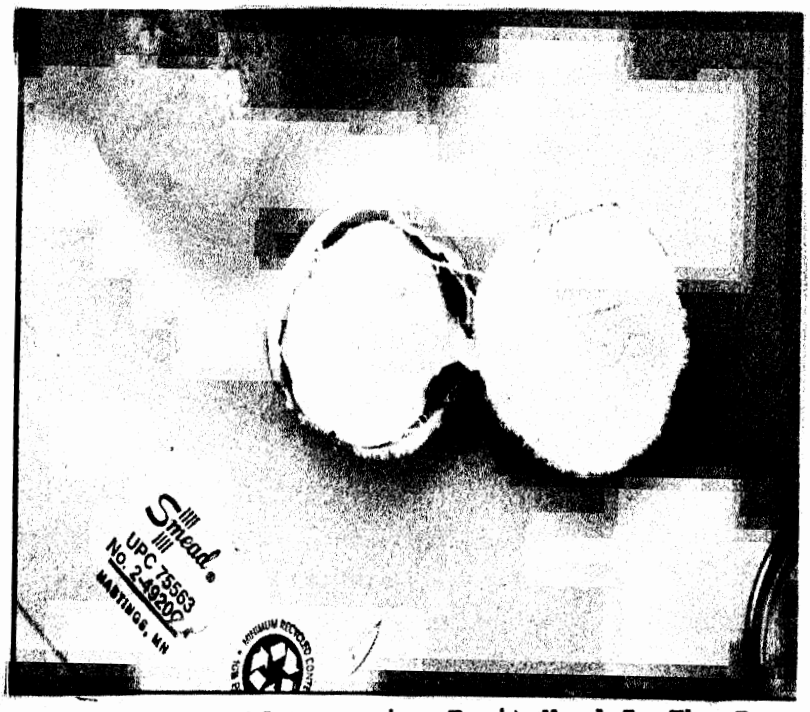


Plate 4 Mesocarp Of Nuxvomica Fruit Used In The Experiment

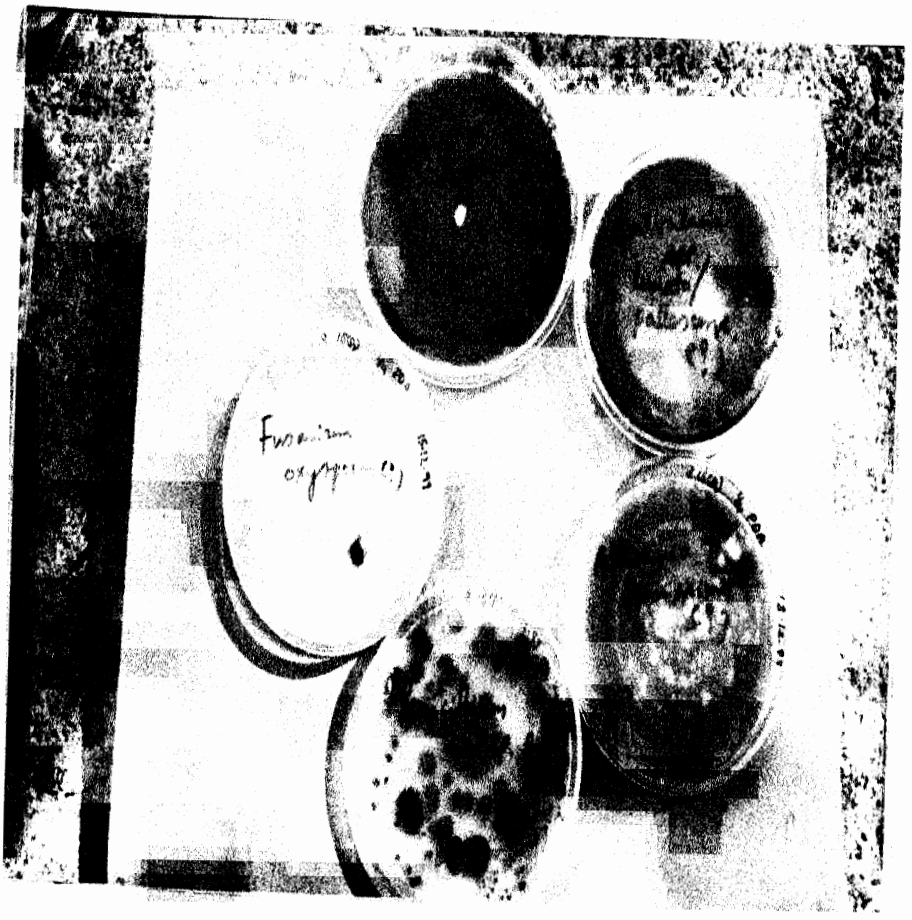


Plate 5 **Entomopathogenic Fungi That Were Isolated From Naturally Killed Field Collected Larvae**

DISCUSSION

In recent years chemical pesticides usage in Agriculture is being discouraged primarily due to the pests developing resistance, killing useful biocontrol agents, pollinators, contamination of food with residues and to avoid environmental pollution. Only possible approach is to devise ecofriendly methods to minimize the use of pesticides and manage the pest problem. In India of the several insect species H.armigera is a notorious, a major constraint in several crops where chemical control proved futile (Armes 1994 , King 1994). The present study was aimed at the management of *Helicoverpa armigera* using microbes and plant derived products.

Among the seventeen bacterial cultures that were tested to evaluate pathogencity against 3 rd instar larvae only five bacterial cultures i.e. BCB111(71.1%), BCB114and BCB117a (58.3), BCB117b and BCB122 (52.8) showed substantial mortality. All other cultures did not show much mortality. All the fungal species that were used *Metarhizium anisopliae, Baeuveria bassiana, Nomuraea riley* showed high mortality. The mortality in case of fungi was observed in 5-6 days after the inoculation. The dead larvae were completely mummified with white mycelia in case of *M. anisopliae, B.bassiana*, and green color sporulation in case of *N.rileyi*. The present studies on pathogenicity are in conformation of Fungal components showed much high mortality than bacteria. The possible reasons may be:

- The larvae have to feed on bacterial inoculated diet in sufficient amounts to get infected and express symptoms (mortality) .

- When the larvae undergo moulting which is frequent in case of growing 3 rd instar larvae, often stop feeding, and when they resume feeding the bacterial cultures may not be effective (Aronson 1991).
- The hydrophobic nature of conidia helps in easy spread of the fungus and results in high mortality, which is not case of bacteria.

The plant-derived products contain allochemicals which act as insect antifeedants, growth regulators (Bernays and Chapman 1977). In this study all the plant products showed deleterious effect on growth. In retardation of growth the plant products were effective in following order Neem (0.0042,0.0016), Nuxvomica (0.0725,0.0715), Datura (0.1170,0.1304) and Calotropis (0.1772,0.1227). Though the plant products were less effective in influencing the life span, their performance was in following order Nuxvomica (53.6%, 53.9%), Datura (55.8%), Neem (71%,60.3%) and (81.2%,73.2%) as shown in table 3. The studies of feeding behaviour in *Helicoverpa zea* and *H.armigera by* Simmonds, Blaney 1984 and the present study are in agreement with their findings. All plant derived products showed reasonable mortality and acted as antifeedants. This may due to presence of some undesirable alkaloids or phytochemicals in the tested plant products. When green chili paste and leaf extract were tested on *H.armigera* they were found in effective in causing the larval growth and mortality. Farmers in some areas of India particularly Eastern Maharashtra are familiar in using green chili fruit extract on *H.armigera* and reported satisfactory control over years which could be due to the application on early instars or the indirect effect of the role of natural enemies in the system which other wise disappear with chemical sprays.

Of all the treatments Neem product showed good effect on larval growth and life span. Neem10% had lower growth (0.0074) and lower life span (59%) when compared to rest of the treatments. The combination of two bacterial cultures used BCB117a+BCB117b showed decrease in life span (58%), though there is a slight decrease in mortality. Neem+117b(0.0046) showed moderate synergistic effect than rest of other neem + bacteria. The reason may be neem acting as antifeedant and bacteria producing the toxin which can kill the weak larvae by lethal septicemia. The life span of the neem treated larvae was 5-6 days because neem activity depends upon the uptake of the food. From the results it was clear that neem along with bacteria were more effective against the control of *H.armigera* larvae. All the control treatments also experienced considerable mortality which could be due to spread of infection during the experiment period.

The second experiment with eighteen cultures and two bacterial cultures were isolated from dead larvae which may be pathogenic to *H.armigera* . Only some of the fungal cultures were identified. The identified cultures were *Penicillum expansum, Aspergillus niger, Fusarium oxysporum, Alternaria tennussima* and *Nomuraea rileyi.* All the isolated cultures were then preserved. The bacteria cultures were preserved using glycerol and stored at 4°C. Glycerol prevents dehydration of the cultures and sub optimal temperature their metabolic activity is greatly reduced and lengthened their survival. Fungal cultures were stored using Paraffin oil at 4°C. Oil is added to reduce the chance of contamination. The fungal cultures when stored at low temperature decrease their metabolic activities are low and persistence of life

is more. Long term preservation for spore forming fungi (*Aspergillus niger, Penicillum expansum*) was done and cultures are viable for long periods.

Of all the treatments used , fungi and neem along with bacteria were effective. In present day agriculture as the insect pests are uncontrollable with the synthetic chemicals, the use of microbes as insecticides is rapidly increasing . Microbials are ideal for use in Integrated Pest Management , because of selectivity and environment safety. Having some constraints there is a need for additional research. And the use of microbials and biopesticides require the attention of pest behaviour, biology and population dynamics. The farmers must be educated to under stand the difference between chemical and biological products and should be encouraged for the use of microbes and biopesticides. This will be possible only when improvements are made in formulation, increased speed kill, longer residual activity and Environmental feasibility.

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SUMMARY

Integrated management of a polyphagous and most devastating pest like *Helicoverpa armigera*, needs more attention. Keeping in view of the indiscriminate use of the synthetic pesticides, the root cause for the resistance,

resurgence and residual activity, on ecofriendly approach for its management, in the form of Biological and Biopesticidal, management was organized during the period Nov 99 to Jan 2000, as a part of IPM research going on at ICRISAT, Patancheru, Andhra Pradesh.

The pathogenicity of Bacterial and fungal components on the larval growth and survival was significant. All the three fungal components *Metarhizium anisopliae* , *Beauveria spps* , *Nomuraea rileyi* have shown a greater mortality than different Bacteria strains. Among the Bacterial strains BCB 111, BCB 117 (a) & BCB114 were found potential against 3rd instar larvae. In the bio-products used Neem & Nuxvomica are most effective in reducing the life span and retarding the growth of *H.armigera* larvae.

Among the combinations tested, addition of neem to bacterial strains do not reveal any advantage. In bacterial combinations used BCB 117 (a) + BCB 117(b) were effective both in reducing the life span and retarding the growth.

A total of 18 fungal cultures and 2 Bacterial cultures were isolated from naturally killed larvae. Among them 5 Fungal cultures viz *Penicillum expansum, Fusarium oxysporum, Alternaria tennuissima* , *Aspergillus niger* and *Nomuraea rileyi.* were identified. All the isolated cultures were preserved both on short term and long term basis.

These studies indicate that synergistic effect of Biorational products can be encouraged as effective tool in plant protection. To be more effective, the future microbial research needs to be strengthened further for answering the existing unsolved questions.