Affectionately dedicated to

my Aaji, Meenakshi,

who loved us dearly and

laid down her life …, three months back ;

and to Aji. C. S.

… Anu

STUDIES ON THE EFFICACY OF ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST *Helicoverpa armigera* (Hub.) Hardwick

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722, DIST. AHMEDNAGAR,
MAHARASHTRA (INDIA)

in partial fulfilment of the requirements for the Degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL ENTOMOLOGY

by

ANUPAMA K. C.
02193

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY,
COLLEGE OF AGRICULTURE,
PUNE 411 005 (MAHARASHTRA)

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Approved by

Dr. A. G. Chandele
(Chairman, Advisory Committee and Research Guide)

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**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY,
COLLEGE OF AGRICULTURE,
PUNE 411 005 (MAHARASHTRA)**

2004

CANDIDATE'S DECLARATION

I hereby declare that this thesis entitled **"Studies on the efficacy of entomopathogenic fungal isolates against *Helicoverpa armigera*" (Hub.) Hardwick**, or part thereof, has not been submitted by me or any other person to any other University or any Institute for Degree or Diploma.

Place : Pune

Date : 1 / 7 / 2004

Anupama ji

(Anupama K. C.)

Dr. A. G. Chandele
Professor of Agril. Entomology,
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Maharashtra State, India.

CERTIFICATE

This is to certify that the thesis entitled, **" STUDIES ON THE EFFICACY OF ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST *Helicoverpa armigera* (Hub.) Hardwick",** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL ENTOMOLOGY**, embodies the results of a piece of *bonafide* research work carried out by **ANUPAMA K. C.,** under my guidance and supervision, and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and the help received during the course of this investigation and sources of literature referred to have been duly acknowledged.

Place : Pune
Date: 1 / 7 / 2004

(A. G. Chandele)
Chairman and Research Guide

Dr. D. L. Sale
Associate Dean and Principal,
College of Agriculture,
Pune-411 005,
Maharashtra, India.

CERTIFICATE

This is to certify that the thesis entitled, **"STUDIES ON THE EFFICACY OF ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST *Helicoverpa armigera* (Hub.) Hardwick"**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL ENTOMOLOGY**, embodies the results of a piece of *bonafide* research work carried out by **ANUPAMA K. C.** under the guidance and supervision of **Dr. A. G. CHANDELE**, Professor of Agricultural Entomology, College of Agriculture, Pune-5 and no part of the thesis has been submitted for any other degree or diploma.

Place : Pune

Dated : 3/JUL2004

(D. L. Sale)

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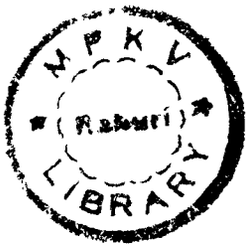
Place :

Date : / /2004 ...*(Anupama K. C.)*

Contents



List of Tables

List of Figures

List of Plates

List of Abbreviations

a. i.	active ingredient
a. m.	ante meridian
DAI	days after incubation
DAT	days after treatment
e. g.	exempli gratia
et al.	*et alli* (and other)
etc.	et cetera (and others)
g	gram (s)
ha	hectare
hrs	hour (s)
i.e.	id est (that is)
kg	kilogram
lit	litre
LC_{50}	Lethal concentration for 50% mortality
LT_{50}	Lethal time for 50% mortality
mg	milligram (s)
ml	millilitre (s)
min	minute (s)
pp	pertaining page
viz.,	videlicet (namely)
^0C	Degree Celsius
%	per cent
@	at the rate of
/	per
>	greater than
<	less than
=	equal

ABSTRACT

STUDIES ON THE EFFICACY OF ENTOMOPATHOGENIC FUNGAL ISOLATES AGAINST *Helicoverpa armigera* (Hubner) Hardwick

By

ANUPAMA K. C.

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

PUNE – 4111 005

2004

Research Guide : Dr. A. G. Chandele

Department : Agricultural Entomology

The investigations on studies of the efficacy of entomopathogenic fungal isolates, against *Helicoverpa armigera* (Hubner) Hardwick was undertaken in the insect pathology laboratory of Entomology Department, College of Agriculture, Pune- 411 005 and MITCON Laboratory, Dr. Manibhai Desai Nagar, NH- 4, Pune, during 2003-2004.

Biological activity of the three isolates of *B. bassiana viz.*, ACENB-1, ACENB-2 and MITCB-1., and two isolates of *M. anisopliae viz.*, ACENM-1 and MITCM-1 were tested. Bioassay studies were conducted using laboratory reared third instar larvae of *H. armigera* as test insect. The efficacy was estimated by calculating LC_{50} values of the conidial concentrations ranging from 1 x 10^4 to 1 x 10^8 condia ml^{-1}. Median lethal time were also calculated for all the possible concentrations tested.

The *B. bassiana* isolates with lowest LC_{50} values (3.049 x 10^4, 9.41 x 10^4 and 9.7 x 10^5 conidia ml^{-1}) were found to be superior in

efficacy than *M. anisopliae* isolates with LC_{50} values (6.3×10^5 and 8.68×10^6 conidia ml^{-1}) in producing mortality in test insect, *H. armigera.* Among the *B. bassiana* isolate, ACENB – 1 (LC_{50}, value, 3.049×10^4 conidia ml^{-1}) was found to be the most effective followed by MITCB-1 and ACENB-2 isolates with LC_{50} values 9.41×10^4 and 9.7×10^5 conidia ml^{-1}. The LT_{50} values reported for the concentration, ranging from 1×10^6 to 1×10^8 conidia ml^{-1}, for the tested isolates were, 240, 228 and 176 hrs respectively for ACENB-1 isolate. For the MITCB-1 isolate this values were 264, 240 and 216 hrs respectively. The respective values for ACENB-2 isolate were 288, 264 and 240 hrs respectively. The *M. anisopliae* isolate ACENM- 1 was having these respective LT_{50} values of 312, 288 and 264 hrs. The LT_{50} value for the conidial concentration 1×10^7 and 1×10^8 conidia ml^{-1} for MITCM-1 isolate were 312 and 288 hrs, respectively.

These values also revealed that the ACENB-1 isolate is followed by MITCB-1, ACENB-2, ACENM-1 and MITCM-2 respectively in their efficacy. The estimated data also supported the fact that *B. bassiana* isolates have a higher efficacy than the average values reported by the earlier workers. Thus this isolates can be utilised in field control of the dreaded pest, *H. armigera*, successfully.

The *M. anisopliae* isolates were having a median efficacy compared to earlier reported experimental findings. Even then, field experiments are needed further to investigate into the aspects like, their efficacy under different temperature and humidity ranges. There is also scope for the experimental studies, like, their compatibility with the other control methods and, the effective additives along with these isolates can be used in the field.

INTRODUCTION

1. Introduction

The genus *Helicoverpa* (Lepidoptera : Noctuidae) is widely distributed over the tropics and subtropics. It is represented by several species, which are among the most dreaded agricultural pests, for they have defied human efforts to check their spread, using chemical insecticides and consequent economic damage to several important crops. Any intervention in the environment towards the elimination of a species brings out parallel changes in the pest to overcome the hurdle. Thus, *Helicoverpa* has overcome almost all the synthetic insecticides (Arms *et al.* 1992, Basavana Goud, 1994). Three international workshops and several national and international conferences held to discuss this genus only reflects its importance.

Among the different species of *Helicoverpa* occurring in India, *Helicoverpa armigera* (Hub.) Hardwick is the most widely prevalent and devastating one. It attacks more than 182 host plants belonging to 47 botanical families in the Indian subcontinent and it is now estimated to feed on more than 200 plant species (Pawar, 1998). This pest is typified by being highly mobile, plasticity in host suitability, fecundity, multivolatine and voracious in its feeding.

H. armigera is known for its extensive host range and severe damage to many food and fibre crops (Anon., 1977). In tomato, the damage caused may range from 40 to 50 per cent (Srinivasan, 1959). The damage due to *H. armigera* is particularly severe in chickpea and pigeonpea in the Indian sub-continent, where 80 per cent of world's chickpea and 90 per cent of the world's pigeonpea crops are grown (Bhatnagar *et al.* 1981). The damage potential of *H. armigera* is so great that an average infestation of one larva per plant of pigeonpea can cause a yield loss of 10 to 15 $kgha^{-1}$ (Reddy and Channabasavanna, 1978). In

sorghum, yield losses of 18-26 per cent have been reported by Rawat *et al.* (1970). While in cotton it was as high as 41 to 56 per cent (Kaushik *et al.,* 1969).

Given the pretext of growing awareness about the failure of chemical pesticides on one hand, and health hazards on the other hand, have compelled the agricultural entomologists to develop economically viable, eco-friendly and sustainable pest management strategies. In this direction, biological control is especially desirable because it is safe, permanent, economical and advocated as the first line of attack. Microbial control is the biological suppression of insect pests employing microbial world. It has the advantage of the higher host specificity, virulence, safety to natural enemies and compatibility with other methods.

Among microbial agents, the fungal pathogens causing diseases to the insects or entomopathogenic fungi, are practically more significant as they are epizootic in nature. Also, they have the advantages of ease of production and contact action, which allow direct penetration of the host cuticle without ingestion (Payne, 1988). Of the 700 species of entomopathogenic fungi currently known, only 10 species are presently being developed for insect pest control, (Keller, S, 1992., Kerwin, J. L, 1992., Mc Coy *et al.*, 1998., Rath, A. C, 1992., Roberts, D. W); which implies scope for this subject.

Agostino Bassi (1835a), who was the first one to demonstrate that entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, could cause an infectious disease in silkworm, also suggested the concept that, an infectious microorganism might be used to control insect pests. About the same time, Metschnikoff (1879) and Krassilstzchik (1886) in Russia, successfully demonstrated the first mass production and field use of an entomopathogenic fungus, *Metarrhizium anisopliae* (Metschnikoff)

Sorokin, to control insect-pest, the grain weevil and the sugar beet curculio.

Beauveria bassiana (Balsamo) Vuillemin, the white muscardine fungus, belongs to subdivison Deuteromycotina, class Hyphomycetes, order Moniliales and family Moniliaceae, is one of the disease producing pathogen in insect pests and is reported to be used against many insects from ancient time for their control (Benham and Miranda, 1953).

Many workers worldwide tested this fungus under laboratory and field conditions and promising results against insect pests were reported. The efficacy of this fungus has been tested against *H. armigera* by several workers including Jayaramaiah, 1981b., Narayanan, 1985., Devaprasad *et al.* 1990a, 1990b., Gowda *et al.* 1992., Sandhu *et al.* 1993a., Kencharaddi and Jayaramaiah, 1997a, 1997b., Saxena and Ahmad, 1997 and found to be effective.

Metarrhizium anisopliae (Metschnikoff) Sorokin, the green muscardine fungi, also belongs to subdivision Deuteromycotina, class Hyphomycetes, order Moniliales and family Moniliaceae. This fungus are known to be attacking over 200 species of insects covering seven orders (Roberts, 1973b.). It is the most widely used entomopathogenic fungi in microbial control attempts (Yendol and Roberts, 1971). The possible use of this fungus in reducing pest populations has been considered by numerous investigators like Latch, 1965., Veen, 1968., Zacharuk and Tinline, 1968 etc. This fungus seems to be pathogenic to a wide range of insect pests of orders Coleoptera, Lepidoptera, Orthoptera and Hemiptera (Alves *et al.* 1996). In India, pathogenicity of *M. anisopliae* has been recorded on termites (Khan *et al.* 1993), mango hoppers (Vyas *et al.* 1993), *Helicoverpa* (Devaprasad *et al.,* 1990a, 1990b, Gopalakrishnan and Narayanan, 1988a, 1988b, 1988c) and on rice bug (Harris, 2000).

The mode of action and symptoms of attack by *B. bassiana* on *H. armigera* were reported by Gardner and Noblet (1978)., Gardner *et al.* (1979)., Sue Perkrul and Grula (1979)., Sandhu (1993) and Sharma *et al.* (1994). Similarly, mode of action and symptoms of attack by *M. anisopliae* on *H. armigera* were reported by Urs and Govindu (1971), Jayaramaiah (1981a) and Gopalkrishnan and Narayanan (1988a, 1988b, 1988c, 1989a, 1989b).

Also, as the *Beauveria* and *Metarrhizium* are facultative pathogens, they have been mass produced both in living insects and in artificial media (Martignoni, 1964., Dulmage and Rodes, 1971., Ignoffo and Hink, 1971., Pandit and Som, 1998).

The success of entomopathogenic fungi as microbial control agents is largely dependent upon the use of highly efficient strains (Daoust and Robert, 1982). Therefore, efficacy studies of the three isolates of *B. bassiana viz.,* ACENB-1, ACENB-2 and MITCB-1; and two isolates of *M. anisopliae viz.,* ACENM-1 and MITCM-1 were undertaken against *H. armigera* to achieve the following objectives.

1. To study the efficacy of entomopathogenic fungal isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarrhizium anisopliae* (Metschinikoff) Sorokin against third instar larvae of *Helicoverpa armigera* (Hubner) Hardwick under laboratory condition.
2. To determine the LC_{50} and LT_{50} values.

REVIEW OF LITERATURE

2. Review of Literature

Testing of entomopathogenic fungal isolates for their efficacy is important in utilizing them in further pest control. The literature pertaining to predetermined aspects such as efficacy of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarrhizium anisopliae* (Metschinikoff) Sorokin against *Helicoverpa armigera* (Hubner) Hardwick against other lepidopteran insect pests is presented below. The review of literature pertaining to LC_{50} and LT_{50} values is also presented in this chapter.

2.1 Efficacy of *Beauveria bassiana* (Bals.) Vuillemin and *Metarrhizium anisopliae* (Metschinikoff) Sorokin against *Helicoverpa armigera* (Hubner) Hardwick

Urs and Govindu (1971) reported that high per cent mortality of larvae of *H. armigera* with *M. anisopliae* var. *anisopliae* may be due to the high susceptibility of the just moulted larvae, since it has been observed that during ecdysis, hyphae and blastospore invade the exuvial fluid and infect the newly formed integument easily (Gopalakrishnan and Narayanan, 1989a).

Gardner and Noblet (1978) and Gardner *et al.*, (1979) reported that in several lepidopteran insects, including *Helicoverpa,* infected with *B. bassiana.,* the haemolymph protein increased upto the last stage of infection and decreased when the insect had ceased feeding prior to death.

Sue Perkrul and Grula (1979) reported that conidia from highly pathogenic mutants of *B. bassiana* germinate quickly (within 18 hrs.) on

the surface of corn earworm larvae (*Helicoverpa* sp.) and immediately begin penetration of the cuticle. Enzymes produced by the penetrating hyphae degrade the cuticle since holes are formed at the point of entry. Clustering of conidia around nodules of larvae is often seen, but penetration is not restricted to such areas. Direct evidence was presented to show that conidia can also germinate inside the spiracle openings and could invade larvae by this route. Once inside the haemocoel, the fungus multiplies extensively; however larval death occurs with only minimal breakdown of internal tissues during mummification, out growth of fungal hyphae occurs first and most extensively in the inter segmental region of larvae.

Agrawal and Rajak (1985) reported that *B. bassiana,* an entomopathogenic fungus was considered to be potentially effective for the control of many insect pests including chickpea borer, *H. armigera* in Jabalpur (M.P.)

Gopalakrishnan and Narayanan (1987) conducted preliminary pathogenicity test by spraying spore suspension of *M. anisopliae* at 1.8 x 10^9 spore ml^{-1} against *H. armigera* which revealed high susceptibility of *H. armigera* to this fungus, recording 80-100% mortality of all the five instars tested with an incubation period ranging from 2 to 14 days.

Narayanan (1988) studied the host pathogen relationship with *B. bassiana* against *H. armigera* by spraying aqueous spore suspension at four different concentrations *viz.,* 1.0 x 10^7, 1.0 x 10^8, 1.0 x 10^9, 1.0 x 10^{10} spores ml^{-1} which revealed that all the larval instars were highly susceptible to the first two concentrations tested, recording 60 to 100 per cent mortality.

Gopalakrishnan and Narayanan (1988 a) conducted, host- pathogen relationship studies with *B. bassiana* against *H. armigera* by spraying

aqueous spore suspension at four different concentrations *viz.*, 1.0 x 10^7, 1.0 x 10^8, 1.0 x 10^9 and 1.0 x 10^{10} spores ml^{-1} and revealed that all the five instars are highly susceptible to the first two concentrations tested recording 60 to 100 per cent mortality with an incubation period ranging from 2 to 15 days.

Gopalakrishnan and Narayanan (1988 b) conducted pathogenicity test by spraying the aqueous spore suspension of the fungus *M. anisopliae* @ 1.8 x 10^9 spores ml^{-1} against all the five different instars of *H. armigera.* It is evident from the results that the fungus was highly virulent inflicting 100 per cent mortality to all the instars except in the case of fifth instar where mortality was 80 per cent with an incubation period ranging from 2 to 5 days.

Gopalakrishnan and Narayanan (1988 c) reported that the infected caterpillars of *H. armigera* with *M. anisopliae* were sluggish and ceased to feed on the third day after inoculation. The body became slightly bent, tough and mummified on the fifth day. Initial growth of the fungus was noticed on the seventh day and on the eight day the whole body was covered with tuft of pure white mycelial growth with green spores covering the entire body of the caterpillar.

Gopalakrishnan and Narayanan (1989) studied the relationship between *H. armigera* and the entomogenous fungus *M. anisopliae* var. *anisopliae* in the laboratory at 27^0C, using fungus derived from larvae of the noctuid collected from tomatoes in Karnataka, India. Treatment with 1.8 x 10^9 conidia ml^{-1} caused 80-100 per cent mortality of larvae in all 5 instars, prepupae and pupae within 2-10 days, with 1^{st} and 2^{nd} instar larvae having 100 and 75% mortality, respectively, within 48 hrs.

Gopalakrishnan and Narayanan (1989a) reported that mortality due to *M. anisopliae* infection followed by sporulation was observed invariably in all the last instars. The fungus infected larvae become hard

and mummified and mycelial growth appeared between the body segments, on the spiracles, and on the appendages. At the advanced stage, the entire body was covered with white mycelial growth leaving an appearance of white cottony cushion.

Zhao- Junsheng *et al.* (1990) carried out an experiment on the control of lepidopterous insect pests with *M. anisopliae* at a dilution rate of 1:10 and 20 in a cabbage *Brassica caulorapa* (Kohlrabi, *Brassica oleracea* var. *gongylodes*) field in Taiwan, Shanxi province, China, in 1997-98. 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 (*Plutella xylostella*) and 77.73 per cent against common cabbage worm (*Pieris rapae*) on kohl rabi and 53.98 per cent against tobacco budworm (*H. armigera*) on tomato higher than those obtained by applying *M. anisopliae* at a dilution rate of 1: 3000.

Agarwal (1990a) reported that eggs of *H. armigera* were susceptible to *B. bassiana* and infected eggs failed to hatch which later became brown and shrunken.

Saxena *et al.* (1990) reported that among the three entomogenous fungi tested, *B. bassiana*, *M. anisopliae* and *N. rileyi.*, *B. bassiana* was the most virulent causing mortality between 65-100 per cent within four days after treatment. *M. anisopliae* and *N. rileyi* showed 50-70 per cent and 40-60 per cent mortality, respectively in seven days after the treatments were imposed. The comparative efficacy of two isolates of *B. bassiana* (CIHNP and IIHR isolates) was studied against *H. armigera.* The percentage larval mortality ranged from 65-100 and 50 to 100 due to CIHNP and IIHR isolates, respectively.

Gopalakrishnan and Narayanan (1990) reported that *B. bassiana* was pathogenic to all stages of *H. armigera* and caused 60 to 100 per cent larval mortality at 10^8 conidia ml^{-1} and 60 per cent pupal mortality at

10^9 conidia $ml^{-1}$and 80 per cent prepupal mortality at 10^{10} conidia ml^{-1}. The fungus was found pathogenic on eggs of *H. armigera* and all the treated eggs failed to hatch when dipped into a suspension of 1.0 x 10^7 conidia ml^{-1}.

Devaprasad *et al.* (1990 a) studied the infectivity of *B. bassiana* on *H. armigera* and reported that Bapatla isolate was found to be most virulent @ 2.17 x 10^5 conidia ml^{-1}.

Devaprasad *et al.* (1990 b) observed the susceptibility of the gram caterpillar *H. armigera* to certain entomopathogenic fungi especially *B. bassiana* and *M. anisopliae.*

Gowda *et al.* (1992) recorded *B. bassiana* (white muscardine fungus) infecting several insect pests including *H. armigera.*

Malik *et al.* (1993) reported that *B. bassiana* gave effective control of *H. armigera* from the 7^{th} day after application.

Sandhu. (1993) reported the dorsal and ventral infection of *B. bassiana* caused cent per cent mortality of *H. armigera* in four days.

Sandhu *et al.* (1993b) stated that conidia of *B. bassiana* showed effectiveness even after 24 months against third instar larvae of *H. armigera* under favourable storage conditions.

Sandhu *et al.* (1993a) studied the conidial viability and virulence of *B. bassiana* against third instar larvae of *H. armigera.* Conidia remained virulent to the larvae to *H. armigera* under favourable condition even after 24 hours.

Sharma *et al. (*1994) in laboratory studies showed that toxins of the entomopathogenus fungus, *B. bassiana* disturbed the physiological balance of *H. armigera.* Reduction in respiratory efficacy led to accumulation of carbohydrates. Toxic metabolites of pathogens appeared to play an important role in decrease of total proteins, amino acids and nucleic acids.

Saxena and Ahmad (1997) conducted field trials to study the effect of *B. bassiana* against *H. armigera* on chickpea. The spraying @ 2.68 x 10^7 spores ml^{-1} was undertaken and recorded 6.8 per cent average pod damage and 2377 kg ha^{-1} yield in treated plot, whereas, in control the average per cent pod damage and yield was 16.5 per cent and 1844 kg ha^{-1} respectively.

Guo- Song- Jing *et al.* (1999) reported that 13.5, 12.0, 8.9, 8.3 and 2.5 per cent larval mortality by natural enemies such as parasitic wasp, nematodes, *Bacillus thuringiensis* and the fungi *B. bassiana* and *M. anisopliae*, respectively in fourth and fifth instar larvae of *H. armigera* on cotton. They also reported that the activity of pathogenic fungi *B. bassiana* and *M. anisopliae* was increased under higher humid condition.

Wadyalkar (2001) evaluated the pathogenicity of *M. anisopliae* using different concentrations from 10^4 to 10^8 spore ml^{-1} of fungal suspension against 2^{nd} instar larvae of *H. armigera* and reported 90 per cent larval mortality in 10^8 spore ml^{-1} concentration at 8^{th} day after treatment.

Varhade (2001) studied the pathogenicity of *M. anisopliae* using different concentrations ranging from 2.26 x 10^9 to 2.26 x 10^6 spore ml^{-1} of fungal suspension against 2^{nd} instar larvae of *H. armigera* and reported 95 per cent larval mortality in 2.26 x 10^9 spore ml^{-1} concentration at 8^{th} day after treatment.

Deshpande *et al.* (2001) reported that entomopathogenic fungi as mycoinsecticides are useful against Lepidopteran pests in pulses. Under the biopesticide programme of Indo-Swiss Collaboration in Biotechnology (ISCB), around 56 different fungal strains have been isolated including *M. anisopliae*, *B. bassiana* and *N. rileyi* from the soil by Galleria Bait Method (GBM), plating on selective medium for the

infected *Spodoptera* and *Heliothis* larvae. Using bioassay with *Heliothis* and *Spodoptera*, four promising strains were isolated on the basis of per cent mortality (> 75% in 4.5 days) for further large scale production and field trials.

2.2 Efficacy of *Beauveria bassiana* (Bals.) Vuillemin and *Metarrhizium anisopliae* (Metschinikoff) Sorokin against Lepidopteran insect pests

Bassi (1835b) reported for the first time that the problems affecting silkworm larvae were actually caused by a fungus that multiplied in and on the body of insects, which was later identified as *B. bassiana.*

Steinhause (1949) reported that, the white muscardine, *B. bassiana* infection started initially on the intersegmental region and then around the spiracle, appendages and finally after death it covered the whole larvae. Ultimately, the body dried up and transformed into mummies due to white muscardine in silkworm larvae.

Anonymous (1956) Tanaka (1964) and McCoy *et al.* (1988) reported that, at an early stage of fungal infection the silk worm larvae showed little or no symptoms except for a few necrotic spots, which might have developed at the invasion sites. At a later stage of infection, the insect generally became restless, less active, their appetite is reduced and they lost coordination. Infected larvae then moved to higher places.

Kodaira (1961) studied, the physiology of the relationship between *B. bassiana* and silkworms. They multiplied as free cells in the blood until the host's death, and then its mycelium gradually invaded the tissues. Infected larvae lost weight principally through water loss.

Tanaka (1964) and Krishnaswamy *et al.* (1973) reported that at later stages of infection, larvae of *Bombyx mori* turn pale and lose their

appetite. The larvae become more inactive, cease movement with frequent vomiting.

Sikura *et al.* (1967) used *B. bassiana* against the coding moth, *Laspeyresia pomonella*. Satisfactory protection of fruits was obtained in Ukraine by three applications of Boverin at 1 kg of preparation to 24 x 10^9 spores per g^{-1} for 1 ha (or in total 7 x 10^{13} conidia) in the Zaporoje region where this pest has two annual generations.

Rogostskaja, (1967) reported spraying branches and trunks of apple trees during the second generation so that the number of imagos will be reduced the following spring.

Ramamurthi *et al.* (1967) reported the occurrence of epizootic due to *Beauveria bassiana* (Balsamo) Vuillemin in *Spodoptera* population on sun hemp in Karnataka.

Rangaswami *et al.* (1968) during systematic survey on the incidence of disease in insect pests in South India, *Aspergillus, Beauveria* and *Metarrhizium* spp. were found to be common on many *Lepidopterous* larvae.

Oblisamy *et al.* (1969) reported the epizootics caused by *B. bassiana* on *Spodoptera litura.*

Suzuki *et al.* (1971) demonstrated the culture filtrate of *M. anisopliae* contains toxins like destruxins A and B causing heavy mortality to the younger instars of silkworm, when compared to the grown up caterpillars.

Hsu *et al.* (1973) and Lewis and Cossentine (1986) reported artificially induced epizootics of the fungal pathogen, *Beauveria bassiana* (Bals.) Vuillemin, in European corn borer *Ostrinia nubilalis* populations.

Guagliumi *et al.* (1974) used *M. anisopliae* in Brazil to control the populations of *Zulia entreriana* and *Aenolomia selecta selecta* in pasture land and *Mahnanarva posticata* in sugarcane plantations. The mortality

rate varied most frequently between 30-40 per cent for nymphs and 20-30 per cent for adults. Insect pest other than frog hoppers were also killed by *Metarrhizium* include, *Diatraea* sp., *Metamacsius hemipterus, Spodoptera frugipedra*, and *Cirphis* sp.

Chitra *et al,* (1975) and Reddy, (1978) reported discharge of digestive fluid from natural openings in larvae of silkworm. The larval mortality occurred in 2 to 10 days.

Paliwal and Jakhamola (1981) during the survey in 1976-1977 on *Prospalta capensis,* a pest of safflower and niger in central India, observed *M. anisopliae* and nuclear polyhedrosis virus infecting *P. capensis* (Lep:Noctuidae) larvae.

El- Sufty *et al.* (1982) reported that the entomopathogenic fungi, *B. bassiana* was effective against *Spodoptera littoralis* (Boisd.) on cotton crop.

Zaz and Kushwaha (1983) reported *B. bassiana* attacking *S. litura* on cabbage and cauliflower.

Carruthers *et al.* (1985) and Feng *et al.* (1985) in an attempt to evaluate the potential of *B. bassiana* as a component of an European corn borer management program in New York, several isolates of *B. bassiana* have been compared under laboratory conditions. One isolate from the People's Republic of China was found to be particularly pathogenic to New York populations of European corn borer larvae.

Deseo *et al.* (1985) evaluated *M. anisopliae* @ 1g lit^{-1} of water by soaking cotton buds in the fungal suspension against *Zeuzera pyrina* L. and reported 95-99 per cent larval mortality of the pest.

Pruett and Colque (1985) reported some entomopathogenic fungi from the Bolivian Orient. Strains of fungi *B. bassiana* and *M. anisopliae* var. *anisopliae* obtained from larvae of the pyralid, *Diatraea rufescens*

collected in Bolivia were shown to be naturally occurring pathogens of *Diatraea* spp. under local conditions in the Bolivian orient.

Vey *et al.* (1986) observed that a strong toxaemic activity of *M. anisopliae* was demonstrated in invertebrates by laboratory tests using 3rd instar larvae of the scarabid *Oryctes rhinoceros* and final instar larvae of *Bombyx mori* as the test insects. Toxic effects of the heamolymph of infected individuals were linked to the production of toxins *viz.*, Destruxins A and B by the pathogen in living insects.

Fargues and Robert (1986) Davidson *et al.* (1995) reported that destruxin from *M. anisopliae* demonstrate a very high insecticidal effect on a wide range of target insects, mainly lepidopteran and dipterans.

Siddaramaiah *et al.* (1986), reported an incidence of infection with *Metarrhizium anisopliae* (Metchnikoff) Sorokin in groundnut in Karnataka, on *Spodoptera litura* larvae. During their eight years of study from 1977 to 1984 on groundnut crop, they observed the larvae of *S. litura* infected with fungi in the range of 2.23, 8.59, 8.19, 12.72, 7.6, 7.2, 5.66 and 6.23 per cent, respectively.

Bordat *et al.* (1988) studied the susceptibility of *Liriomyza trifolii* and *L. sativae* to 11 strains of entomogenous fungi in the laboratory. Pupae were placed in peat infected @ of about 10^8 conidiospore g^{-1} with suspensions of *B. bassiana* (4 strains), *M. anisopliae* (3 strains), *Paecilomyces farinosus* (1 strain) and *P. fumosoroseus* (3 strains). At 25^0C, *L. trifolii* was susceptible to *P. farinosus* (23% adult emergence) and 2 strains of *P. fumosoroseus* (2.5 and 4% adult emergence). An ambient temperature of 20^0C slowed metamorphosis and so contributed to mycosis development. *L. sativae* was generally less susceptible than *L. trifoli* to the tested strains. *M. anisopliae* 78 and *P. farinosus* 46 were highly efficient as adults emerged from only 23.5 per cent and 27.5 per cent of pupae.

Patel *et al.* (1988) stated that entomogenous fungus *Metarrhizium anisopliae* (Metch) Sorokin var. *anisopliae* was found pathogenic to the larvae of *Agrotis segetum* (Schiff). Soil application of fungus @ 1.2 x 10^7 spore g^{-1} of soil inflicted 45 per cent mortality in last instar larvae of *A. segetum.*

Prasad and Kushwaha (1990) conducted the field survey in Rajasthan, during 1975-76. Larvae of *Spodoptera litura* Fab. infesting cauliflower and cabbage were found infected with *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *Streptococcous* sp., *M. anisopliae* and *Entomophthora* sp. The mortality of *S. litura* on cauliflower due to these pathogens was 50 per cent.

McDowell *et al.* (1990a) conducted studies to determine the effects of a commercial *B. bassiana* spore preparation (ABG- 6178, Abbott Laboratories) on larvae of *Elasmopalpus lignosellus* (Lepidoptera : Pyralidae) *B. bassiana* was virulent to first and third instars in bioassays in which inoculum was applied to leaf substrates. First instars were more susceptible than third instars. Larvae treated with *B. bassiana* continued to develop and consume food at normal rates until died or pupated. Higher conidial levels were required to cause mortality when inoculum was mixed into larval soil habitat.

Agarwal (1990b) reported that eggs of teak leaf skeletonizer *Hyblaea puerea* (Cramer) (Lep: Hyblacidae) were susceptible to *B. bassiana* and the infected eggs failed to hatch which later became brown and shrunken.

Raghavaiah and Jayaramaiah (1990) reported that when eight races of *Bombyx mori* were infected with entomogenous fungus, *B. bassiana* at 10^1 and 10^9 spores per ml^{-1}, no cocoons were formed at 10^8 and 10^9 concentrations. The percentage of cocoon formation was in the range of 48 to 78 per cent in the remaining concentrations of the fungus.

Santiago (1991) determined the potential of *M. anisopliae,* for the control of insect pests of cabbage in the laboratory and field condition. Out of 28 tested strains of *M. anisopliae* strain Fm_8 and Fm_{12} showed high virulence to the larvae of *Plutella xylostella* (L.) and *Crocidoloma binotalis,* but were found ineffective against the larvae of *P. xylostella* (Z.) and on the basis of larval mortality and yield, strain Fm_8 was judged more potent as compared to strain 12.

Patel *et al.* (1991) collected 500 larvae of *Agrotis ipsilon* (Hufn.), *Agrotis segetum* (Schiff.) and *A. spinifera* from potato field in Gujarat during 1986 reared in the laboratory and observed the mortality due to various natural enemies, 1.6 per cent larvae were found infected with *M. anisopliae.*

Shi-Yih Hung and Boucias (1992) reported that 5^{th} instar of *Spodoptera exigua* (Hubn.) larvae were found to be highly susceptible to haemocoelic challenge of low dosages (50-500 cells/larvae) of *B. bassiana* blastospores. At higher dosages ($5x10^3 - 5x10^4$ cells/larvae), fungal challenge cause cessation in larval development and death within 2-3 days post injection. A dosage of $5x10^2$ blastospores/larvae, producing synchronous larval mortality within 72 hrs was selected for phagocytic studies. Total and differential haemocyte counts revealed that infection by *B. bassiana* caused a dramatic reduction in the major hemocyte class, the granulocyte, by 36 hrs post challenge. Fungal infection was also observed to inhibit filopodial formation and spreading of granulocytes by 24 hr post challenge. At intervals during the injection cycle, the phagocytic competence of circulating haemocytes were evaluated with a second injection of fluorescent labeled fungal cells. Results of these assays demonstrated that as the disease progressed, an increasing number of haemocytes were unable to phagocytose labelled fungal cells. In summary, *B. bassiana* appears to possess a multifaceted capability for

both suppressing and eluding the cellular defense response of *S. exigua* larvae.

Maniaina (1992) determined the pathogenicity of *B. bassiana* against second instar larve of *Chilo partellus* L. and 5th and 6th instar larvae of *Busseola fusca* L. (stem borer). The fungus caused 3 to 100 per cent mortality of larvae of *C. partellus* and 30 to 84 per cent mortality of *B. fusca.*

Maniaina (1992 a) assessed several strains of *M. anisopliae* and *B. bassiana* in the laboratory to determine their pathogenicity against second instar larvae of *Chilo partellus* (Swinh.) and 5th and 6th instar larvae of *Busseola fusca* (L.). The strains of *M. anisopliae* ICIPE 18 and 30 showed high level virulence to both the tested insects causing 65-100 per cent larval mortality. The isolates of *B. bassiana* caused 30-100 per cent larval mortality in *C. partellus* and 30-80 per cent larval mortality in *B. fusca.* Easwaramoorthy and Santhalakshmi (1993) observed for the first time *B. bassiana* infecting sugarcane root borer, *Emmalocera depressella* (Swinh.) from Yamuna nagar, Haryana. They recorded 100% mortality of sugarcane root borer, in 5.3 days by spraying fungal suspensions (10^7 spores ml^{-1}) of *B. bassiana.*

Timothy and Subhash (1993) tested the susceptibility of 5 fungal strains of *B. bassiana* against eastern tent caterpillar (*Malacosoma americanum* Wlk.). All fungal strains were from either the ARS culture collection (Peoria, IL) on the USDA – ARS collection of Entomopathogenic fungi cultures, Boyce Thompson Institute at Cornell University (Ithaca, Ny). Healthy larvae of *M. americanum* were placed in plastic dishes in groups of 10 and inoculated with an 800 µl suspension containing 6 x 10^7 spores in water (i.e. 7.2 x 10^7 spores ml^{-1}.). All strains of *B. bassiana* were effective against *M. americanum,* resulting in the death of all treated caterpillars within 4 days. In some cases

M. americanum caterpillars exhibited signs of distress (inactivity and melanization of cuticle) within 6 hrs of exposure of *B. bassiana* spores. The surface of the caterpillars was thickly covered with hairs, which may enhance the adsorption and retention of spores and promote rapid infection.

Ibrahim and Low (1993) conducted studies to examine the possibility of using entomogenous fungi to control *Plutella xylostella* (L.) in Serdong, Selangor, Malaysia. Three applications of spore suspensions of *B. bassiana* and *Paecilomyces fumosoroseus* each applied at a dilution of 1 x 10^8 spores ml^{-1} at a rate of 3.75 x x 10^{13} spores ha^{-1}, were required to cause significant reduction ($P< 0.05$) in the larval population. The virulence of both fungal species was maintained throughout the duration of the experiment.

Li, H.K. (1993) reported *B. bassiana, M. anisopliae, Isaria farinosus* (*Paelilomyoces farinosus*), *Cordyceps* sp. *and 7 Fusarium* spp. infecting *Chilo hyrax* (*C. niponella*), *C. luteellus, Proceras venosata* (*C. sacchariphagus sacchariphagus*), *Phragmataecia castaneae, Sesamia inferens* Wlk., *Sesamia* sp. and *Scirpophaga nivella* on the reeds *Miscanthus sacchariflorus* and *Phragmites communis* (*P. australis*).

Chiuo and Hou (1993a) also reported *B. bassiana* and *M. anisopliae* to be effective against eggs, larvae and pupae of *Ostrinia furnacalis* (Guence).

Chiuo and Hou, (1993b) reported that granular formulation of *B. bassiana* at 2 x 10^8 conidia g^{-1} was as effective as carbofuran in screen house tests against Asian corn borer *Ostrina furnacalis* Guenee (Lep: Pyralidae).

Maniana *et al.* (1994) tested 2 strains each of *M. anisopliae* and *B. bassiana* with conidial suspension @ 1.0 to 2.0 x 10^{11} conidia ha^{-1} against *Chilo partellus* (Swinhoe) on maize. Two applications were given

in one plot and only one application given in another plot. The significant reduction in the larvae of *C. partellus* and the performance in both the plots were reported. There was significant reduction in the number of *C. partellus* larvae even with one application.

Martinez *et al.* (1995) described the results of comparative bioassays under laboratory conditions for determining the susceptibility of eggs, larvae and pupae of the sugarcane pest, *Diatraea saccharalis* (Fabricius) to different isolates of the entomopathogenic fungi *B. bassiana* and *M. anisopliae.* Variance analysis were carried out and the means were compared by Newman Keuls test. TL_{50} and DL_{50} were determined for more aggressive isolates. The results demonstrated that only eggs of the pyralid were not susceptible to isolates used in this study.

Loc (1995) observed that the mortality due to *B. bassiana* ranged from 66.67 to 100 per cent in second instar larvae, 52.50 to 85.00 in third instar larvae and 0 to 5 per cent in the fourth instar larvae of *Spilosoma oblique* (Walker).

Gutierrez *et al.* (1995) studied the effect of *M. anisopliae* and chitin inhibitior trilflumuron and diflubenzuron alone and in combination against *Spodoptera frugiperda* a pest fungus, was applied at 4.11×10^{10}, 4.11×10^{8}, 4.11×10^{6} (S. & A.) and 4.11×10^{2} conidia ml^{-1} and was found effective against 7 days old larvae of the pest. Higher concentration of the fungus gave 100% mortality after 5 days but failed to control the noctuid in combination with the chitin inhibitor.

Pandit and Samanta (1995) tested the efficacy of two entomogenous fungi *B. bassiana* and *M. anisopliae* against the larvae of *Spilosoma oblique* (Walker) and reported 74-78 per cent and 75-91 per cent larval mortality of the pest, respectively.

Fuentes and Carballo (1995) opined that *B. bassiana* isolates 447 was most effective against *P. xylostella* at 2.2×10^{5} conidia ml^{-1}. The

above author tested *B. bassiana* at different concentrations *viz.*, 10^7, 10^6, 10^5 and 10^4 spore ml^{-1} of which 10^7 spores ml^{-1} recorded least pod damage followed by 10^0, 10^5 and 10^4.

Lezama *et al.* (1996) evaluated the virulence of several strains of entomopathogenic fungi *M. anisopliae, B. bassiana, Nomuraea rileyi, Paecilomyces fumosoroseus* and *P. javamicus* against the eggs and neonate larvae of *Spodoptera frugiperda* (S. & A.) under laboratory condition. They have reported tha *M. anisopliae* @ 1.0 x 10^8 spore ml^{-1} concentration and other were highly pathogenic to both eggs and neonate larvae of the pest, except the isolates of *N. rileyi* which were pathogenic to larvae only.

Verma *et al.* (1996) studied the pathogenicity of *B. bassiana*, *Fusarium oxysporum* and *M. anisopliae* var. *anisopliae* against eleven different insect pests of sugarcane under laboratory condition. Out of these, *B. bassiana* and *M. anisopliae* were pathogenic to larvae of *Chilo auricilius* (Dudgeon), *C. infuscatellus, Sesamia inferens* (Wlk.), adults of *Phytoscaphus* spp., *Lepropus lateralis* and nymphs and adults of *Pyrilla purpusilla*(Wlk.).

Jean – Marc *et al.* (1996) evaluated the impact of 3 control methods on larval European corn borer, *Ostrinia nubilalis* Hubner, dynamics on corn, *Zea mays* (L.) was evaluated under field conditions at Versailles, France. The control methods studied were a chemical insecticide, *Beauveria bassiana* Vuillemin (Deuteromycotina: Hyphomycete) and a transgenic corn hybrid. The experimental study showed that *B. bassiana* control was similar to chemical control.

Andreas *et al.* (1997) studied the effects of *M. anisopliae* infection and three different secondary metabolites released by the fungus, destruxin A and E and cytochalasin D, on the morphology and cytoskeleton of plasmotocytes of the Greater wax moth *Galleria*

mellonella. Plasmatocytes isolated from *M. anisopliae* infected larvae exhibited impairment of attachment, spreading and cytoskeleton formation accompanied with the occurrence of blebbing and pycnotic nuclei. Plasmotocytes treated with destruxin *in vitro* exhibit similar morphological and cytoskeleton alterations. The corresponding effects were characterized by inhibition of attachment, spreading and filopodia formation as well as by impared formation of acting filaments and microtubules. Cytochalasin was shown to affect plasmatocytes *in vitro* in a different manner than destruxin A and E. The results of comparative study strongly suggested that the morphology and cytoskeleton alterations of plasmatocytes observed in *M. anisopliae* infected larvae were predominantly caused by destruxins released by the fungus during mycosis.

Bischoff and Reichmuth (1997) tested strains of the entomopathogenic fungi *M. anisopliae, B. bassiana* and *Paecilomyces farinosus* against *Plodia interpunctella* (Hubner) and *Ephestia kuehniella* (Zeller). The pathogenicity of each strain was assessed by conducting experiments investigating the different virulent factors assessed the pathogenicity of each strain. It was concluded that several strains could serve as potential biological control agents of these stored products pests.

Vanninen and Hokkanen (1997) studied the efficacy of entomopathogenic fungi against *Argyresthia conjugella.* The efficacy of *Paeciolomyces fumosoroseus, P. farinosus, B. bassiana, M. anisopliae* against larvae and pupae of *Argyresthis conjugella* was studied. All species were highly pathogenic to larvae in petridishes.

Samson *et al.* (1997) evaluated two isolates, F 1332 and F 1454 of the fungal pathogen *M. anisopliae* as soil application against the larvae of sugarcane soldierfly *Inopus rubriceps* in ratoon crop of sugarcane. The treated soil was found infective to larvae, in bioassay, but poor results

were achieved in the field test. They had suggested that the low infectivity of isolates in field test was probably due to application difficulties in established crop and low probability of contact with spore by the larvae, which were small and may not move much in soil.

Rao and Reddy (1997) collected several groundnut leaf miner larvae infected by the fungus in the field at Patancheru, India druing 1996. The fungus was identified as *M. anisopliae* and multiplied on PDA medium. The pathogen was tested against field collected healthy groundnut leaf miner larvae and it was reported that the larvae died within 24 hrs and sporulation began on 7^{th} day.

Smith *et al.* (1998) carried out preliminary investigations into the potiential entomopathogenic fungi for the control of pests or stored maize. They collected the *Sitophilus zeamais* and *Tribolium* sp. from maize stores in Kenya and isolated the entomopathogenic fungus *Beauveria* sp.

Ramesh *et al.* (1999) tested *B. bassiana* against cotton leaf roller *Sylepta derogata* F. under field condition by broadcasting of 2.7×10^{12} spores which resulted 40 times more average yield in tested block than control block.

Ekesi *et al.* (2000) evaluated the pathogenicity of three isolates of *M. anisopliae* three of *B. bassiana* and one of *N. rileyi* to adult *Sitotroga cerealella* in the laboratory. All the isolates were pathogenic to the moth at three concentrations of conidia but pathogenicity varied. The most pathogenic isolates were *M. anisopliae* CPD 6 (89-100% mortality) followed by *M. anisopliae* CPD 5 (42-94%) *B. bassiana* CPD 3 (48-96%) and *N. rileyi* CPD 1 (33-95%) at 7 days after treatment. The mortalities caused by all these isolates at the highest concentration of 2.6×10^{9} conidia 50 g^{-1} of sorghum did not differ significantly from mortality caused by pirimiphos methyl at 10 ppm. At the same concentration of

conidia, *M. anisopliae* CPD 6 had a more rapid action then the other isolates with an LT_{50} of 2.1 days at 7 days after treatement. All isolates reduced the number of F_1 progency of *S. cerealella*.

Zade (2000) evaluated the pathogenicity of *M. anisopliae* using different concentrations from 0.52 x 10^6 to 8.25 x 10^6 spore ml^{-1} of fungal suspension against 2nd instar larvae of *S. litura* and reported 86.66 per cent larval mortality in 8.25 x 10^6 spore ml^{-1} concentration at 7th day after treatment.

Padmaja and Kaur (2001) recorded the pathogenicity of entomopathogenic fungus *Metarrhizium anisopliae* (Metsch.) Sorokin for the first time on rice leaf folder, *Cnaphalocrocis medinalis* (Guence) (Lepidoptera : Pyralidae). Effective control of the pest was recorded under field conditions after application of spore suspension of *M. anisopliae* in gelatin (1%) at 1 x 10^8 spores ml^{-1} on the infested rice crop. Between 5 to 7 days after treatment, 60-70 per cent mortality was recorded.

Babu *et al.* (2001) studied the toxicity of neem seed kernel extract (NSKE) and combinations of NSKE and the entomopathogenic fungus, *B. bassiana* was examined on the tobacco cutworm *Spodoptera litura* Fab. In laboratory trials. NSKE or *B. bassiana* applied separately or in combination decreased the mean larval weight and retarded the larval growth of *S. litura*. The total duration and the number of survivors were significantly decreased by an increase in the concentration of NSKE and the pathogen. Development deformities such as larval-pupal and pupal-adults intermediates were observed in NSKE and fungal treatment. The results revealed the potential for including *B. bassiana* and neem in an overall management programme of *S. litura*.

Viji and Bhagat (2001) conducted studies to determine the efficacy of three neem products (Neemax, Achook, NSKP), two

entomopathogenic fungi, *Metarrhizium anisopliae* (Mets) Soro., *Beauveria bassiana* (Bals.) Vuill., two insecticidal dusts (Fenvalerate 2% D, Chlorpyriphos 1.5% D) and three seed treatment chemicals (Accephate 75 sp, Chlorpyrifos 20 EC, Imidacloprid 70 WS) against *Agrotis ipsilon* (Hufn.) in the field and laboratory. In *M. anisopliae* and *B. bassiana* treatement each @ 5 x 10^{12} spores ha^{-1}, the damage was higher in the earlier stages of seedling growth whereas in later stages they gave good protection to the crop.

2.3 Studies on LC_{50} and LT_{50} values of *Beauveria bassiana*)Vuillemin and *Metarrhizium anisopliae* (Metschinikoff) Sorokin *against Helicoverpa armigera*(Hubner) Hardwick

Prasad, *et al.* (1990) bioassayed five entomopathogenous fungi for their infectivity to 2nd instar larvae of *H. armigera* by spraying them with conidial suspension, of which *B. bassiana* was found to be most virulent in recording the lowest LC_{50} at 2.1 x10^5 conidia ml^{-1} and susceptibility decreased with the increased age of the larvae.

Prasad *et al.* (1990a) conducted bioassays of 2nd, 3rd, and 4th, instar larvae of *H. armigera* against *B. bassiana* and reported decrease in sucsceptibility with age of the larvae in terms of both LC_{50} at LT_{50} at 10^7 spores ml^{-1} of *B. bassiana.*

Kencharaddi and Jayaramaiah (1997a) studied the dosage mortality response data for the borer species *H. armigera* treated wih spore suspensions of *B. bassiana,* showed a progressive increase in the dose required to cause 50 per cent mortality, with increasing larval age. The LC_{50} values for first, third and fifth instars larvae of *H. armigera* were 4.75 x 10^2, 2.31 x 10^4 and 1.4 x 10^8 spores ml^{-1} respectively. They also

shown that the median lethal concentration of *M. anisopliae* for the first instar larvae of *H. armigera* was 6.07 x 10^4 spores per ml^{-1} and it increased to 6.15 x 10^5 spores ml^{-1} for the third instar and it was highest (1.10 x 10^8 spores ml^{-1}) for the fifth instar.

Hassani (2000) studied the effect of entomopathogenic fungi against third instar larvae of *Spodoptera littoralis* (Boisd.) and *H. armigera.* He reported that for *H. armigera* the highest mortality was recorded by *M. a.* 79 followed by *B. ba* 108, *M. a.* 144 and *M. a.* 74. Regardless of the isolates, mortality in *H. armigera* ranged from 39 to 98 per cent while for *S. littoralis* from about 28 to 59 per cent. Medium lethal time (MLT) for *H. armigera* was 6.1 to 11.0 days and 9.7 to 12.8 days for *S. littoralis.* Mortality of *H. armigera* started on day 2 and reached about 60% on day 6 in *M. anisopliae* and *B. bassiana.*

Parmar (2001) conducted pathogenicity test of *B. bassiana* against third instar larvae of *H. armigera* and obtained 30.0 -96.66% larval mortality within 8 days in the laboratory condition. He also reported LC_{50} for the same at 1.90 x 10^5 spores ml^{-1} of fungal suspension.

Udar (2002) undertook pathogenic studies with 2.23 x 10^9, 2.23 x 10^8, 2.23 x 10^7, 2.23 x 10^6 and 2.23 x 10^5 conidia ml^{-1} concentrations of *B. bassiana* against second, third and fourth instar larvae of *H. armigera* and larval mortality were recorded on 24, 48, 72, 96, 120, 144, 168, 216 and 240 hrs after treatment and LC_{50} values 4.16 x 10^4, 2.23 x 10^5 and 4.67 x 10^6 spores ml^{-1} were calculated for second, third and fourth instar larvae of *H. armigera* as per standard procedure of probit analysis. The results of probit analysis also revealed that LT_{50} was 114.81 hrs for 2.23 x 10^9, 123.02 hrs 2.23 x 10^8, 131.82 hrs for 2.23 x 10^7, 147.91 for 2.23 x 10^6 and 181.97 hrs for 2.23 x 10^5 spores ml^{-1} concentration for the second instar., and 131.82 hrs for 2.23 x 10^9, 144.54 hrs for 2.23 x 10^8, 154.88 hrs for 2.23 x 10^7, 177.82

hrs for 2.23 x 10^6 and 245.47 hrs for 2.23 x 10^5 spores ml^{-1} concentration for third instar and., 154.88 hrs for 2.23 x 10^9, 173.78 hrs for 2.23 x 10^8, 204.17 hrs for 2.23 x 10^7, 239.88 hrs for 2.23 x 10^6 and 301.99 hrs for 2.23 x 10^5 spores ml^{-1} concentration for fourth instar larvae of *H. armigera*.

Pandurang (2002) studied the efficacy of effective isolates *B. b.* 127, *M. a.* 121 and N. r. 114 under laboratory conditions against *H. armigera* 1 x 10^4 to 1 x 10^8 spores ml^{-1}. In case of *B. bassiana* the mortality range was between 23.33 to 86.66 per cent with LD_{50} value 0.48 x 10^6 spores ml^{-1}. The *M. anisopliae* showed mortality range between 33.33 to 96.66 per cent with LD_{50} value at 0.9 x 10^5 spore ml^{-1}.

Patil (2002) reported that the critical dose for effecting 50 per cent mortality in 2nd instar larvae of *H. armigera* was 1.47 x 10^5 spore ml^{-1} with fiducial limit 4.78 x 10^3 – 4.57 x 10^6 spore ml^{-1}. The heterogeneity test was significant with χ^2 values of 0.32. The regression equation was y = 3.24 + 0.34 x. The critical time required to effect 50% larval mortality (LT_{50}) was observed at 79.43 hrs after spray, when *M. anisopliae* was sprayed at a concentration of 2.28 x 10^{10} spore ml^{-1}. Also for 2.28 x 10^9, 2.28 x 10^8, 2.28 x 10^7 and 2.28 x 10^6 spore ml^{-1} concentrations, the LT_{50} was 85.11 hrs. 97.72 hrs, 104.71 hrs and 123.02 hrs, respectively.

2.4 Studies on LC_{50} and LT_{50} values of *B. bassiana* and *M. anisopliae* against other Lepidopterous insect pests

Riba *et al.* (1983) evaluated the effect of different strains of *M. anisopliae* and other entomogenouos fungi against the eggs and larvae of maize pyralid, *Ostrinia nubilalis* by spraying on dipping the

larvae in fungal suspension. Strain No. 139 of *M. anisopliae* was reported pathogenic to the eggs, the lethal dose being less than 10^5 spore ml^{-1}.

Devaprasad *et al.* (1989) tested the three isolates of *B. basianna* (New Delhi, Bangalore, Bapatla isolates) for their infectivity to *Spodoptera litura* (F.). Of them, *B. bassiana* (Bapatla isolates), was found to be the most virulent, recording the lowest LC_{50} of 19.90 x 10^5 conidia ml^{-1} The LC_{50} values of Bangalore and New Delhi isolates of *B. bassiana* ranged from 5.55 x 10^5 to 5.58 x 10^9 conidia ml^{-1}. Bioassay of second, third and fourth instar larvae of *S. litura* showed that susceptibility decreases with increase in age of the larvae in term both LC_{50} and LT_{50}.

McDowell *et al.* (1990b) conducted studies to determine the effects of a commercial *B. bassiana* spore preparation (ABG- 6178, Abbott Laboratories on larvae of *Elasmopalpus lingosellus* (Lepidoptera: Pyralidae). LC_{50} values reported for first and third instar larvae were 1.21 x 10^1 (1.93 x 10^{-1} - 6.05 x 10^0) and 5.07 x 10^1 (1.82 x 10^2 – 1.85 x 10^0) CFU cm^{-2} leaf surface, respectively. LT_{50} (± 95% CM) values for first and second instar larvae when treated with 10, 50, 100, 500 and 1000 CFU cm^{-2} concentrations were 14.3 (17.3 – 12.2), 8.3 (9.7 – 7.2), 6.6 (7.9-4.9), 5.7 (6.3 – 5.0), 4.4 (5.1 – 3.5) days and 19.6 (31.5 – 15.7), 12.4 (15.7- 10.6), 12.6 (14.5 – 11.4), 10.2 (12.0 – 8.7), 6.3 (8.1 – 3.3) days respectively.

Brousseau *et al.* (1996) demonstrated that destruxin from *M. anisopliae* were toxic to third, fourth and fifth instar larvae of *Choristoneura fumiferana* Clemens. Lethal doses 50 and 95% were respectively 0.116 and 0.414 µg $larva^{-1}$ for third instar larvae, 0.356 and 1.088 µg $larva^{-1}$ for fourth instar larvae, and 1.520 and 4.088 µg $larva^{-1}$ for firth instar larvae.

Patel (1997) reported that LC_{50} values against second and third instar larvae of *S. obliqua* were 1.10 X 10^5, 6.25 x 10^6 conidia ml^{-1} of *B. bassiana.*, and 1.65 x 10^6, 2.21 x 10^7 conidia ml^{-1} of *M. anisopliae*, respectively. The corresponding LT_{50} values at 10^7 conidia ml^{-1} of *B. bassiana* were 116.90, 131.86 hrs against second and third instar larvae, respectively, while in case of *M. anisopliae* the values were 128.30, 162.66 hrs respectively. Early instar larvae were most sensitive to fungi than the later instar. Similar results have been reported against storage insect pests (Patel and Kanaujia, 1997)

Kencharadii and Jayaramaiah (1997b) observed the dosage mortality response data for the borer species of *Adisura atkinsoni* treated with spore suspension of *B. bassiana* showed a progressive increase in the dose required to cause 50 per cent mortality, with increased larval age. The median lethal concentration of *B. bassiana* required for the first instar larvae of *Adisura atkinsoni* were 2.07 x 10 spore ml^{-1} and it increased to 2.13 x 10^4 spores ml^{-1} for third instar and 1.39 x 10^5 spores ml^{-1} for fifth instar larvae. They also reported that the median lethal concentration of *M. anisopliae* for the first instar of larvae *Adisura atkinsoni* larvae was 1.96 x 10^3 spores ml^{-1} and it increased to 1.22 x 10^4 spores ml^{-1} for third instar and 1.21 x 10^7 spores ml^{-1} for fifth instar larva.

Zhang *et al.* (1997) reported that 2 strains (M_2 and M_4) of *M. anisopliae* spp. were pathogenic to fourth instar larvae of *Dendrolimus punctatus.* LD_{50} of M_4 strain was 4.75 x 10^7 spore ml^{-1} and LT_{50} was 6.19 -12.20 days at the concentration of 1.0 x 10^7 to 1.0 x 10^{11} spore ml^{-1}.

Ramkumar (1998a) reported that the LC_{50} values of *M. anisopliae* estimated for second, third and fourth instar larvae were 18.07 x 10^5, 39.74 x 10^5 and 211.05 x 10^5 conidia ml^{-1}. LT_{50} values for second instar

larvae treated with 1 x 10^8, 1 x 10^6 and 1 x 10^5 conidia ml^{-1} suspension were 159.97, 192.42, 228.50, and 290.60 hrs., respectively. LT_{50} values for third instar larvae treated with the same dose were 200.49, 224.67 and 249.97 hrs respectively. In case of fourth instar larvae 50 per cent mortality was obtained in 197.63, 231.14 and 274.79 hrs for the dose of 1 x 10^8, 1 x 10^7 and 1 x 10^6 conidia ml^{-1} respectively. Time mortality was not calculated for 1 x 10^5 conidia ml^{-1} as the mortality was less than 50 per cent.

Ramkumar (1998b) reported that the LC_{50} values of *B. bassiana* calculated for second, third and fourth instar larvaeof *Spodoptera litura* (F.) were 16.20 x 10^5, 69.09 x 10^5 and 179.60 x 10^5, conidia ml^{-1} with fiducial limits of 5.14 x 10^5 to 44.46 x 105, 20.88 x 105 to 285.34 x 105 and 52.22 x 10^5 to 1190.57 x 10^5 conida ml^{-1} respectively. The LT_{50} values for second instar larvae treated with 1 x 10^8, 1 x 10^7, 1 x 10^6 and 1 x 10^5 conidia ml^{-1} suspension, were 152.36, 183.60, 242.40 and 273.27 hrs, respectively. The LT_{50} values for third instar larvae treated with same dose as above were 184.76, 203.14 and 249.49 hrs, respectively. And, in the case of fourth instar larvae the LT_{50} value were 198.89, 249.40 and 270.00 hrs, respectively.

Easwaramoorthy *et al.* (2001) studied the occurrence of *M. anisopliae* var. *anisopliae* on sugarcane internode borer, *Chilo sacchariphagus indicus* Kapur. *Metarrhizium anisopliae* var. *anisopliae* is found on the larvae of sugarcane internode borer, *Chilo sacchariphagus indicus* Kapur under field conditions at Coimbatore. In laboratory tests, the fungus caused 20.0 to 83.3 per cent mortality in third instar larvae and 10.0 to 90.0 per cent mortality in fourth instar when treated with different doses ranging from 10^4 to 10^9 spores ml^{-1}. The time

taken to kill the larval varied from 5.6 to 13.1 days in the third instar and 5.9 to 9.9 days in fourth instar. The mean number of spores produced per dead larva varied from 0.17 x 10^9 to 0.48 x 10^9 and 0.92 x 10^9 to 1.52 x 10^9in fourth instar.

MATERIAL AND METHODS

3. Material and Methods

The present investigation on the efficacy of entomopathogenic fungal, isolates of *Metarrhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin against third instar larvae of *Helicoverpa armigera* (Hubner) Hardwick, was carried out in the insect pathology lab of Entomology Department, College of Agriculture, Pune-411 005 and MITCON Laboratory, BAIF Development Research Foundation Camp, Dr. Manibhai Desai Nagar, N. H.-4, Pune. The biological activities studies were carried out during the period of 2003 to 2004.

The material used and methodologies adopted for the experimentation are presented hereunder. Three isolates, *viz.,* ACENB-1, ACENB-2 and MITCB-1 of *B. bassiana* and two isolates, *viz.,* ACENM-1 and MITCM-1 of *M. anisopliae* were tested. ACEN isolates were obtained from Entomology Department, College of Agriculture, Pune, and MICT isolates from MITCON laboratory, BAIF Development Research Foundation Camp, Dr. Manibhai Desai Nagar, NH-4 Pune. All these fungi were maintained on Sabouraud's maltose agar + yeast (SMA +Y) medium. The fungi were subcultured in MITCON laboratory and the efficacy studies of the fungal isolates at different concentrations against laboratory reared third instar larvae of *Helicoverpa armigera* (Hubner) Hardwick, were conducted at the Department of Entomology, College of Agriculture, Pune-5.

3.1 Material

3.1.1 Material used for the maintenance of fungal cultures

Throughout the experiment Borosil petriplates, test tubes, conical flasks and beakers of different capacities, pipettes, measuring cylinders, glass rods, non absorbent cotton and sealing caps were used in laboratory.

Other material *viz.,* spirit lamp, inoculating needle etc., were used for inoculating the fungus on medium. Phase contrast microscope and standard haemocytometer (Improved Neubauer double ruling, Germany) were used for counting the spore ml^{-1} of the fungal suspension.

Different chemicals *viz.,* maltose, bactopeptone, yeast, agar-agar and distilled water were used for the preparation of Sabouraud's maltose agar+ yeast (SMA + Y). Wetting agent Tween 80 (0.1%) was used for making spore suspensions.

3.1.2 Material used for the maintenance of *H. armigera* culture

Material needed for the maintenance of egg stage includes petriplates, plastic tubs, double layered black muslin cloth, twines, fine camel hairbrush etc. Glass jars, black muslin cloth pieces, twines etc were needed for rearing neonate larvae on chickpea seedling. Transparent plastic vials of 3.5 x 3.2 cm dimension was used for the individual rearing of larvae either on semisynthetic media or on bhendi fruit pieces. Petriplates and sterilized soil were used for keeping the pupae for emergence in the adult emergence cage (65.2 cm x 22.6 cm x 46.0 cm). Moth collecting tubes were utilized for transferring the newly emerged adults to the mating and oviposition cage (65.2 cm x 22.6 cm x 46.0 cm). Non-absorbent cotton and twines were used for hanging the adult diet inside both the adult emergence cage and the mating and oviposition cage. Enamel trays were used for pouring freshly prepared semi synthetic diet.

3.1.2.1 Adult diet composition

Ten per cent honey solution enriched with multivitamins, prepared in sterile distilled water was used as adult feed (Sharma and Chaudhary, 1985).

3.1.2.2 Semi synthetic diet composition for individual rearing of *H. armigera* larvae

The semi-synthetic diet developed by Dang et al. (1970) and modified by Nagarkatti and Satyaprakash (1974) was used for mass rearing of the larvae in laboratory for bioassay studies. The components of this semi-synthetic diet include, kabuli gram flour (*Cicer arietinum*), ascorbic acid, methyl para hydroxy benzoate, sorbic acid, yeast, streptomycin sulphate, vitamin E capsule, multivitamin tablets, agar-agar, 10% formaldehyde and distilled water.

3.1.3 Material used for bioassay studies

Inoculation needles or sterilized metal spatula for scratching the conidia from the culture tubes, Tween 80 (0.1%), distilled water, micropipettes, small plastic vials, forceps with blunt end to hold the larvae while dipping in conical suspension, beakers, glass beads, glass rods, filter paper (Whatman No. 1), phase contrast microscope, Neubauer haemocytometer etc, were used for conducting bioassay.

3.2 Methods

3.2.1 Composition and method of preparation of media

Sabouraud's maltose agar + yeast (SMA + Y) medium was used for maintaining the fungal cultures of both *M. anisopliae* and *B. bassiana* in laboratory.

3.2.1.1 Components

Maltose	:	40.00g
Bactopeptone	:	10.00 g
Yeast	:	10.00 g
Agar-agar	:	20.00 g
Distilled water	:	upto 1000 ml

3.2.1.2 Procedure

Sabouraud's maltose agar + yeast (SMA + Y) medium was prepared by adding maltose 40 g, Bactopectone 10 g, Yeast 10 g in 1000 ml of distilled water and boiling the contents. After boiling, 20 g agar agar was added and continuously stirred with glass rod. Thus prepared medium was strained through muslin cloth and equal quantity of medium was taken in the conical flasks and plugged with non-absorbent cotton. Such flasks containing media were sterilized in autoclave at 1.05 kg cm^{-2} pressure at 160^0C temperature for 30 minutes which were further used for inoculation of fungi by transferring into petriplates as per necessity.

3.2.2 Sterilization of glasswares, medium and other materials

The glasswares were sterilized by keeping in cleansing solution (60 g of potassium dichromate and 60 ml of concentrated sulphuric acid mixed in 1000 ml of water) for 24 hours followed by washing with tap water, rinsed twice in distilled water and air-dried. Then they were autoclaved at 1.05 kg cm^{-2} pressure at 160^0C temperature for 2 hrs. Pipette, measuring cylinder and media were autoclaved at 1.05 kg cm^{-2} pressure for 20 minutes in autoclave. To avoid contamination, the isolation chamber was disinfected by keeping laminar airflow unit in working condition for about 30 minutes before inoculation.

3.2.3 Maintenance of cultures of *B. bassiana* and *M. anisopliae* isolates

Sabouraud's maltose agar + yeast (SMA + Y) medium was used to maintain the fungal cultures. These cultures were maintained in the laboratories by subculturing them at an interval of one month. The sterilized media taken out from the autoclave unit was brought to the working table of laminar airflow unit. The media was then transferred to already sterilized petriplates and test tubes under aseptic conditions.

These petriplates and test tubes (in slanting position), containing media were kept for solidification.

3.2.4 Inoculation of fungal cultures into petriplates and test tubes

The petriplates (10-15 no each) containing sterilized media were taken into isolation chamber and inoculated with the isolates of *B. bassiana* and *M. anisopliae* separately. Care has been taken to avoid contamination of one fungal isolate by the other. The inoculation was done by inserting the flame sterilized inoculating needle with fungal conidia at the centre of the petriplate. Then these inoculated petriplate were incubated at 25 $\pm 1^0$C temperature for growth and development of the fungi on the medium (Khan *et al.* 1993). Same procedure was repeated to make the culture slants in test tubes also. All these steps were carried out in aseptic condition, under laminar air flow unit (Microfilt make) to avoid contamination. Thus enough cultures were maintained to carry out the laboratory evaluation of *B. bassiana* and *M. anisopliae* isolates against 3rd instar larvae of *H. armigera.*

3.2.5 Estimation of germination percentage

Conidia from 14 days old cultures of both the fungi were harvested using sterile metal spatula and conidial suspensions were made using sterile, distilled water and a few drops of Tween 80 as wetting agent. The conidial suspensions were diluted with one per cent sucrose solution in test tube until the required concentration was obtained. A drop of suspension from each suspension was put on separate cavity slides and covered with coverslip. Melted paraffin was used to seal the coverslip to prevent contamination. These slides were incubated for 48 hrs at 25 $\pm$ 1^0 C. The per cent germination was calculated based on count of germinated conidia and non-viable conidia. Conidial suspensions with more than 95 per cent germination were used in the bioassay studies.

3.2.6 Standardization of the conidial count

Conidial count was observed at 14 days after inoculation (DAI), by counting the conidia ml^{-1} of fungal suspension. For standardizing the conidial count, 20 g of inoculated medium with homogenous fungal growth was suspended in 70 ml distilled water using a rotary mixer for 20 minutes. The homogenate was passed through muslin cloth followed by Whatman No. 1 filter paper. Adding sufficient quantity of sterilized distilled water made final volume of 100 ml, was made.

Fungal suspension obtained as stock solution was diluted upto 1: 1000, by serial dilution method, and observed under phase contrast microscope for conidial spore count with the help of Neubauer haemocytometer. The conidia were counted from 80 small squares from each counting chamber. The number of conidia ml^{-1} of the fungal suspension was worked out by using the following formula.

$$\text{Conidial count ml}^{-1} = \frac{X}{80} \times \frac{1}{400} \times \frac{1}{10} \times 1000 \times \text{dilution factor}$$

Where,

X = Reading of haemocytometer

$\frac{1}{400}$ = Volume of fluid standing on each small square

1000 = Conversion factor of 1 mm to 1 ml

10 = depth factor

3.2.7 Estimation of conidial count

The engraved haemocytometer consist of 9 big squares. Each big square is further subdivided into 25 middle squares. Each middle square is again subdivided into 16 small squares. The specification for depth and size of the square is given below.

Depth = 0.1 mm

Area of big square = 1 mm^2

Area of middle square = 0.04 mm^2 (0.2 mm x 0.2 mm)

Area of small square = 0.0025 mm^2 (0.05 x 0.05mm)

The haemocytometer was cleaned with swabed alcohol. After a series of dilution (1:1000), 0.1 ml spore suspension from different media was placed in the centre of haemocytometer with the help of sterilized micropipette and covered with specially designed coverslip. The conidia were allowed to settle for 5 minutes. These were then counted in each of the four corners and in a central unit of 16 small squares under a phase contrast microscope.

Calculation was done by using the formula given below:

$$\text{Conidial count ml}^{-1} \text{ of fungal suspension} = \frac{N \times 1000}{X}$$

Where,

N = Total no. of conidia counted per number of squares

X = Volume of solution between the coverglass and above the squares (Area of squares x depth of chamber)

3.2.8 Rearing of test insect, *H. armigera* in the laboratory

Mass rearing of test insect, *Helicovepa armigera* (Hubner) Hardwick, was done to produce healthy 3rd instar larvae of high and consistent quality in required numbers. These 3rd instar larvae of uniform size and quality were used as test insect for assessing the efficacy of entomopathogenic fungal isolates of *M. anisopliae* and *B. bassiana.*

The 2nd, 3rd, 4th and 5th instar larvae of *H. armigera* were collected from pigeonpea crop of a farmer's field from Sinhagad area of Pune, Maharashtra; during the 1st week of January 2004. They were reared in

the insect-pathology laboratory of Department of Entomology, College of Agriculture, Pune, to avoid any infection from entomopathogens. To avoid cannibalism, larvae were reared individually in sterilized plastic containers (3.5 x 3.2 cm) on Kabuli gram flour based semisynthetic diet, which has been developed by Dang *et al.* (1970) and modified by Nagarkatti and Satyaprakash (1974). Ingredients or contents of the same for the preparation of 1 kg diet are given below

Kabuli flour (*Cicer arietinum*)	105.00 g
Ascorbic acid	3.25 g
Methyl para hydroxy benzoate	2.00 g
Sorbic acid	1.00 g
Yeast	10.00 g
Streptomycin sulphate	0.25 g
Vitamin E capsule	0.25 g
Multivitamin tablets	12.75 g
Agar-agar	2.00 ml
10% Formaldehyde	2.00 ml
Distilled water	780.00 ml

3.2.8.1 Method of preparation of semisynthetic diet

The above contents of the diet were mixed thoroughly with every addition of the ingredient (except agar) in 390 ml of water and mixed well by grinding in mixer grinder for 5-10 minutes. In a separate container, agar was dissolved by boiling in 390 ml of water at 100^0C. Then it was cooled and agar-agar solution was blended with ingredients like ascorbic acid, yeast, vitamin E and multivitamin tablets taken in the mixer grinder. Streptomycin sulphate was added finally and the entire

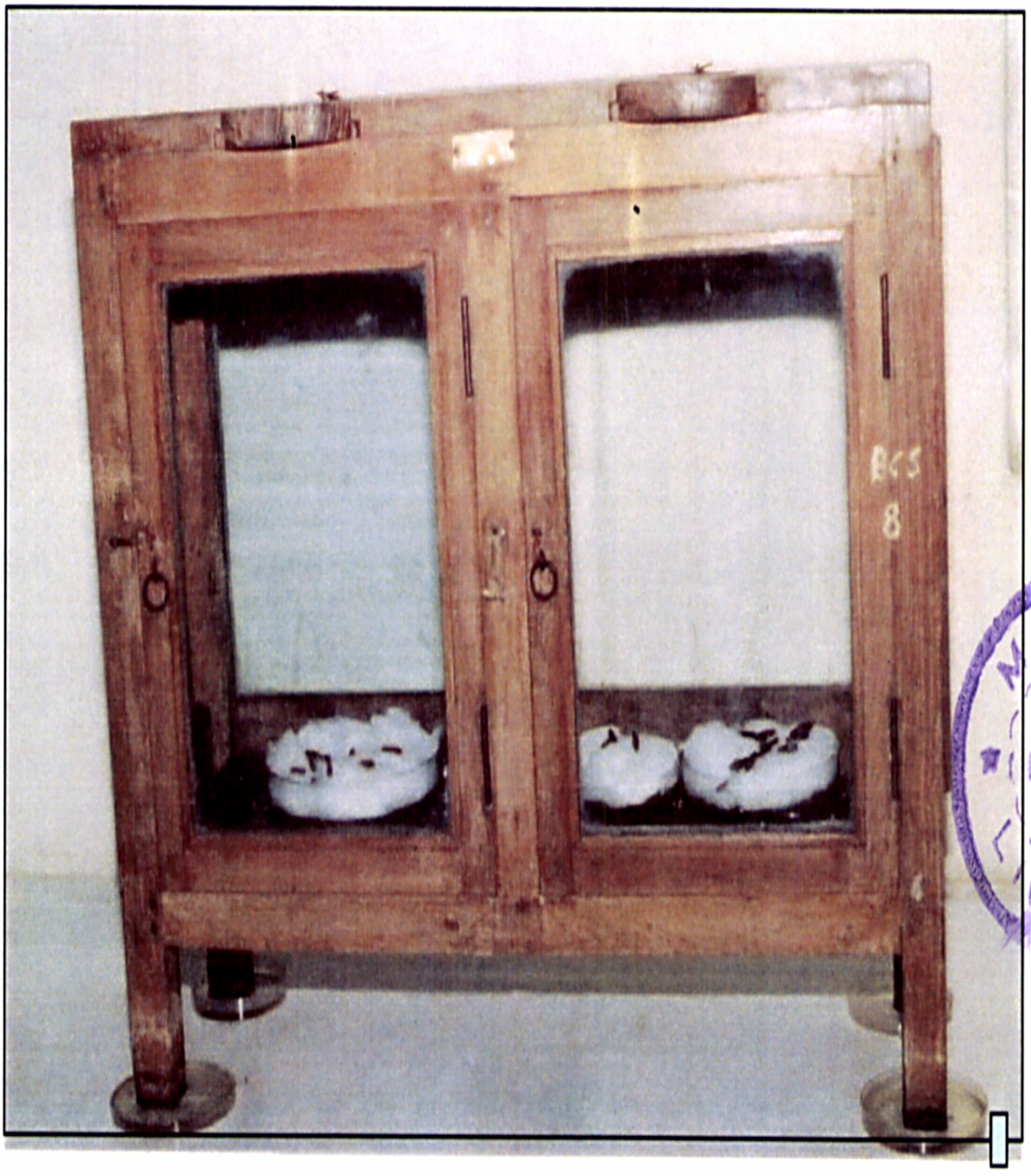


Plate1. Pupae of *H. armigera* kept for adult emergence

solution was stirred again for about 2 to 3 minutes. The blended content was immediately poured into enamel trays at a thickness of 1-1.5 cm. After cooling, these diet trays were stored in refrigerator until use.

The field-collected larvae were reared on this artificial diet until pupation. The pupae were obtect and broadly rounded from anterior side and tapering posteriorly. Freshly formed pupae were greenish in the beginning and later turned brown. After discarding the feeble and diseased pupae, the healthy ones were taken in petriplates (10 cm diameter) containing sterilized soil. The pupae were sexed observing the following characters; the distance between the genital and anal pore in female was more than double of that in the male. And also for the female pupae, on the either side of genital pore, 'V' shaped depression or fold extending upto the 10^{th} segment was visible. Supplementary characters like average length of genital and anal pores and abdominal length which were also observed with an ordinary hand lens.

These petriplates containing pupae were kept in the moth emergence cage (65.2 cm x 22.6 cm x 46.0 cm diameter) for eclosion. Adult emergence cage is a tetrapod wooden box having glass door in the front, which provides sufficient light. Adult food was given through a sliding opening on the top portion of the box. Moths emerged were also collected through this opening daily, with the help of moth collecting tube.

Pupal period was for 7-14 days. The freshly emerged female and male in 10: 13 ratio (Gopali, 1998) was released into mating and oviposition chamber. 10 : 13 female to male sex ratio is needed for higher fecundity and higher hatchability due to better zygotic condition. Mating and ovipositon chamber is having the same structure and dimensions as adult emergence chamber (65.2 cm x 22.6 cm x 46.0 cm).

Plate 2. Adult mating and oviposition chamber of *H. armigera*

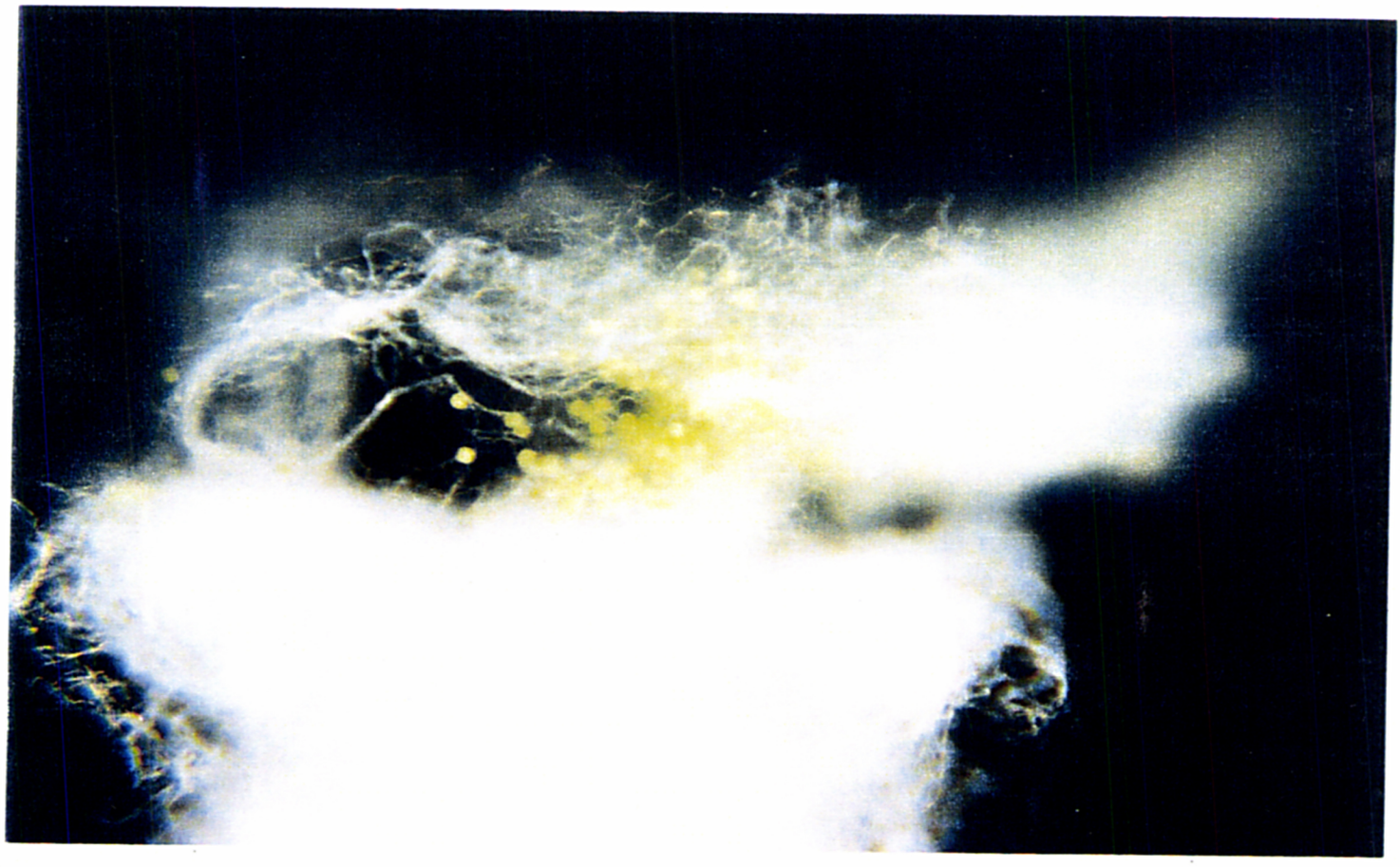


Plate 3. Eggs of *H. armigera* kept for eclosion

Plate 4. Third instar larvae of *H. armigera* reared on artificial diet

Legs of the chamber were placed immersed in water taken in petridishes to avoid crawling of ants and to give adequate humidity for mating and ovipositon. Cotton swab immersed in honey solution (10%) enriched with multivitamins was kept at hanging position inside the chamber as adult feed. The same honey solution was also poured into small vials filled with cotton so that cotton will remain in a soaked condition throughout the day. Fresh honey solution was provided daily till the death of moths.

Young maize cobs'silk (specially arranged) were kept at erect position in small plastic vials with water inside. By this the silk remained fresh for egg laying. Mating took place during early morning hours (3.00 – 6.00 a. m.), one or two days after emergence. Yellowish white eggs were laid along the exposed silk, 2-5 days after mating. Maize cobs were changed daily in the morning and eggs were collected using fine camel hair brush dipped in 0.02% sodium hypochlorite solution, into clean sterilized plastic tubs. These tubs with eggs were covered with double layered black muslin cloth and kept for eclosion. Optimum humidity was also provided. Care was also taken to see that the humidity does not exceed the optimum as it can cause fungal growth over the egg surface preventing the eggs from eclosion. Colour of egg was changed from yellowish white to deep yellow one day after laying and further turned greyish black one day before hatching. Most of the eggs hatched within 3-4 days. Neonate larvae were provided with chickpea seedling. They seemed to be crawling aimlessly for few hours without feeding despite the food being made available. The chickpea seedling were found to be best for early settlement, highest survivability and uniform growth.

So they were reared on chickpea seedling for 4 days and afterwards, the grown up larvae were reared individually in vials. Artificial diet or Bhendi fruits pieces of 1 cm thickness was provided as

food. Feed was changed and excreta removed daily. Third instar larvae, 9.4 mm x 2.83 mm dimension and 7.7 days old (Kashyap and Dhindsa, 1989) were used for bioassay. Precaution undertaken during the entire period of larval rearing is given under appendices.

3.2.9 Bioassay studies

Before bioassay in laboratory, the procedure suggested by Daoust and Roome (1974) for bracketing of pathogen was followed

3.2.9.1 Preliminary trial

For checking the efficacy and for deciding the final hypothetical concentrations, a preliminary trial was conducted using the concentration of 1 x 10^5, 1 x 10^6, 1 x 10^7 and 1 x 10^8 conidia ml^{-1} of all the fungal isolates. Ten laboratory reared 3^{rd} instar larvae were used for each treatment. Mortality was observed from 2^{nd} day onwards. After registering the mortality range between 20-80 per cent the concentrations were decided for final bioassay studies.

3.2.9.2 Final testing

For the final bioassay, stock solutions were made initially by scraping conidia from 14 days old culture in 0.1 per cent Tween 80 of the fungal cultures. To suspend the hydrophobic conidia, the conidial suspensions were mixed with sterile glass beads and shaken vigourously. Then the conidial count was taken, using standard haemocytometer (Improved neubaur double ruling, Germany) under phase contrast microscope. The stock solution of *B. bassiana* and *M. anisopliae* fungal isolates was around 2 x 10^8 to 5 x 10^9 conidia ml^{-1}. From the stock solution the serial dilution of 1 x 10^8, 1 x 10^7 , 1 x 10^6, 1 x 10^5 and 1 x 10^4 conidia ml^{-1} were prepared for each isolate using distilled water and 0.1 per cent Tween 80, as per the formula given below

Plate 8. Bioassay of *B. bassiana* and *M. anisopliae* isolates using third instar larvae of *H. armigera*

$$X = \frac{\text{Required concentration} \times \text{Final volume needed}}{\text{Counted concentration}}$$

X = Volume in ml of spore suspension to be added to final volume.

Larval dip method was used to test the efficacy of the isolates to the test insect. Fifteen ml conidial suspension of desired concentration was taken in 50 ml beaker. The third instar larvae of *H. armigera* were dipped individually in the conidial suspensions for 5 second by holding the larvae using blunt ended forceps. After treatment each larva was kept in separate plastic vials containing moist, Whatman No. 1 filter paper and allowed to feed on disinfected okra pieces and incubated at room temperature. Three replications of 10 larvae were used for all the treatments including control. Larvae dipped in 0.1 per cent Tween 80 solution was kept as control.

The mortality was recorded from 24 hrs after treatment, at 24 hrs interval upto 14 days. Larvae that had died before 24 hrs after treatment, with no evidence of mycosis were assumed to have been killed during handling and therefore were excluded from analysis. Moribund larvae were considered as dead. All the recorded larvae were having the visual symptoms of respective fungal attacks on *H. armigera* larvae as described by Urs and Govindu., 1971; Sue Perkrul and Grula, E. A., 1979., Jayaramaiah, 1981a and Gopalakrishnan and Narayanan., 1988a, 1988b, 1989a.

Dead larvae were kept in separate plastic vials containing Whatman No. 1 filter paper for allowing mycelial growth over the cadaver. After sporulation, the fungi were reisolated to observe under phase contrast microscope.

Precautions

- The conidia of the fungus are reported to cause allergic reactions. It was seemed better to use facemask and hand gloves while handling the fungal conidia.
- Necessary precautions like time and space gap were also given so that the conidia of different fungal isolates never mixed.

3.2.10 Statistical analysis

Mortality data was corrected using Abbott's formula (Abbott, 1925) given below.

$$\text{Corrected mortality (\%)} = \frac{\text{\% mortality in treatment - \% mortality in control}}{\text{100- \% mortality in control}}$$

The data was subjected to probit analysis (Finney, 1964) and the LC_{50} values were worked out accordingly. The LT_{50} values were calculated according to the formula suggested by Biever and Hostetter, 1971, for the calculation of median lethal doses from the bioassay using microorganisms. This formula is given below

$$LT_{50} = a + \frac{e\,(c - b)}{d}$$

where,

a = The number of hours from the initiation of the test until the reading made just before the 50% value was reached

b = The total number of larvae dead at the reading just before the 50% value was reached

c = Fifty per cent of the total number tested

d = The number of larvae dying in the 24 hr period during which the 50% mortality was reached

e = The number of hours between mortality counts

EXPERIMENTAL RESULTS AND DISCUSSION

4. Experimental Results and Discussion

Studies on the biological activity of three isolates of *B. bassiana viz.,* ACENB-1, ACENB-2 and MITCB-1 and two isolates of *M. anisopliae viz.,* ACENM-1 and MITCM-1 were carried out during 2003-2004. Efficacy was assayed for each isolate using conidial concentrations ranging from 1 x 10^4 to 1 x 10^8 conidia ml^{-1}, using third instars larvae of *H. armigera* as test insect. Probit analysis (Finney, 1964) was done to determine the LC_{50} values for each isolate. Using the formula suggested by Biever and Hostetter (1971) for the calculation LT_{50} values of bioassays using microorganisms, the corresponding LT_{50} values were also calculated for the fungal isolates, for the concentrations ranging from 10^6 to 10^8 conidia ml^{-1}. The total mortality observed for the *M. anisopliae* isolate MITCM-1, was below 50 per cent and hence its median lethal time was not calculated. The results obtained during the investigations are presented, and discussed in the light of available pertinent literature in this chapter.

4.1 Study of the efficacy of *B. bassiana* isolates.

Three *B. bassiana* isolates *viz.,* ACENB-1, ACENB-2 and MITCB-1 were tested for determining the biological activity on the third instar larvae of *H. armigera.* All the isolates were found to be effective to produce disease in *H. armigera* larvae. The infected caterpillars with *B. bassiana* isolates, became sluggish and ceased to feed. About 24 to 60 hrs after death of the caterpillars, white mycelial spots were observed first on the prolegs, intersegmental regions and spiracular areas. Later the entire body surface was covered with white fluffy mycelial growth. The cadavers got tough and mummified. These results were found to be in conformity with the findings of Sue-Perkrul and Grula, 1979.,

Plate 5. Initial symptom of attack by *B. bassiana*

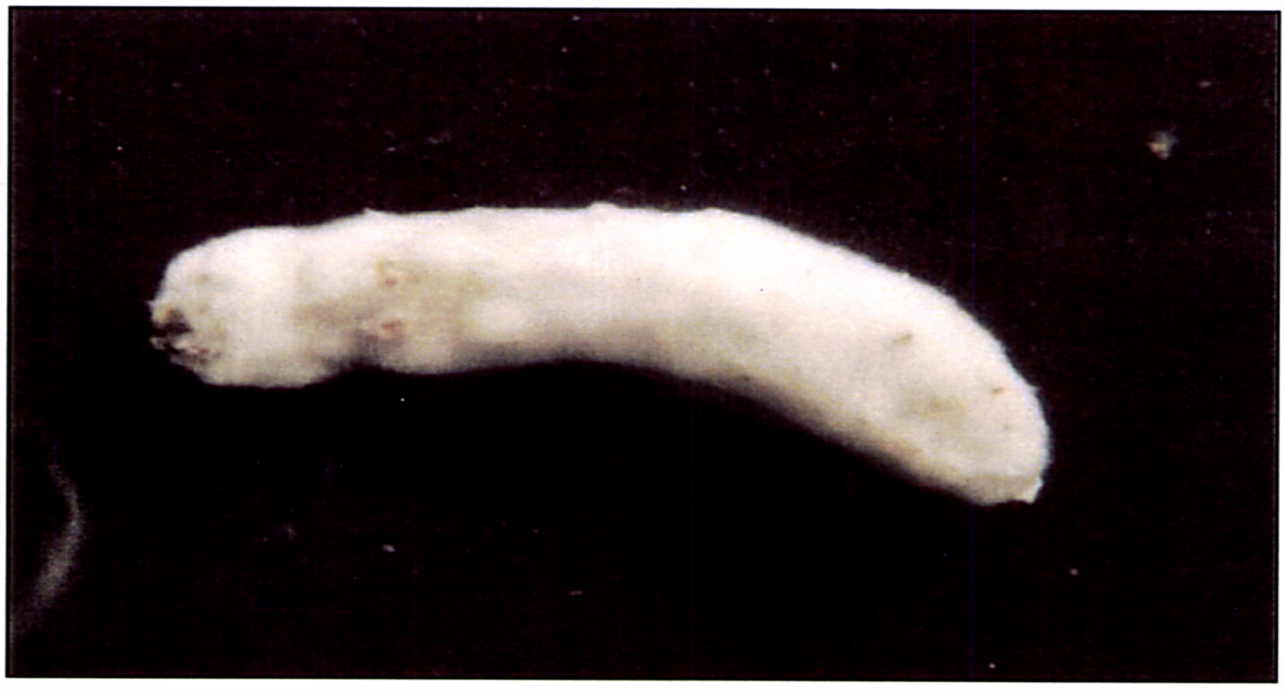


Plate 6. Final symptom of attack by *B. bassiana*

Gopalakrishnan and Narayanan, 1988a, 1989 b. The already established pathogenicity of the tested isolates were reaffirmed by this observation.

A mortality range of 33.33 to 93.33 percent was observed for the *B. bassiana* isolates. This finding was supported by the observations of Pandurang (2002) who reported a mortality range between 23.33 to 86.66 per cent to *B. bassiana* isolates.

4.1.1 Determination of LC_{50} values of *B. bassiana* isolates

4.1.1.1 LC_{50} value of ACENB-1 isolate

The tested conidial concentrations and mortality data of ACENB-1 is presented in Table 1. The graphical relationship between log concentration and probit kill is depicted in Fig-1.

The results presented in Table 1 revealed that the mortality ranged between 43.33 to 93.33 per cent, for the concentrations ranging from 1×10^4 to 1×10^8 conidia ml^{-1}. The LC_{50} value was estimated to be 3.049×10^4 conidia ml^{-1} with upper and lower fiducial limits of 2.00×10^5 conidia ml^{-1} and 5.011×10^3 conidia ml^{-1} respectively. The probit regression equation was $y = 3.296 + 0.38 x$, the slope being 0.38. The line was found to be the best fit for the data. The heterogeneity test was significant with χ^2 value 0.8752.

4.1.1.2 LC_{50} value of MITCB-1 isolate

Conidial concentration and mortality data of MITCB-1 is presented in Table 2. The graphical relationship between log concentration and probit kill is presented in Fig 2.

From the results presented in Table 2, it became clear that the mortality ranged between 36.66 to 86.66 per cent for the concentrations ranging from 1×10^4 to 1×10^8 conidia ml^{-1} tested against third instar larvae of *H. armigera.* The LC_{50} value estimated was 9.41×10^4 conidia ml^{-1}, with upper and lower fiducial limits of 5.0×10^5 conidia ml^{-1} and

Table : 1 Determination of LC_{50} value of ACENB-1 isolate against third instar larvae of *H. armigera*

Concentration (conidia ml^{-1})	Log Concentrations	Larval mortality (%)	Corrected mortality (%)	Empirical probit
1×10^4	4	43.33	43.33	4.8321
1×10^5	5	56.66	56.66	5.1687
1×10^6	6	70.00	70.00	5.5244
1×10^7	7	83.33	83.33	5.9673
1×10^8	8	93.33	93.33	6.5009
Control	-	0.00	0.00	-

Regression equation $y = 3.296 + 0.38\ x$

Wherein,

y = Probit kill or empirical probit

x = Log concentration

Slope = 0. 38

LC_{50} = 3.043×10^4 conidia ml^{-1}

Fiducial limits :

Upper = 2.00×10^5 conidia ml^{-1}

Lower = 5.011×10^3 conidia ml^{-1}

Heterogeneity (χ^2) = 0.8752

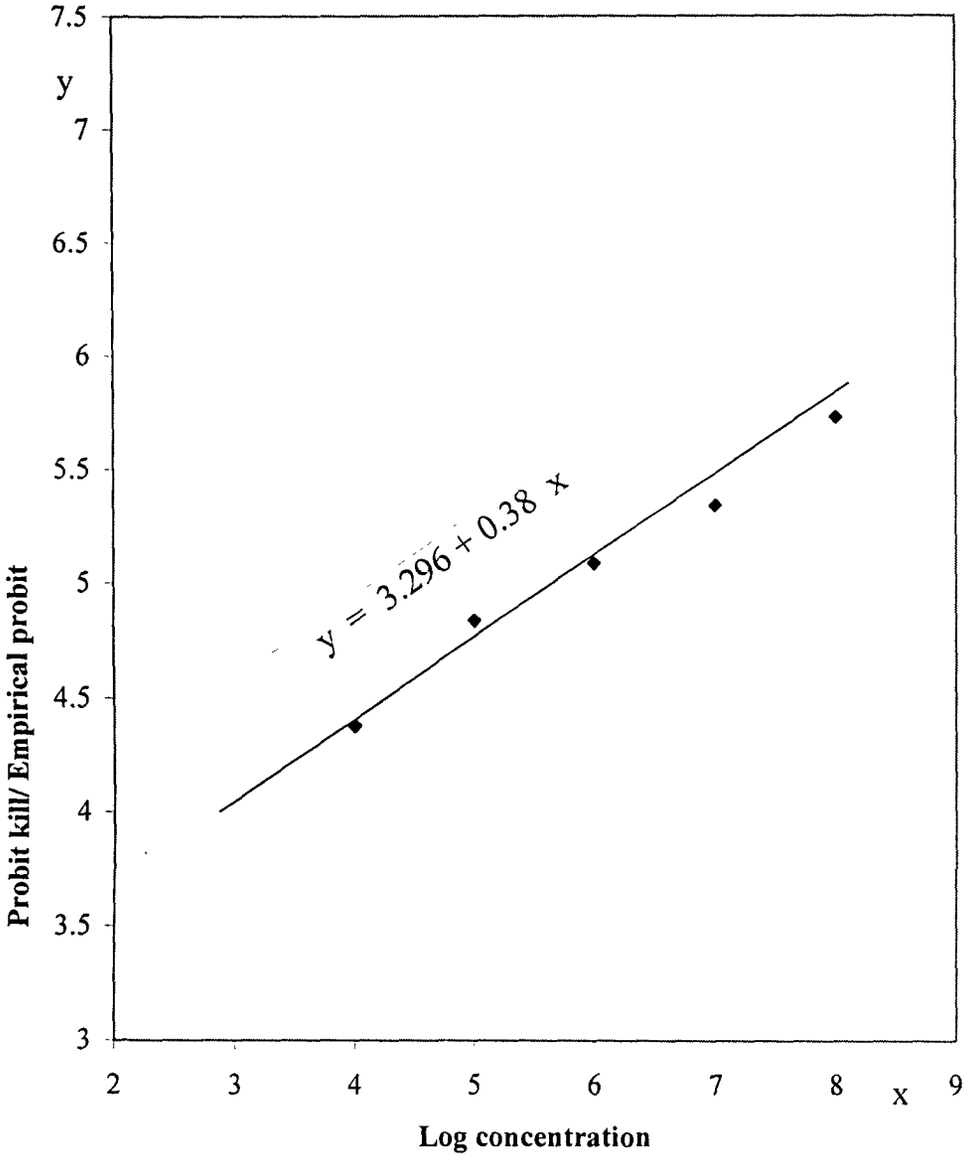


Fig. 1 Log concentration probit kill regression line for ACENB-1 isolate of *B. bassiana*

Table : 2 Determination of LC_{50} value of MITCB-1 isolate against third instar larvae of *H. armigera*

Concentration (conidia ml^{-1})	Log Concentrations	Larval mortality (%)	Corrected mortality (%)	Empirical probit
1×10^4	4	36.66	36.66	4.6602
1×10^5	5	50.00	50.00	5.000
1×10^6	6	66.66	66.66	5.4316
1×10^7	7	76.66	76.66	5.7290
1×10^8	8	86.66	86.66	6.1123
Control	-	0.00	0.00	-

Regression equation $y = 3.1946 + 0.363\ x$

Wherein,

y = Probit kill or empirical probit

x = Log concentration

Slope = 0. 363

LC_{50} = 9.41×10^4 conidia ml^{-1}

Fiducial limits :

Upper = 5.00×10^5 conidia ml^{-1}

Lower = 1.995×10^3 conidia ml^{-1}

Heterogeneity (χ^2) = 2.52

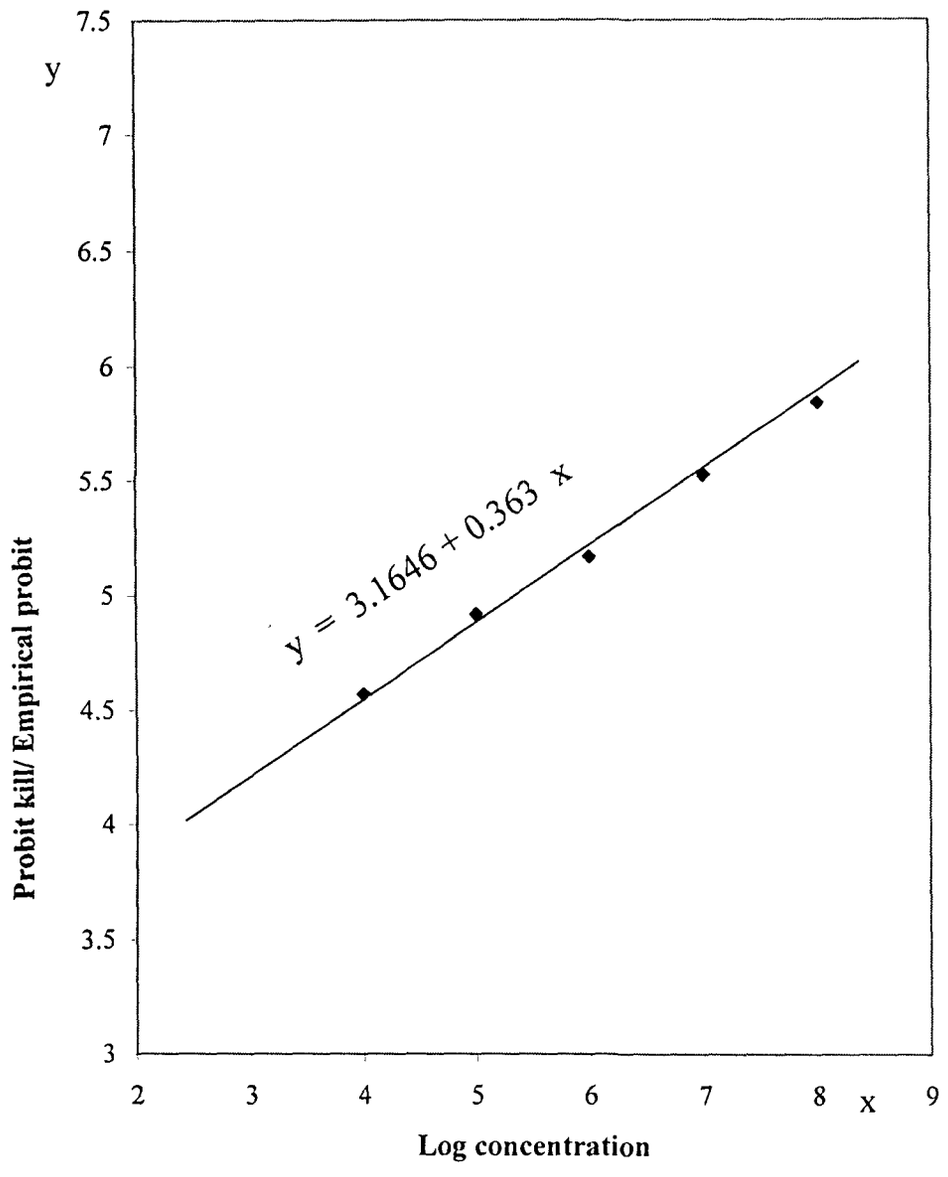


Fig. 2 Log concentration- probit kill regression line for MITCB-1 isolate of *B. bassiana*

1.995 x 10^4 conidia ml^{-1} respectively. The probit regression equation was y = 3.1946+ 0.363 x. The slope was 0.363. The line was found to be the best fit for the data. The heterogeneity test was significant with χ^2 value 2.52.

4.1.1.3 LC_{50} value of ACENB-2 isolate

Conidial concentrations and mortality data of ACENB-2 isolate are presented in Table 3. The graphical relationship between log concentration and probit kill is presented in Fig. 3.

The result presented in Table 3 revealed that the mortality has ranged between 33.33 and 80.00 percent, for the concentrations ranging between 1 x 10^4 and 1 x 10^8 conidia ml^{-1}. The calculated LC_{50} was 1.88 x 10^5 conidia ml^{-1}, with upper and lower fiducial limits of 9.7 x 10^5 conidia ml^{-1} and 3.6 x 10^4 conidia ml^{-1} respectively. The probit regression equation was y = 3.312 + 0.32 x. The slope was 0.32. The line was found to be the best fit for the data. The heterogeneity test was significant with χ^2 value 0.636.

From the results presented above it is inferred that the ACENB-1 isolate was the most effective one among the *B. bassiana* isolates tested. The lower LC_{50} value of 3.049 x 10^4 conidia ml^{-1} revealed its higher efficacy. Kencharaddi and Jayaramaiah (1997a) reported a median lethal concentration value of 2.31 x 10^4 conidia ml^{-1} for the third instar larvae of *H. armigera*. They also reported an LC_{50} value of 2.13 x10^4 conidia for third instar larvae of an another lepidopteran borer species, *Adisura atkinsoni* Moore. Thus, the present findings were found to be in line with these reports.

MITCB-1 isolate having an LC_{50} value of 9.4 x 10^4 conidia ml^{-1} was the second most effective *B. bassiana* isolate tested. Parmer (2001) tested the efficacy of *B. bassiana* isolates against the third instar larvae

Table : 3 Determination of LC_{50} value of ACENB-2 isolate against third instar larvae of *H. armigera*

Concentration (conidia ml^{-1})	Log Concentrations	Larval mortality (%)	Corrected mortality (%)	Empirical probit
1×10^4	4	33.33	33.33	4.5692
1×10^5	5	46.66	46.66	4.9172
1×10^6	6	56.66	56.66	5.1687
1×10^7	7	70.00	70.00	5.5244
1×10^8	8	80.00	80.00	5.8416
Control	-	0.00	0.00	-

Regression equation $y = 3.312 + 0.32 x$

Wherein,

y = Probit kill or empirical probit

x = Log concentration

Slope = 0. 32

LC_{50} = 1.88×10^5 conidia ml^{-1}

Fiducial limits :

Upper = 9.7×10^5 conidia ml^{-1}

Lower = 3.6×10^4 conidia ml^{-1}

Heterogeneity (χ^2) = 0.636

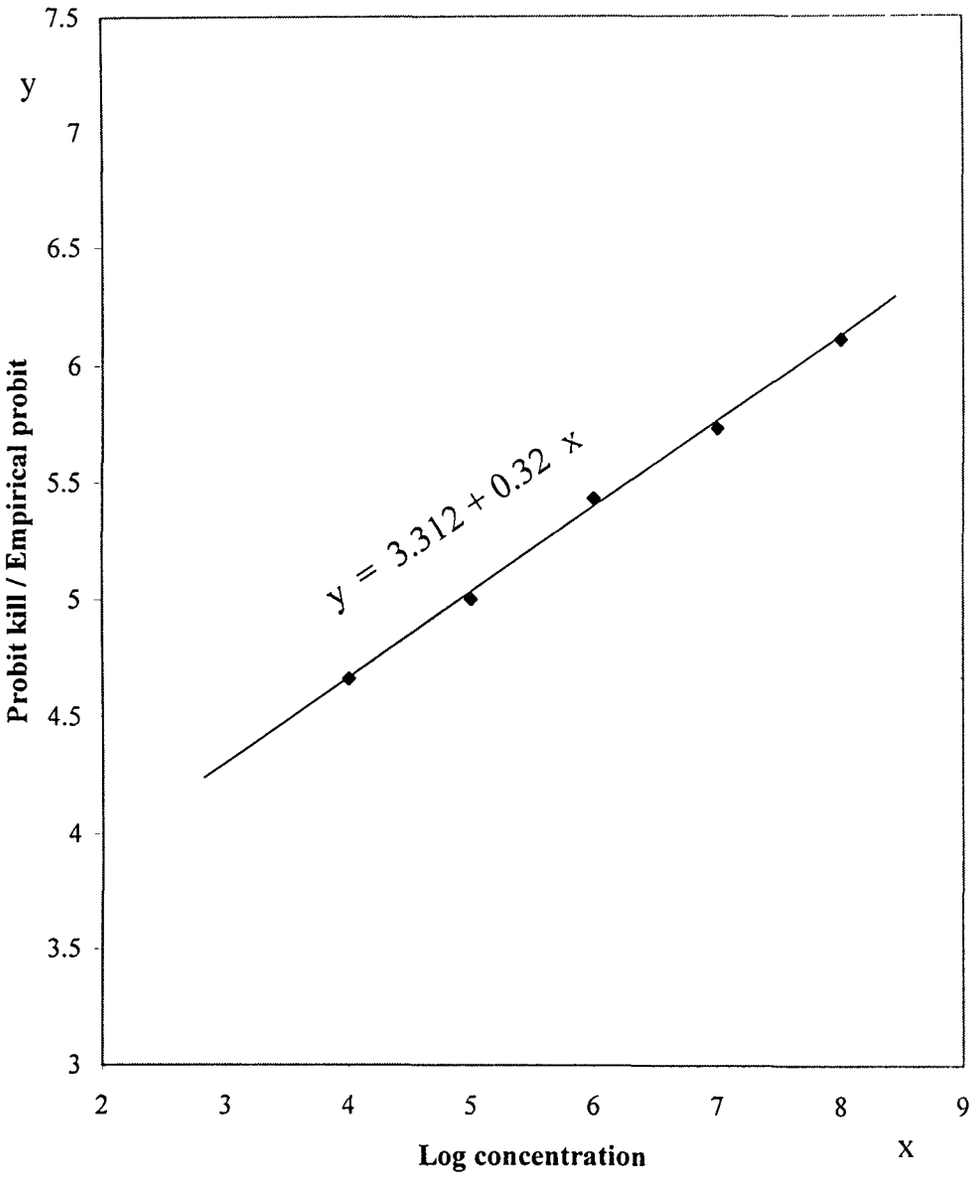


Fig. 3 Log concentration-probit kill regression line for ACENB-2 isolate of *B. bassiana*

of *H. armigera* and reported a median lethal concentration of similar order, i.e. 1.9 x 10^5 conidia ml^{-1}.

ACENB-2 isolate with a calculated LC_{50} of 6.3 x 10^5 conidia ml^{-1} was the least effective against 3rd instar larvae of H. armigera. Udar (2002) reported a similar LC_{50} value of 2.23 x 10^5 for the 3rd instar larvae of *H. armigera.*

The other median lethal concentrations tested on the third instar larvae of *H. armigera* for *B. bassiana* included, 4.8 x 10^5 conidia ml^{-1}, for an isolate *viz., B. ba.* 127 by Pandurang, 2002; and 1.9 x 10^5 conidia ml^{-1} for an isolate *viz., B. ba.* 108 by Hassani (2000).

Devaprasad *et al.* (1989) reported a median lethal concentration value of 1.9 x 10^5 conidia ml^{-1} to the 2nd instar larvae of *S. litura* for Bapatla isolate of *B. bassiana.* They also reported that the LC_{50} values have ranged between 5.55 x 10^5 and 5.58 x 10^9 conidia ml^{-1} for Bangalore and New Delhi isolates. Ramkumar (1998a) also recorded an LC_{50} value of 1.62 x 10^6 conidia ml^{-1}, and 6.9 x 10^6 conidia ml^{-1} respectively for second and third instar larvae of *S. litura.*

Comparing these reported median lethal concentrations with the results of the present findings, it can be inferred that, all the isolates of *B. bassiana* tested are having an efficacy, higher than the average. Among the *B. bassiana* isolates checked, ACENB-1 with the lowest LC_{50} value of 3.049 x 10^4 conidia ml^{-1} was found to be the most effective, followed by MITCB-1 isolate (LC_{50} : 9.41 x 10^4 conidia ml^{-1}) and ACENB-2 isolate (LC_{50} : 1.88 x 10^5 conidia ml^{-1}).

4.1.2 Determination of LT_{50} values of *B. bassiana* isolates

The time needed to kill 50 per cent of the test population was calculated for the concentrations ranging from 1 x 10^6 to 1 x 10^8 conidia ml^{-1}. LT_{50} values obtained for all the three *B. bassiana* isolates are presented below.

4.1.2.1 LT_{50} value for ACENB-1 isolate

The results revealed that critical time required to effect 50 per cent larval mortality for the conidial concentration of 1 x 10^8 conidia ml^{-1} was observed at 176 hrs after inoculation. For the conidial concentration of 1 x 10^7 conidia ml^{-1}, it was observed after 228 hrs of inoculation. The recorded LT_{50} value for the concentration, 1 x 10^6 conidia $ml^{-1}$was 240 hrs.

4.1.2 2 LT_{50} values for MITCB-1 isolate

The calculated value showed that the time required to effect 50 per cent larvae mortality for conidial concentration 1 x 10^8 conidia ml^{-1} was observed at 216 hrs. The median lethal time for the concentration 1 x 10^7 and 1 x 10^6 conidia ml^{-1} was, 240 hrs and 264 hrs, respectively.

4.1.2.3 LT_{50} values for ACENB-2 isolate

The calculated time required to effect 50 per cent larval mortality for conidial concentration 1 x 10^8 conidia ml^{-1} was observed at 240 hrs. The LT_{50} values for the concentration 1 x 10^7 and 1 x 10^6 conidia ml^{-1} were recorded at 264 hrs and 288 hrs respectively after inoculation.

Mortality was found to occur from 48 hrs after inoculation for the conidial concentration of 1 x 10^8 of ACENB-1 isolate. For the other concentrations *viz.*, 1 x 10^7 and 1 x 10^6 conidia ml^{-1} the inoculation period was found to be 72 hrs and 96 hrs., respectively. For the MITCB-1 isolate the incubation period was 72 hrs, 96 hrs and 96 hrs, for 1 x10^8, 1 x 10^7 and 1 x 10^6 conidia ml^{-1} concentrations, respectively. Sue-Perkrul and Grula (1979) stated that, in ideal conditions *B. bassiana* takes at least 18 hrs to germinate and begins to penetrate in the larval cuticle. Gopalkrishnan and Narayanan (1987) also reported an incubation period ranging from 2 to 15 days after inoculation for *B. bassiana* . These two observations supported the present findings in this respect.

All the mortality data revealed that, the increased larval mortality was directly proportional to spore concentration of the fungal suspension. It has already been established that there is a positive correlation between the number of infective spores and the mortality by mycosis (Ferron, 1978).

The results revealed that the median lethal time required to kill 50 per cent test population by all the conidial concentrations of ACENB-1 were the lowest. The values were 176 hrs for 1 x 10^8 conidia ml^{-1}, 228 hrs for 1 x 10^7 conidia ml^{-1} and 240 hrs for 1 x 10^6 conidia ml^{-1}. The LT_{50} values of 1 x 10^8 conidia ml^{-1} (176 hrs.) was found to be the lowest compared to the LT_{50} values (216 hrs and 240 hrs) of the same conidial concentration of other two fungal isolates *viz.,* MITCB-1 and ACENB-2. Similarly the LT_{50} value of 1 x 10^7 conidia ml^{-1} (228 hrs) was the lowest when compared to the LT_{50} values (240 hrs and 246 hrs) of the same conidial concentrations of other two fungal isolates. Same results were observed for the LT_{50} values of the conidial concentration of 1 x 10^6 conidia ml^{-1} i.e. 240 hrs for ACENB-1, 264 hrs for MITCB-1 and 288 hrs for ACENB-2 isolates. Present findings are supported by the findings of Udar (2002) on third instar larvae of *H. armigera.* Ramkumar (1998a, 1988b) also reported similar LT_{50} values for third instar larvae of *Spodoptera litura* (Fabricius).

All these observations revealed the higher efficacy of ACENB-1 isolate, followed by MITCB-1 and ACENB-2 isolate.

4.2. Study of the efficacy of *M. anisopliae* isolates

The two *M. anisopliae* isolates *viz.,* ACENM-1 and MITCM-1 were tested to assess the their biological activity to third instar larvae of *H. armigera.* All the isolates were found be efficient to control *H. armigera* larvae. Caterpillars infected with *M. anisopliae* isolates

Plate 7. initial and final symptoms of attack by *M. anisopliae*

became sluggish and ceased to feed. Twenty four to 48 hrs after death, white mycelial spots were observed on the cadavers. Later the body got covered with tuft of pure white mycelial growth with green spores covering the entire body of the caterpillar. Similar symptoms were reported by Gopalakrishnan and Narayanan, (1988c, 1989b) in *H. armigera* larvae infected with *M. anisopliae*. The above made observations reaffirmed the already established pathogenecity of *M. anisopliae* isolated tested.

The mortality was found to be ranging between 20 to 76.66 per cent and this finding was supported by the observation of Hassani (2000).

4.2.1 Determination of LC_{50} values of *M. anisopliae* isolates.

4.2.1.1 LC_{50} values of ACENM-1 isolate

The tested conidial concentrations and mortality data of ACENM-1 isolate is presented in Table 4. The graphical relationship between log concentration and probit kill is presented in Fig 4.

The results presented in Table 4, revealed that the mortality ranged between 26.66 to 76.66 per cent, for the concentrations ranging from 1×10^4 to 1×10^8 conidia ml^{-1}. The LC_{50} value calculated was 6.3×10^5 conidia ml^{-1}, with upper and lower fiducial limits of 1.43×10^6 conidia ml^{-1} and 2.79×10^5 conidia ml^{-1}, respectively. The probit regression equation was $y = 3.248 + 0.302 x$, the slope being 0.302. The line was found to be the best fit for the data. The heterogeneity test was significant with χ^2 value of 0.888.

4.2.1.2 LC_{50} values of MITCM-1 isolate

The tested concentrations and mortality data of MITCM-1 isolate is presented in Table 5. The graphical relationship between log concentration and probit kill is presented in Fig 5.

Table : 4 Determination of LC_{50} value of ACENM-1 isolate against third instar larvae of *H. armigera*

Concentration (conidia ml^{-1})	Log Concentrations	Larval mortality (%)	Corrected mortality (%)	Empirical probit
1×10^4	4	26.66	26.66	4.3781
1×10^5	5	43.33	43.33	4.8321
1×10^6	6	53.33	53.33	5.0836
1×10^7	7	63.33	63.33	5.3406
1×10^8	8	76.66	76.66	5.7290
Control	-	0.00	0.00	-

Regression equation $y = 3.248 + 0.302\ x$

Wherein,

y = Probit kill or empirical probit

x = Log concentration

Slope = 0. 302

LC_{50} = 6.3×10^5 conidia ml^{-1}

Fiducial limits :

Upper = 1.43×10^6 conidia ml^{-1}

Lower = 2.79×10^5 conidia ml^{-1}

Heterogeneity (χ^2) = 0.888

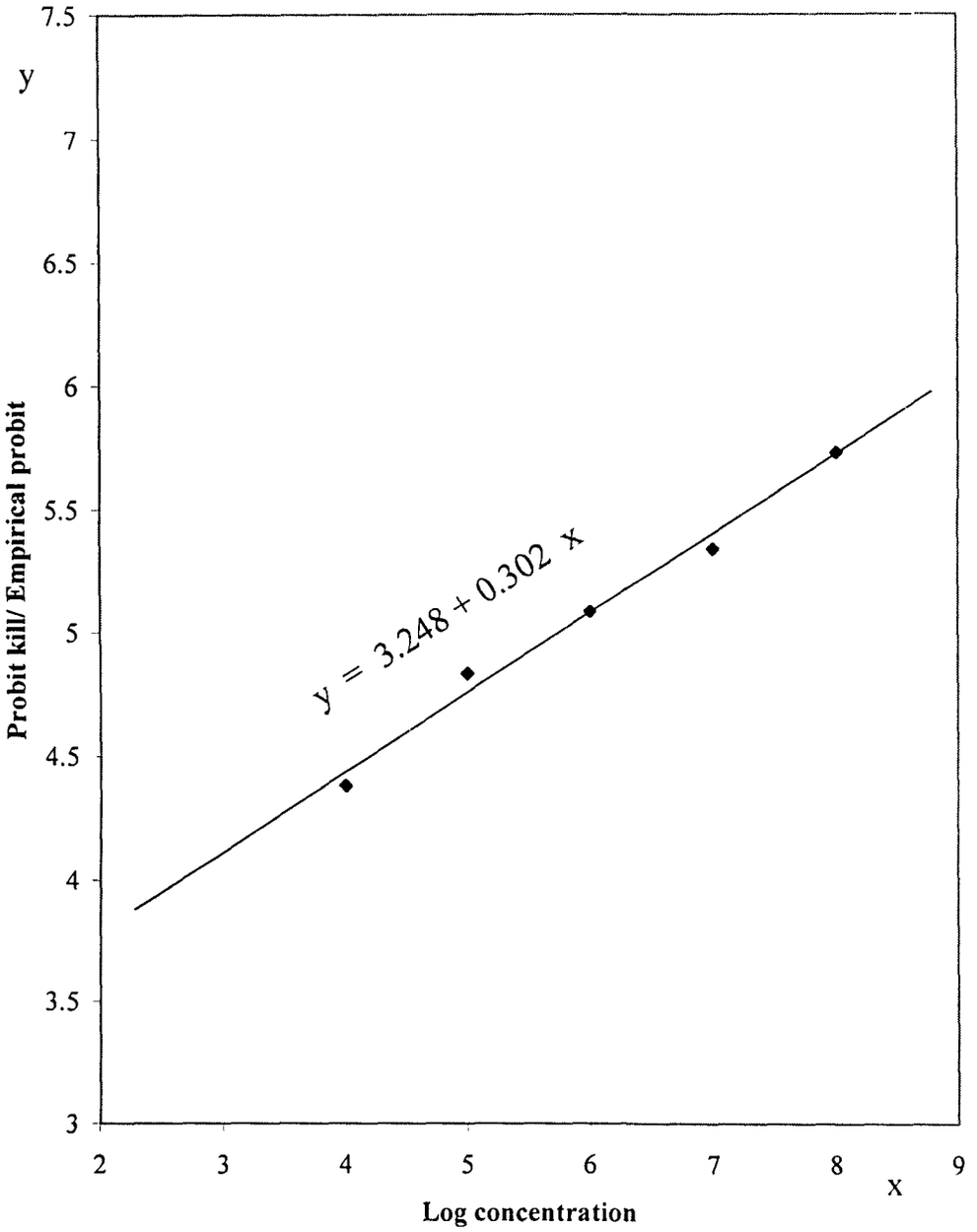


Fig. 4 Log concentration- probit kill regression line for ACENM-1 isolate of *M. anisopliae*

Table : 5 Determination of LC_{50} value of MITCM-1 isolate against third instar larvae of *H. armigera*

Concentration (conidia ml^{-1})	Log Concentrations	Larval mortality (%)	Corrected mortality (%)	Empirical probit
1×10^4	4	20.00	17.24	4.0553
1×10^5	5	36.66	34.48	4.6011
1×10^6	6	46.66	44.82	4.8698
1×10^7	7	56.66	55.17	5.1307
1×10^8	8	70.00	68.97	5.4959
Control	-	3.33	-	-

Regression equation $y = 3.283 + 0.2475 x$

Wherein,

y = Probit kill or empirical probit

x = Log concentration

Slope = 0. 2475

LC_{50} = 8.68×10^6 conidia ml^{-1}

Fiducial limits :

Upper = 8.4×10^7 conidia ml^{-1}

Lower = 8.91×10^5 conidia ml^{-1}

Heterogeneity (χ^2) = 2.264

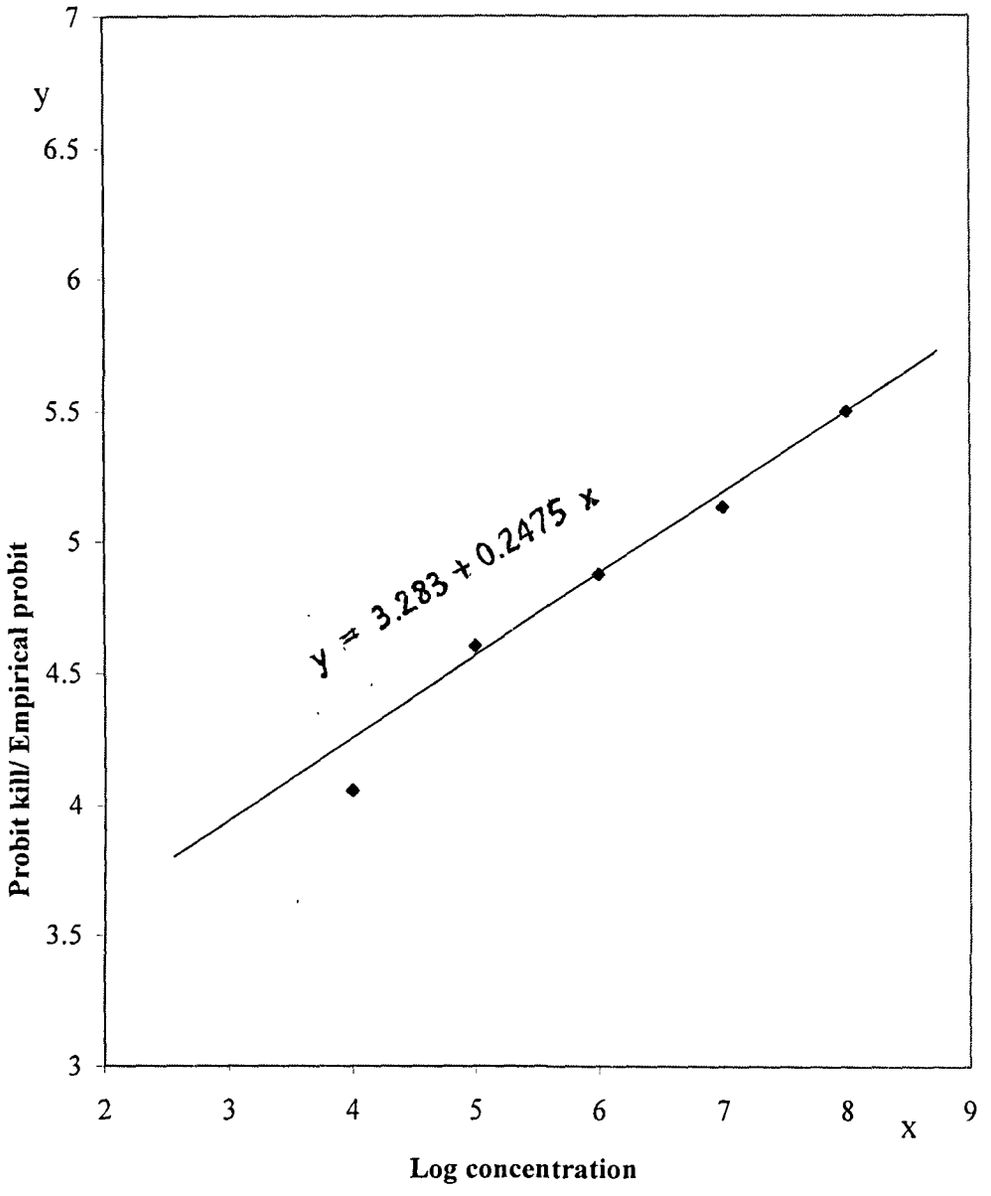


Fig. 5 Log concentration- probit kill regression line for MITCM-1 isolate of *M. anisopliae*

The results presented in Table 5 revealed that, mortality ranged between 17.24 to 68.97 per cent for the concentrations ranging from 1 x 10^4 to 1 x 10^8 conidia ml^{-1}. The LC_{50} value calculated was 8.68 x 10^6 conidia ml^{-1}, with upper and lower fiducial limits 8.4 x 10^7 conidia ml^{-1} and 8.91 x 10^5 conidia ml^{-1} respectively. The probit regression equation was y= 3.284 + 0.2475x, the slope being 0.2475 the line was found to be the best fit for the data. The heterogeneity test was significant with χ^2 value 2.264.

From the results presented above, it can be inferred that ACENM-1 isolate was the most effective among *M. anisopliae* isolates tested. The lower LC_{50} value of 6.3 x 10^5 conidia ml^{-1} revealed its higher efficacy in comparison with MITCM-1 isolate (LC_{50} : 8.68 x 10^6 conidia ml^{-1}). Kencharaddi and Jayaramaiah (1997a) reported an LC_{50} value of 6.15 x 10^5 conidia ml^{-1} for the third instar larvae of *H. armigera*. Pandurang (2002) also reported a median lethal concentration of 0.9 x 10^5 spore ml^{-1} for *M. anisopliae* isolate, M. a. 121. Another report in accordance with these reports was made by Patil (2002), who found that the critical dose for effecting 50 per cent mortality in second instar larvae of *H. armigera* was 1.47 x 10^5 conidia ml^{-1}. All these observations are in the line with the present finding of LC_{50} value for ACENM-1 isolate.

Hassani (2000) reported an LC_{50} value of 1.6 x 10^6 conidia ml^{-1} on the third instar larvae of *H. armigera* to an *M. anisopliae* isolate M. a. 79. This observation supports the LC_{50} value of MITCM-1 isolate, recorded.

Also all these reported median lethal concentration values revealed that, the bioassayed *M. anisopliae* isolates are having a normal biological activity in par with the earlier reports.

4.2.2 Determination of LT_{50} values

The time needed to kill 50 per cent of the test population was calculated for the concentrations ranging from 1 x 10^6 to 1 x 10^8 conidia

ml^{-1}. The total mortality observed for the 1 x 10^6 conidial concentration of *M. anisopliae* isolate MITCM-1 was found to be less than 50 per cent ; hence median lethal time cannot be calculated. LT_{50} values obtained for both the *M. anisopliae* isolates are presented below :

4.2.2.1 LT_{50} values for ACENM-1 isolate

The results revealed that critical time required to effect 50 per cent larval mortality for the conidial concentration of 1 x 10^8 conidia ml^{-1} was observed at 312 hrs after inoculation. For the conidial concentration of 1 x 10^7 conidia ml^{-1} it was observed at 288 hrs. The recorded LT_{50} value for the conidial concentration of 1 x 10^6 conidia ml^{-1} was at 264 hrs after inoculation.

4.2.2.2 LT_{50} values for MITCM-1 isolates

The calculated median lethal time for the conidial concentration of 1 x 10^8 conidia ml^{-1} was at 288 hours. The median lethal time for the conidial concentration 1 x 10^7 conidia ml^{-1} was observed at 312 hours after inoculation.

The mortality was found to occur from 144 hrs after inoculation for the conidial concentration of 1 x 10^8 conidia ml^{-1} of ACENM-1 isolate. For the conidial concentration of 1 x 10^7 conidia ml^{-1}, it was at 168 hrs after inoculation and for the conidial concentration 1 x 10^6 conidia ml^{-1} it was observed at 144 hrs after inoculation. For the MITCM-1 isolate the LT_{50} values for 1 x 10^8 and 1 x 10^7 conidial concentration ml^{-1} were 168 and 144 hrs after inoculation. These findings are supported by the observations made by Gopalakrishnan and Narayanan (1987) who reported an incubation period of 2 to 14 days the third instar larvae of *H. armigera* infected with *M. anisopliae*. Gopalakrishnan and Narayanan (1988b) also reported an incubation period of 2 to 5 days to the larvae of *H. armigera.*

Difference in the biological activity of different strains of same fungus can be due to one or more of the following reasons.

- The different fungus strain may have been collected from preferred or secondary insect hosts.
- The different culture media or storage methods may affect virulence.
- Optimum environmental factors for each strain are probably different.
- Length of time the fungus strain maintained on an artificial medium may be different (Prasertphon, 1963).

4.3 Comparison of efficacy of *B. bassiana* and *M. anisopliae* isolates

The median lethal concentration values of all the *B. bassiana* isolates (3.049 x 10^4, 1.88 x 10^5, 9.4 x 10^4 conidia ml^{-1}) were found to be less than the LC_{50} values of *M. anisopliae* isolates (6.3 x 10^5 and 8.68 x 10^6 conidia ml^{-1}). These results revealed that the *B. bassiana* isolates were more effective than *M. anisopliae* isolates

All the mortality data revealed that, the increased larval mortality was directly proportional to spore concentration of fungal suspension. The positive correlation between the number of infective spores and the mortality by mycosis has been established already by Ferron, 1978.

The results of LT_{50} values revealed that the median lethal time required to kill 50 per cent test population by all the conidial concentrations of ACENM-1 were the lowest. The values were 264 hrs for 1 x 10^8 conidia ml^{-1}, 288 hrs for 1 x 10^7 conidia ml^{-1} and 312 hrs for 1 x 10^6 conidia ml^{-1}, respectively. The LT_{50} values for the MITCM-1 isolate were 288 hrs for 1 x 10^8 conidia ml^{-1} and 312 hrs for 1 x 10^7 conidia ml^{-1}, respectively. These observations revealed that ACENM-1 isolate has higher biological activity compared to MITCM-1 isolate.

Also the LT_{50} values for *B. bassiana* isolates were found to be less than the LT_{50} values of the corresponding concentrations. These results

also revealed that *B. bassiana* isolates *viz.,* ACENB-1, MITCB-1, ACENB-2 were more effective than the *M. anisopliae* isolates *viz.,* ACENM-1 and MITCM-1.

These findings were supported by Saxena *et al.* (1990) who reported that among the three entomopathogenic fungal isolates tested, including *B. bassiana* and *M. anisopliae.*, the *B. bassiana* isolate was found to be the most effective. The findings of Kencharaddi and Jayaramaiah (1997a, 1997b) also agreed with the present findings. The observations of Hassani (2000) were also in line with the present finding. Also a broader host range of about 500 species of insects was reported to *Beauveria* sp., especially from the order lepidoptera; than *M. anisopliae* (Anonymous, 2002), whereas there was only a host range of more than 200 insect species belonging to the order coleoptera, lepidoptera, orthoptera and hemiptera has been reported to the less effective *M. anisopliae.*

SUMMARY AND CONCLUSION

5. Summary and Conclusions

During the present investigation, the laboratory experiments were carried out to evaluate the efficacy of three, *B. bassiana* isolates *viz.,* ACENB-1, ACENB-2 and MITCB-1 and two *M. anisopliae* isolates *viz.,* ACENM-1 and MITCM-1. The two aspects of studies were the calculation of LC_{50} values of these fungal isolates and the calculation of LT_{50} values for the possible conidial concentrations. Laboratory studies were conducted at MITCON Laboratory, Dr. Manibhai Desai Nagar, N.H. 4 Pune, and at Insect- Pathology lab, Entomology Department, College of Agriculture, Pune, during 2003-2004. The findings of these studies are summarized and included in this chapter.

5.1 Summary

5.1.1 Studies on the efficacy of *B. bassiana* isolates

All the tested *B. bassiana* isolates produced disease symptoms in accordance with the findings of earlier workers. Thus the pathogenicity is reaffirmed. The bioassay studies revealed the efficacy of all the *B. bassiana* isolates. The mortality ranged from 43.33 to 93.33; 36.66 to 86.66 and 26.66 to 76.66 per cent respectively for ACENB-1, MITCB-1 and ACENB-2 isolates.

5.1.1.1 Determination of LC_{50} values

Bioassay was conducted using serial dilutions ranging from 1×10^4 to 1×10^8 conidia ml^{-1} of all the *B. bassiana* isolates. The LC_{50} values recorded were 3.049×10^4, 9.41×10^4 and 9.7×10^5 conidia ml^{-1} respectively for ACENB-1, MITCB-1 and ACENB-2 isolates. It was inferred from these median lethal concentration values that ACENB-1, was the most efficient isolate among *B. bassiana* isolates, followed by MITCB-1 and ACENB-2.

5.1.1.2 Determination of LT_{50} values

Time required to effect 50 per cent mortality of the test population were calculated for the serial dilutions ranging from 1 x 10^6 to 1 x 10^8 conidia ml^{-1}; for all the bioassayed *B. bassiana* isolates. The calculated LT_{50} values of ACENB-1 for the conidial concentrations 1 x 10^6, 1 x 10^7 and 1 x 10^8 conidia ml^{-1} were 240, 228, and 176 hrs respectively. The LT_{50} values for corresponding conidial concentrations of MITCB-1 were 264, 240 and 216 hrs respectively. And the LT_{50} values for ACENB-2 isolate were 288, 264 and 240 hrs respectively for conidial concentrations 1 x 10^6, 1 x 10^7 and 1 x 10^8 conidia ml^{-1}.

In all these observations made, mortality data increased in positive proportion with the conidial concentration. The LT_{50} values of ACENB-1 isolate were the least, compared to the corresponding conidial concentrations of other two isolates of *B. bassiana* This revealed its higher efficacy against the test insect *H. armigera.* On comparing the LT_{50} values, it was also inferred that ACENB-1 isolate is followed by MITCB-1 and ACENB-2 isolate in its efficacy.

5.1.2 Studies on the efficacy of *M. anisopliae* isolates

The two tested *M. anisopliae* isolates produced disease symptoms in accordance with the findings of earlier workers. Thus pathogenicity is reaffirmed and bioassay studies were conducted. The results revealed the efficacy of both the *M. anisopliae* isolates. The mortality ranged between 26.66 and 76.66 per cent for ACENM –1 isolate and 17.24 and 68.97 for MITCM-1 isolate.

5.1.2.1 Determination of LC_{50} values

Bioassay was conducted using serial dilutions ranging from 1 x 10^4 to 1 x 10^8 conidia ml^{-1} of both the *M. anisopliae* isolates. LC_{50} values recorded were 6.3 x 10^5 and 8.68 x 10^6 conidia ml^{-1} respectively for

ACENM-1 and MITCM-1 isolates. The lowest LC_{50} value revealed the higher efficacy of ACENM-1 isolate compared to MITCM-1 isolate.

5.1.2.2 Determination of LT_{50} values

The median lethal time was calculated for the concentrations 1 x 10^6, 1 x 10^7 and 1 x 10^8 conidia ml^{-1} for ACENM-1 isolate and found to be at 312, 288 and 264 hrs after inoculation, respectively. The LT_{50} values of the conidial concentrations 1 x 10^7 and 1 x 10^8 conidia ml^{-1} were 312 and 288 hrs respectively. These LT_{50} values revealed the higher efficacy of ACENM-1 isolate compared to MITCM-1 isolate.

5.1.3 Comparison of the efficacy of *B. bassiana* and *M. anisopliae* isolates

The respective LC_{50} values for ACENB-1, MITCB-1, ACENB-2, ACENM-1 and MITCM-1 isolates tested were 3.049 x 10^4, 9.41 x 10^4, 9.7 x 10^5, 6.3 x 10^5 and 8.68 x 10^6 conidia ml^{-1}. It can be inferred from these values that the efficacy also follows the same order.

The LT_{50} values for the 1 x 10^8 conidial concentration were 176 hrs for ACENB-1, 216 hrs for MITCB- 1, 240 hrs for ACENB-2, 264 hrs for ACENM-1 and 288 hrs for MITCM-1. These estimated values also supported the above mentioned order of efficacy of isolates. Again the same observations were made for the conidial concentrations 1 x 10^7 and 1 x 10^6 conidia ml^{-1}. For the conidial concentration, 1 x 10^7 conidia ml^{-1}, the LT_{50} values were, 228 hrs for ACENB -1, 240 hrs for MITCB-1, 264 hrs for ACENB-2, 288 hrs for ACENM-1 and 312 hrs for MITCM-1 isolate. The LT_{50} values for the conidial concentration of 1 x 10^6 conidia ml^{-1} were 240 hrs for ACENB- 1, 264 hrs for MITCB-1, 288 hrs for ACENB-2 and 312 hrs for ACENM-1, respectively.

5.2 Conclusions

- All the *B. bassiana* and *M. anisopliae* isolates tested were found to be effective against the test insect *H. armigera*
- Among the *B. bassiana* and *M. anisopliae isolates* tested; *B. bassiana* isolates with lowest LC_{50} values (3.049×10^4, 9.41×10^4 and 1.88×10^5 conidia ml^{-1}) were found to be superior in their efficacy. LT_{50} values reported also supported this finding.
- The most efficient isolate among the *B. bassiana* isolate was ACENB- 1 with the lowest LC_{50} of 3.049×10^4 conidia ml^{-1} LT_{50} values were also found to be the lowest (216, 240, 264 hrs) for this isolate. Thus this isolate was found to be effective for incorporation into IPM programmes for the control of *H. armigera* species.
- Although all the tested isolates showed higher *efficacy* than the average performance, further field studies are needed to confirm its field efficacy in controlling the test insect, *H. armigera.* Field studies under different temperature and humidity ranges have to be further carried out.

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* Originals not seen

APPENDIX

7. Appendix

The following precautions were undertaken during the entire rearing period of *H. armigera*

1. The rearing room was initially disinfected with formaldehyde fumes (potassium permanganate crystals were taken in petriplates and treated with equal proportion of formaldehyde (40%) and the room was kept closed for two days).
2. The containers, equipments, vials etc were thoroughly cleaned in water and dipped in 0.2% formalin for one hour to eliminate the surface contaminants.
3. Keeping the rearing cages and vials in bright sunshine for 2-3 hours also helped in eliminating any living forms as surface contaminants.
4. Care was taken to see that there was minimum handling of the larvae.
5. Filter paper was cut and put at the bottom of rearing vials which helped in the easy removal of excreta and excess moisture.
6. Caterpillars showing diseased symptoms, malformed pupae and weak adults were immediately discarded from healthy culture.
7. Distilled water was used for preparing adult and larval diet.
8. Chickpea seedlings were cleaned thoroughly and dipped in disinfectant before feeding the larvae.
9. For rearing healthy insects, 2 ml of formalin was added to one kg of artificial diet.
10. To maintain proper sanitation, restriction of personnel inflow was resulted into the rearing room.
11. Rearing cages were kept away from ants by keeping antwells below the base.

VITA