

**CULTURAL AND MORPHOLOGICAL  
CHARACTERIZATIONS OF *Beauveria bassiana***

**THESIS**

**Submitted to  
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola  
in partial fulfilment of the requirements  
for the Degree of**

**MASTER OF SCIENCE  
IN  
AGRICULTURE  
(PLANT PATHOLOGY)**

**By**

**WARGANE VILAS SUKHADEO**

**SECTION OF PLANT PATHOLOGY  
COLLEGE OF AGRICULTURE, NAGPUR**

**DR. PANJABRAO DESHMUKH KRISHI VIDYAPEETH,  
KRISHINAGAR PO, AKOLA (MS) 444 104**

**Enrolment Number – LL-157**

**2019**

## DECLARATION OF STUDENT

I hereby declare that the experimental work and its interpretation of the thesis entitled “**CULTURAL AND MORPHOLOGICAL CHARACTERIZATIONS OF *Beauveria bassiana***” or part there of has neither been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis publication of any University or scientific organization. The source of materials used and all assistance received during the course of investigation have been duly acknowledged.

Place: Nagpur

Date : 06 /06 / 2019

**Wargane Vilas Sukhadeo**

(Enrolment No. LL-157)

## CERTIFICATE

This is to certify that the thesis entitled "**CULTURAL AND MORPHOLOGICAL CHARACTERIZATIONS OF *Beauveria bassiana***" submitted in partial fulfilment of the requirement for the degree of "**Master of Science in Agriculture (Plant Pathology)**" of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola is a record of bonafide research work carried out by **WARGANE VILAS SUKHADEO** under my guidance and supervision.

The subject of the thesis has been approved by the student's advisory committee.

**Shri. R. L. Parate**

Chairman,  
(Advisory Committee)

Place: Nagpur  
Date: 06 /06 / 2019

### Countersigned

Associate Dean,  
College of Agriculture, Nagpur.  
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE  
INCLUDING EXTERNAL EXAMINER (AFTER VIVA-VOCE)

1. Chairman	(Shri. R. L. Parate	.....
2. Member	(Dr. S. B. Bramhankar)	.....
3. Member	(Dr. P. N. Rakhonde)	.....
4. Member	(Dr. N. V. Lavhe)	.....
5. External Membe	( Dr. M. J. Patil)	.....

## **ACKNOWLEDGEMENT**

“Committed to the lord whatever do and your plan will succeed” start with the name of almighty that is most beneficent and merciful. I am greatly thankful to God for giving me blessing and always illuminating me with the height of success.

It is a moment of great pleasure to put in record my heartfelt gratitude and indebtedness to honorable guide and chairman of my advisory committee Shri. R. L. Parate, Assistant Professor, Department of Plant Pathology, College of Agriculture, Nagpur for able magnificent guidance, inspiration, constructive criticism and encouragement during the course of investigation and preparation of the thesis.

I acknowledge my sincere thanks to my advisory committee members Dr. S. B. Bramhankar, who taught me the systematic way of conducting experiments and to overcome the difficulties without giving up. It is indeed due to his constant encouragement that this work has materialized. Dr. P. N. Rakhonde, Assistant Professor of Plant Pathology, Anand Niketan College of Agriculture, Warora and Dr. N. V. Lavhe, Assistant Professor of Entomology, College of Agriculture, Nagpur for their kind co-operation and guidance during the various stages of this investigation.

I wish to express my sincere thanks to Dr. K. D. Thakur, Professor and Head, Plant Pathology Section, College of Agriculture, Nagpur, for providing necessary facilities required for the present investigation.

I am also highly indebted to Dr. D. M. Panchbhai, Associate Dean, College of Agriculture, Nagpur for providing necessary facilities to carry out my research work.

I sincerely acknowledge, my cordial thanks and, kind co-operation and regular inspirations to Dr. S. R. Potdukhe, Associate Professor of Plant Pathology, late Dr. G. D. Mate, Dr. T. S. Pillai, Dr. D. D. Guldekar and Dr. M. J. Patil Assistant Professors of Plant Pathology, College of Agriculture, Nagpur.

I am also thankful to Dr. C. J. Gaikwad, Librarian, College of Agriculture, Nagpur for his valuable guidance and to provide all the facilities related to thesis work.

I am also thankful to Ajay Pofale Agriculture assistant, Shri. Jaikumar Jain, Mrs. Snehal, Miss. Shobana, Mr. Soni, Mr. Ramesh Kadwate, Mr. Subhash and all supporting staff of Plant Pathology Section for their kind co-operation and timely assistance in field and laboratory work.

I sincerely acknowledge to most helping, loving and caring nature person respected Neha mam for her huge help related to my thesis work.

A special thanks to my besties Bhushan, Sachin, Kajal, Sharath, Vaibhav, Vivek, Santosh, Ankush, Vishal, Shubham, Gitanshu, Sagar, Pavan, Dnyanu, Umesh, Ramesh, Vasudev, Bhagwat, Vilas, Ajay, Datta, Shrilekh and Hemant for stimulating discussion, working together and for all the fun we had in the last few years. I am indebted to my seniors Pravin Gawande, Santosh Shingane, Vivek Tathe, Pramod Gayke, Shubham Pattekar, Avinash Parihar, Gopal Giri, Ganesh Jawle (G), Sagar Band, Shubham Kakad, Ajay Labsetwar, Sagar Soudar and juniors Rushi, Akshay, Swapnil, Suraj, Bodhit, Priya, Anjali, Shital, Ashvini, Supriya, Ravali, Snehal and all UG juniors for providing and stimulating fun filled environment.

Today I feel that my parent's hard work and dreams have been blossomed. I would like to pay high regards to my grandparents, my mother Mrs. Kalindibai, my father Shri. Sukhadeo, my sister Pooja and my brothers Gajanan and Dipak for their sincere encouragement and inspiration throughout my research work.

I wish to express my cordial thanks and profound gratitude to all authors whose literature has been cited in this investigation.

Inadvertently I might have forgotten to mention the names of invisible hand for their help, I am thankful to them as well.

Place: Nagpur

Date: 06/06/2019

**(WARGANE VILAS SUKHADEO)**

## Table of Contents

Sr. No.	Particular	Page
	Declaration of student	i
	Certificate	ii
	Acknowledgement	iii
A	List of tables	vi
B	List of figures	vii
C	List of plates	viii
D	Abbreviations	ix
E	Thesis abstract	x
I	Introduction	1
II	Review of literature	6
III	Material and methods	17
IV	Results and discussion	27
V	Summary and conclusions	48
VI	Literature cited	51
	Vita	57

**(A) LIST OF TABLES**

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
1.	Isolates of <i>Beauveria bassiana</i>	27
2.	Pathogenicity of different isolates of <i>Beauveria bassiana</i> on 2 <sup>nd</sup> instar larvae of <i>Spodoptera litura</i> at 10 days after treatment	29
3.	Mean cumulative per cent mortality of 2 <sup>nd</sup> instar larvae of <i>Spodoptera litura</i> with <i>Bauveria bassiana</i> at different concentration	30
4.	Different media tested for growth and sporulation of <i>Beauveria bassiana</i>	34
5.	Effect of different temperature regimes on growth and sporulation of <i>Beauveria bassiana</i>	36
6.	Effect of different humidity levels on growth and sporulation of <i>Beauveria bassiana</i>	39
7.	Effect of different pH levels on growth and sporulation of <i>Beauveria bassiana</i>	42
8.	Effect of selective insecticides on radial mycelial growth of <i>Beauveria bassiana</i>	45

**(B)****LIST OF FIGURES**

<b>Figure No.</b>	<b>Title</b>	<b>Page No.</b>
1.	Effect of different temperature regimes on growth and sporulation of <i>Beauveria bassiana</i>	38
2.	Effect of different humidity levels on growth and sporulation of <i>Beauveria bassiana</i>	41
3.	Effect of different pH levels on growth and sporulation of <i>Beauveria bassiana</i>	44

**(C) LIST OF PLATES**

<b>Plate No.</b>	<b>Caption</b>	<b>Page No.</b>
1	Rearing of <i>Spodoptera litura</i>	31
2	Pathogenicity studies on 2 <sup>nd</sup> instar larvae of <i>Spodoptera litura</i> by <i>Beauveria bassiana</i>	32
3	Effect of <i>B. bassiana</i> on the growth and development of <i>S. litura</i> larvae	32
4	Different media tested for growth and sporulation of <i>Beauveria bassiana</i>	35
5	(A) Conidia of <i>B. bassiana</i> (B) Germinated conidia with 45x	35
6	Effect of different temperature regimes on growth and sporulation of <i>Beauveria bassiana</i>	37
7	Effect of different humidity levels on growth and sporulation of <i>Beauveria bassiana</i> .	40
8	Effect of different pH levels on growth and sporulation of <i>Beauveria bassiana</i>	43
9	Effect of selective insecticides on radial mycelial growth of <i>B. bassiana</i>	46

**(D)****ABBREVIATIONS**

%	Per cent
/	Per
Conc.	Concentration
C.D.	Critical difference
CFU	Colony forming unit
DAI	Days after inoculation
DAT	Days after Treatment
<i>et al.</i>	et alia (and associate)
<i>etc.</i>	et. Cetra
Fig.	Figure
<i>i.e.</i>	idest (that is)
ml	mililitre
mm	millimeter
hrs	Hour
R.H.	Relative Humidity
°C	Degree Celsius
PDA	Potato Dextrose Agar
SDA	Sabouraud Dextrose Agar
BOD	Biological Oxygen Demand
S.E.± (m)	Standard error of mean
EC	Emulsifiable Concentrate
SC	Soluble Concentrate
SL	Soluble Liquid
<i>Var</i>	Variety
<i>Viz.,</i>	Namely
@	At the rate
Sig	significant
GT <sub>50</sub>	Time for 50% to germinate

**(E) THESIS ABSTRACT**

- a) **Title of the Thesis** : " **CULTURAL AND MORPHOLOGICAL CHARACTERIZATIONS OF *Beauveria bassiana*** "
- b) **Full name of student** : **Wargane Vilas Sukhadeo**
- c) **Name and address of Advisor** : **Shri. R. L. Parate,**  
Assistant Professor,  
Department of Plant Pathology,  
College of Agriculture, Nagpur.
- d) **Degree to be awarded** : M. Sc. (Agriculture) in Plant Pathology
- e) **Year of award of degree** : 2019
- f) **Major subject** : Plant Pathology
- g) **Total number of pages in the Thesis** : 46
- h) **Number of words in the abstract** : 317
- i) **Signature of the student** :
- j) **Signature, Name and Address of forwarding Authority** : **Dr. K. D. Thakur,**  
Professor and Head,  
Plant Pathology Section,  
College of Agriculture,  
Nagpur.

---

**ABSTRACT**

An investigation entitled "CULTURAL AND MORPHOLOGICAL CHARACTERIZATIONS OF *Beauveria bassiana*" was carried out during 2018-2019. Effects of nutrition, pH, temperature and humidity

were assessed on the basis of radial growth. Influence of different spore concentrations viz.,  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  and  $10^9$  spores per ml were tested for the pathogenicity of *B. bassiana* against second instar larvae of *Spodoptera litura*. Similarly compatibility of *B. bassiana* was evaluated with commonly used insecticides viz., spinosad 45 SC, imidachloprid 200 SL, chloropyriphos 20 EC and indoxacarb 14.5 SC.

The mummified bodies of lepidopteran pest larvae with symptoms of fungal infection were collected from field conditions from different places. Detailed morphological studies of effective isolate (Bb3) show that the fungus grew well on SDA (sabouraud dextrose agar). The mycelium on this medium, appeared white, dense and powdery. The colonies appeared raised in the center and developed radial furrows. The culture was turned yellowish white and powder type. Spore structures was globose to sub-globose.

Two different semi synthetic media namely sabouraud dextrose agar and potato dextrose agar were evaluated for better growth of *B. bassiana* and it was observed that SDA medium (41.50 mm) support the growth of isolate. Significantly higher radial growth was however, seen in media on the basis of these results, pH value 6.0, temperature of 25°C and 95 per cent relative humidity was considered as optimum for growth and sporulation of *B. bassiana*.

In terms of larval mortality,  $10^7$  spores per ml was recorded highest mortality of *S. litura* followed by  $10^8$  spores per ml, least larval mortality was observed at  $10^5$  spores per ml after 3, 5, 7 and 10 days of treatment. Compatibility of *B. bassiana* against commonly used insecticides in plant protection was tested. Imidachloprid were non-toxic to *B. bassiana*, as no significant reduction in radial growth was noticed. The insecticide chloropyriphos recorded 77.20 per cent inhibition of growth and was toxic to *B. bassiana*.

## Chapter I

### INTRODUCTION

#### 1.1 Background information

*Beauveria bassiana* is an entomopathogenic deuteromycete which infect a variety of insects. Its hosts are predominantly members of the insect orders lepidoptera and coleoptera, including a number of economically important agricultural insects inhabiting soil or crop plants. Realization of the impact of naturally occurring epizootics of the mycosis in pest populations and the potential of artificial dissemination of fungal conidia to increase the inoculum is of historical significance in the use of insect pathogens as pest control agents. The fungus generally infects its host through the host integument. The germ tubes from conidia on the surface of the insect penetrate the cuticle. After crossing the integument, the fungus develops as blastospores within the hemocoel. Histopathological studies indicate that toxins released by the fungus kill the host. After host death, mycelia grow through all tissues. The fungus grows outside the cadaver and develops conidiophores under conducive environmental conditions.

The origins of the microbial pest control date back to the early nineteenth century, when the Italian scientist Agostino Bassi spent more than 30 years studying white muscardine disease in silkworms (*Bombyx mori* L.). He identified *Beauveria bassiana* (Bals.-Criv). Vuill., named in his honour, as the cause of the disease. The *Beauveria bassiana* is a parasite of many arthropods, including more than 200 species of insects and acaridae. The disease caused by the fungus is called white muscardine disease (Fargues *et al.* 1997). When spores of the fungus come into contact with the body of an insect host, they germinate, enter the body, and grow inside, eventually killing the insect. Approximately 750 species of fungi, two species such as *Beauveria bassiana* and *Metarhizium anisopliae* are mostly used for controlling harmful insects and as of 2007. A total of 58 biological preparations were produced by using fungus *Beauveria bassiana* are

being broadly used for controlling harmful insects in rangelands, forests, crop fields and greenhouses (Marcos and Wraight, 2007). Fungus *Beauveria bassiana* has not been studied and biological preparation of this fungus is not common to used in our country. It has been important to investigate the possibility of isolation of this fungus species and applicability of such preparation for controlling harmful insects. Entomogenous fungi are potentially the most versatile biological control agents, due to their wide host range that often results in natural epizootics. An attractive feature of these fungi is that infectivity is by contact and the action is through penetration (Nadeau *et al.* 1996). These fungi comprise a heterogenous group of over 100 genera with approximately 750 species, reported from different insects. Many of these offer a great potential in pest management. The most important fungal pathogens are *Metarhizium spp.*, *Beauveria spp.*, *Metarhizium rileyi*, *Lecanicillium lecanii* and *Hirsutella spp.*

Fungus isolation on the surface of leaves and in soil is common in pathogen on plants, but not in pathogen on insects. Pathogen *B. bassiana* on insects is isolated from the leaf surface of brambly plants *Crataegus monogyna*, needle like plants *Urtica dioica* and weed *Dactylis gomerata* and *Elytrigia repens*. As explained, *B. Bassiana* from the leaf surface is proven virulent to leaf insects, while isolate from soil is virulent to soil insects. Isolation *B. bassiana* in habitat of paddy was virulent to *Leptocorixa acuta*. Characterization of isolate *B. bassiana* from soil is taken as the first step in order to produce effective isolate in controlling insects in soil, as well as isolate on leaf surface to insects on leaf.

*B. bassiana* isolated from larva *S. litura* will virulent to larva *S. litura*, including isolated from *H. armigera* will virulent to larva *H. armigera*. These two kinds of pests were taken because of their significant influence in tomatoes. Larva *S. litura* and *H. armigera* are proven difficult to be controlled with synthetic insecticide due to their resistance to all conventional active insecticide in all plant ecosystems. Isolation of *B. bassiana* from leaf surface of tomatoes and soil where

the plant roots are housed is considered as advanced step in developing entomopathogenic fungi as biological agents.

The availability of *B. bassiana* virulence as myco-insecticide or pathogen which naturally survives in the field is the first step in development of controlling agents to biological elements. Virulence isolate can be obtained through selection using one to three characters such as biological, molecular or phenotypic in colony morphology. Selection of fungus isolate according to phenotypic characters in colony morphology is easier and more affordable compared with that of biological and molecular characters. Various isolates of *B. bassiana* indicate that phenotypic character in colony morphology is correlated to virulence isolates (Afandhi *et al.* 2012).

Insects, constituting the largest class in animal kingdom and whole living world, are the friend as well as foes of human being. But unfortunately the role of insects as harmful organism is much wider than the beneficial. The tobacco caterpillar, *Spodoptera litura* is a polyphagous sporadic pest with high mobility and reproductive capacity. It is serious of various economically important crops such as cotton, groundnut, chilli, tobacco, castor and pulses *etc.* Chemicalization is known to destabilize the ecosystem by destroying the rich fauna of predators, parasitoids, pollinators, soil builders, birds, lizards, small animals as well as earthworms and microbial diversity (Gupta and Kumar 2014).

In culture *B. bassiana* grows as white mould. On most common culture media, it produces many dry, powdery conidia in distinctive white spore balls. Each spore ball is composed of a cluster of conidiogenous cells. The conidiogenous cells of *B. bassiana* are short, ovoid and terminate in a narrow apical extension called a rachis. The conidia is single celled, haploid and hydrophobic.

The major issues involved in mass production and utilization of mycopathogens are selection of effective strains, development of cost effective methods for mass rearing, development of effective methods

for storage and shipment and production of effective formulation. Environmental factors like temperature, humidity and sunlight play a profound role on the field persistence of entomopathogenic fungi.

Thus, conservation and periodic enhancement of efficacy of biological control agents will help in crop protection and in producing agricultural commodities free from pesticide residues. There is a general feeling that, the development and spread of biological control will empower the poor farmers to manage their pest problems in an eco-friendly ways.

## **1.2 Objectives of research**

1. To study cultural and morphological characteristics of *Beauveria bassiana*.
2. To assess compatibility of *Beauveria bassiana* with insecticides.

## **1.3 Scope and limitation**

In the majority of cropping system total emphasis is still placed on single technology aspects such as the use of pesticides, host plant resistance and bio-control, consideration rarely being given to their interaction. However, an important approach that could be taken in integrated pest management (IPM) programme is the use of biological pesticides together with a rational use of chemical pesticides. Biological control in particular when accomplished by entomopathogens, is technique that should be considered as an important pest population density reduction factor in integrated pests management (IPM) programme. Therefore, the conservation of such entomopathogens, whether they occur naturally or when they are applied or introduced to control insects, is an interesting practice. However, the use of incompatible insecticides may inhibit the development and reproduction of these pathogens, affecting IPM (Anderso and Roberts 1983). On the other hand, the utilization of selective insecticides in association with pathogens can increase the efficiency of control, allowing the reduction of the amount of applied

insecticides, minimizing environmental contamination hazards and expression of pest resistance (Quintela and McCoy 1998).

The environmental factors like humidity, temperature, *etc.*, plays an important role in the growth and development of entomopathogenic fungi. The mortality of insect pest by fungal infection takes more time and they also have a short shelf life so proper storage facility is a must.

#### **1.4 Hypothesis**

Regular use of a wide spectrum of insecticides can result in their accumulation in the field environment and this may impede natural epizootics. Enhanced effectiveness can be achieved by joint action of prevalent fungal entomopathogens and compatible insecticides, which will reduce not only the cost of protection, but also reduce risk and gravity of resistance to insecticides. Combination of synthetic insecticides and microbial insecticides has been used to stress the host thus making them more susceptible to disease. This knowledge of compatibility between *B. bassiana* and insecticides is crucial, so that growers may select appropriate compounds to scheduled treatments accordingly in order to minimize any deleterious effects in bio-control efficiency.

## Chapter II

### REVIEW OF LITERATURE

The literature pertaining to research investigation is reviewed under the following major head.

#### 2.1 Morphology of fungus

Wagner *et al.* (2000) studied that the light and electron microscopy were used to describe the mode of penetration by *B. bassiana*. Long hyphal structures were observed to follow the leaf apoplast in any direction from the point of penetration. A few hyphae were observed within xylem elements.

Hassanloui *et al.* (2006) studied the several morphological and physiological characteristics of *B. bassiana* were evaluated for a possible link to virulence against both insect species diamondback moth (DBM) and colorado potato beetle (CPB). Special mean diameter (2.45-3.58  $\mu\text{m}$ ) and conidia size (5.72-10.25  $\mu\text{m}^2$ ) were significantly different among isolates. The time for 50% of conidia to germinate (GT<sub>50</sub>) varied from 11 to 21 hrs depending on isolate.

Diane *et al.* (2007) reported three types of single cell propagules produced by the *B. bassiana*. Atomic force microscopy (AFM) revealed the presence of bundles or fascicles in aerial conidia absent from *in vitro* blastospores and submerged conidia. These analysis indicated that the cell surfaces of aerial conidia were hydrophobic, whereas those of blastospores and submerged conidia were hydrophilic.

Castrillo *et al.* (2010) studied the genetic characterization of *B. bassiana* using seven microsatellite markers showed that most of the emerald ash borer (EAB) derived strains clustered with bark or soil derived strains collected from the same site, indicating the indigenous nature of most strains isolated from EAB. More soil samples contained

*B. bassiana* colony forming units than bark samples, suggesting that soil serves as the primary reservoir for fungal inocula.

Afandhi *et al.* (2012) reported that the *B. bassiana* isolated from the three natural habitats involving larva *S. litura* and *H. armigera*, the soil and surface of tomato leaves. Isolate of insect with curved colony character represented higher growth rate than that of the leaves surface or the soil with raised and bowl shaped colony character

Kulu *et al.* (2015) studied that 60 soil samples was taken from nearby mustard plant root, 30 samples from monoculture field and 30 samples from polyculture field. There are no significant differences between *B. bassiana* isolates which isolated from monoculture and polyculture based on morphological and molecular characterization according PCR techniques.

## **2.2 Pathogenicity test**

Sivasankaran *et al.* (1990) reported that, second and third Instar larvae of the shoot borer *Chilo infuscatellus* Snell., were more susceptible (51.47 to 65.2 %) to the fungus, *Beauveria bassiana* even at a low dosage ( $10^5$  or  $10^6$  spores/ml), at  $10^7$  spores/ml, the mortality observed was 68.53-75.93 per cent. Mortality of the larvae decreased with increase in larval age or decrease in the concentration of the fungus.

Quintela and McCoy (1997) reported that, fungal concentrations of  $1 \times 10^6$  and  $1 \times 10^7$  conidia per ml of *B. bassiana* and *M. anisopliae* affected larval development, movement and resulted in 90-100 per cent larval mortality.

Godonou *et al.* (2009) studied the biological agents to control the diamondback moth (DBM, eight isolates of *B. bassiana* and *M. anisopliae* virulence against larvae of the insect. The isolate Bba5653 caused 94 per cent mortality of DBM larvae, and the mortality was significantly higher than that caused by any of the other isolates.

Vijayavani *et al.* (2009) reported that the pupae of *S. litura* treated with *B. bassiana* (SBT#11 and SBT#16). Were highly pathogenic causing 100 per cent mortality in *S. litura* which is conidial concentration dependent SBT#11 was more virulent with a LT<sub>50</sub> of 5.1 and 6.0 days in laboratory for SBT#16 and SBT#11 respectively.

Malarvannan *et al.* (2010) studied tobacco caterpillar, *S. litura* was reared in semi synthetic diet. *B. bassiana* was sub cultured on potato dextrose agar (PDA). Four different spore concentrations ( $2.4 \times 10^7$ ,  $2.4 \times 10^6$ ,  $2.4 \times 10^5$ ,  $2.4 \times 10^4$  conidia/ml) were prepared from the 15 day old culture of the fungi. A preliminary study on *B. bassiana* against *S. litura* larvae was done. The least pupation (43.33 per cent) was observed in larvae treated with the highest spore concentration ( $2.4 \times 10^7$ ) of *B. bassiana*.

Prasad *et al.* (2010) reported that control pest population of *H. armigera* with an ecosafe entomopathogen *B. bassiana*. When four different concentrations (0.1, 0.125, 0.2 and  $0.25 \text{ ml} \times 10^8$  spores/ml) were sprayed topically against most damaging IV<sup>th</sup> instar larvae of *H. armigera*, a dose dependent mortality was observed that went up to 76.7 per cent with highest dose of  $0.25 \text{ ml} \times 10^8$  spores/ml.

Kaur *et al.* (2011) studied that the control of insects belonging to various orders. The virulence of *B. bassiana* was tested against second, third and fourth instar larvae of *S. litura* using three concentrations *i.e.*,  $2.03 \times 10^8$ ,  $4.03 \times 10^6$  and  $1.47 \times 10^5$  spores/ml. All the treatments resulted in significantly higher mortality than control.

Moorthi *et al.* (2011) reported that the isolates of the entomopathogenic mitosporic ascomycete, *B. bassiana* were evaluated against *S. litura* using leaf spray method. Mortality of 66.67, 73.33 and 80.0 per cent with respect to the isolates Bb2, Bb9 and Bb10, respectively, was obtained four days after treatment. The isolate Bb10 was the most virulent with potential for the management of *S. litura*.

Revathi *et al.* (2011) studied the *B. bassiana* isolates from fields showed greater than 70 per cent mortality of *H. armigera* in the bioassay the per cent mortality declined, there was a decrease in the enzyme activities of *B. bassiana*.

Ummidi *et al.* (2013) studied the relationship between rate of conidial germination of the two entomopathogenic fungi *B. bassiana* and *M. anisopliae* and their virulence against the insect pest *S. litura* larvae. Rate of germination in terms of TG<sub>50</sub> (time taken for germination of 50 per cent of conidia) was estimated by calculating the germinated and ungerminated conidia at every 2 hrs intervals up to 48 hrs from the SDAY (sabouraud dextrose agar + yeast extract) plates inoculated with 3x10<sup>6</sup> conidia/ml. Virulence of *B. bassiana* and *M. anisopliae* was elucidated by performing bioassay with *S. litura* larvae at 2<sup>nd</sup> instar stage. The study demonstrated that the strains of both *B. bassiana* and *M. anisopliae* with low TG<sub>50</sub> values were more virulent towards *S. litura* larvae.

Gupta and Kumar (2014) studied the bioefficacy of *B. bassiana* against 3<sup>rd</sup> instar larvae of *S. litura*. Spore suspension of three different concentrations 0.1, 0.125 and 0.2x10<sup>8</sup> were prepared by serial dilution. When these concentrations of spores were applied on 3<sup>rd</sup> instar larvae of *S. litura*, it increases the per cent of mortality.

Younas *et al.* (2016) reported that the reduction in the larval population of *H. armigera* and pod infestation increased chickpea yield and the highest yield in both seasons and the maximum yield was obtained in the *B. bassiana* 3.21x10<sup>6</sup>+chlorantraniliprole treatment.

Fatima *et al.* (2017) studied that the different spore concentrations of *B. bassiana* 1x10<sup>7</sup> spores per ml was found to be more effective and promising concentration. This was evidenced by the fact that 1x10<sup>7</sup> spores per ml concentration of *B. bassiana* in general was found promising in terms of increased per cent larval mortality, reduced pupation and reduced per cent adult emergence of

both *S. litura* and *P. xylostella*. When five different concentrations viz.,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  and  $1 \times 10^9$  spores per ml were used against *S. litura* and *P. xylostella*, a dose dependent mortality was observed that has resulted in about to 85 per cent larval mortality in case of *S. litura* and 100 per cent in case of *P. xylostella*.

Swathi *et al.* (2017) studied the susceptibility of second instar *H. armigera* to seven strains of *B. bassiana*. The highest virulence (100 per cent) was recorded in strain-4 with  $LC_{50}$  value ( $2.02 \times 10^5$  spores  $ml^{-1}$ ) followed by strain-1 (92.50 per cent) with  $LC_{50}$  value ( $2.48 \times 10^5$  spores  $ml^{-1}$ ) at 120 hrs after treatment.

Indriyanti *et al.* (2017) studied the different doses treatment 1 g  $100 ml^{-1}$ , 2 g  $100 ml^{-1}$ , 4 g  $100 ml^{-1}$ , 8 g  $100 ml^{-1}$  and 0 g  $100 ml^{-1}$  (as control). Each treatment used 10 larvae and repeated five times. The result showed that *B. bassiana* with 8 g  $100 ml^{-1}$  conc. was more effective to kill *S. litura* larvae than others doses.

Ismail *et al.* (2017) studied that *B. bassiana* ( $1.3 \times 10^6$  spores/ml) and three new chemistry insecticides [emamectin benzoate (Timer®), flubendiamide (Belt®) and chlorantraniliprole (Coragen®)] alone and in mixtures at dose less than the 2 folds of recommended field dose were tested against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. litura*, under laboratory conditions using larval immersion and leaf dip method. Interaction of *B. bassiana* and new chemicals showed the higher per cent of mortality in both tested instars larvae as compared to alone treatment. Bb\* Coragen® (91.43%, 86.14%) interaction showed the highest larval mortality followed by Bb\*Belt® (84.60%, 76.88%) and Bb\*Timer® (74.60%, 70.86%) for 2<sup>nd</sup> and 4<sup>th</sup> instar larvae respectively.

### 2.3 Cultural studies

Senthamizhselvan *et al.* (2010) studied that the two culture media tested both potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) recorded maximum mycelial growth in *B.*

*bassiana* isolates. Maximum growth was observed in the isolates of BbMtKKL 2107 (68.02 mm) and BbMdKKL 2106 (66.91 mm). Minimum growth was noticed in the isolates BbMtKKL 2107 (65.16 mm) and BbMdKKL 2106 (63.44 mm) in PDA media.

Mabelle *et al.* (2011) studied *B. bassiana* grown on three liquid media containing casaminoacids, corn steep liquor or peptone. After incubation, the blastospore counts reached  $6.38 \times 10^9$  blastospores/ml, in the medium containing sucrose and corn steep liquor, which was significantly higher than the obtained with media containing casaminoacids or peptone.

Gangwar *et al.* (2013) evaluated different substrates *viz.* sorghum, maize, pearl millet, barnyard millet (*Echinochloa frumentacae*) and cow dung for mass multiplication of *B. bassiana*. Sorghum grains were found to be best and exhibited maximum population ( $6.32 \times 10^9$  cfu) followed by maize (each grain broken into 8-10 pieces) with  $5.77 \times 10^9$  cfu and barnyard millet ( $5.08 \times 10^9$  cfu), respectively.

Shanmugam *et al.* (2017) studied that maximum growth *B. bassiana* has supported by sabouraud dextrose agar medium by recording the mycelial growth of 85.3 mm.

Kumar and Kumar (2017) studied the cultured using different nutrient media to determine the type best suited for isolation and mass production of *B. bassiana*. It was identified that rate of mycelial formation occurred in potato dextrose agar over a 7 days period under a photoperiod of 16 hrs light and 8 hrs of darkness at a relative humidity of 85 per cent.

Deb *et al.* (2017) studied the growth of *B. bassiana* was tested in different solid media namely SDA (sabouraud dextrose agar), PDA (potato dextrose agar), SDAY (sabouraud dextrose agar + yeast extract), CDA (czapek dox agar), OMA (oat meal agar), PCA (potato carrot agar) and RBA (rose bengal agar) on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day after

incubation. Maximum colony diameter of 45.33 mm was observed in SDYA followed by SDB (43.00 mm).

Singh *et al.* (2017) tested solid and liquid media were tested for mass multiplication of *B. bassiana*. Significantly producing spore per ml recorded on 7 day after the day after inoculation of various treatments. SDB (sabouraud dextrose broth) was the best treatment by bringing down the *B. Bassiana* production up to 48.3 spore/ml in liquid medium.

#### **2.4 Influence of temperature on the growth and sporulation of *B. bassiana***

Hallsworth *et al.* (1996) studied the growth and conidial physiology of the entomopathogenic fungi *Beauveria bassiana* under the different range of temperature (5 to 35°C). Growth of isolate was optimal between 20 and 35°C depending on the isolate.

Ying and Feng (2006) studied that the conidia produced on sabouraud dextrose medium at 15-30°C, pH 4.0-8.0. The culture conditions were optimized as 25°C and pH 5.0-6.0.

Wang *et al.* (2011) studied new virulent *B. bassiana* isolate (*B. bassiana*-CYT5) and demonstrated high heat tolerance (60-100 per cent relative germination) between 1 hrs and 2 hrs exposure at 37°C, 38°C, 39°C and 40°C, respectively. *B. bassiana*-CYT5 suggested could be a new efficient biocontrol agent against *Frankliniella occidentalis*.

Ahmad *et al.* (2016) studied that *in vitro* effect of several temperatures (15, 20, 25, 30 and 35°C) on growth rate, spore production and spore germination of two different isolates of the fungus *B. bassiana*. The optimum temperature was 25°C without significant differences.

Kim *et al.* (2018) reported that low thermotolerance in *B. bassiana* is the main impediment to their industrialization. Two morphologically different colonies (BbHet1 and BbHet2) were isolated from the pairing. BbHet2 conidia had 60.7% germination after exposure to 45°C for 60 min (the others had < 15 per cent).

Alali *et al.* (2019) reported that *B. bassiana* isolated for different biological parameters (*i.e.*, growth rate, sporulation and spore germination) at growing temperatures ranging from 20°C to 35°C. Among these isolates (eight from insects and 11 from soil samples), the five with the highest growth rate, spore production and germination at 30°C.

## **2.5 Influence of humidity on the growth and sporulation of *B. bassiana***

Shipp *et al.* (2003) investigated the influence of different humidity levels on per cent of infection by *Beauveria bassiana* on greenhouse insect and mite and their commercially available biological control agents. A humidity of 97.5% relative humidity resulted in significantly higher per cent of infection (60-88.8%), while at 75 and 80% relative humidity only 15.3-43.9% of the pest and insects were infected *in vitro*.

Padmini *et al.* (2010) studied the thirty isolates of *Beauveria spp.*, at five different relative humidities at a constant temperature of 30°C. Only twenty isolates could grow at 60%, 63%, 75% and 85% relative humidities, while 91% relative humidity did not permit growth of ten isolates. On the other hand, eleven isolates, out of thirty displayed sporulation. Optimum relative humidity for *Beauveria* isolates for growth and sporulation appeared to be 60%.

Kotwal *et al.* (2012) evaluated the effect of different parameters on growth and sporulation of *Metarhizium anisopliae*. During the incubation of mass culture relative humidity of 85 per cent favours the growth and sporulation.

Mishra *et al.* (2015) reported that the *Beauveria bassiana* HQ917687 virulence to housefly larvae and adult was assessed at different relative humidity, (50, 75, 90, and 100 %) at the fungal dose of  $10^8$  conidia/ml. Depending on the relative humidity regime tested, difference in mortality rates of housefly adult and larvae were detected. During assay on adult housefly, 100 per cent mortality was achieved at relative humidity 90 and 100 per cent. Lethal time,  $LT_{50}$  was 2.9 days at 100 per cent relative humidity.

## **2.6 Influence of different pH level on the growth and sporulation of *B. bassiana***

Padmavathi *et al.* (2003) studied the relation of the pH tolerance and optimum range of an isolate of *B. bassiana* to its phenotypic characters. All isolates with a chalky type colonial morphology had an intermediate growth rate and showed a wide optimum range of pH 5.0-13.0 or 5.0-14.0. All isolates with dusty type colonial morphology were found to have a high growth rate and a wide pH tolerance range of 4.0-14.0. Both chalky and dusty colonial morphology are due to a growth pattern characterized by iterated conidiation with a very brief hyphal stage.

Ying and Feng (2006) studied that the conidia produced on media sabouraud dextrose medium at pH 4.0-8.0. The culture conditions were optimized as pH 5.0-6.0.

Karthikeyan *et al.* (2008) studied optimum pH and growth pattern of *Beauveria bassiana* on different media such as ground nut cake broth, coconut water broth and potato dextrose broth. The pH 6.0-8.0 was found to be the most suitable for growth and spore formation. The highest growth was obtained in potato dextrose broth.

Pham *et al.* (2009) studied the optimal liquid culture conditions in shake flasks for maximal sporulation of *B. bassiana*. The optimal initial pH for the spore production of *B. bassiana* using potato dextrose broth was 5.2.

## 2.7 Compatibility of chemical pesticides with *B. bassiana*

Alizadeh *et al.* (2007) studied the compatibility of *B. bassiana* with imidaclopride, flufenoxuron, teflubenzuron + phuzalon, endosulfane and amitraz and effect of these pesticides on conidial germination, vegetative growth and sporulation of the fungus. The results indicated that flufenoxuron is not compatible with *B. bassiana* and it caused complete or strong inhibition in its development. The compatible formulation with *B. bassiana* (isolate DEBI008) was imidacloprid.

Rajanikanth *et al.* (2010) reported that the compatibility of six strains of *B. bassiana* with insecticides, *viz.*, imidacloprid, spinosad, indoxacarb and chlorpyrifos. All the strains were compatible with imidacloprid and spinosad. Indoxacarb showed no significant inhibition of radial growth of *B. bassiana*. Chlorpyrifos was found to be incompatible with all the strains.

Amutha *et al.* (2010) studied compatibility of *B. bassiana* in the laboratory condition with twelve commonly used insecticides. Among the insecticides tested for their compatibility, only chlorpyrifos 20 EC was rated as relatively less toxic to *B. bassiana*, while, spinosad (45 % SC), econeem (1%), quinalphos (25 EC), acetamprid (20%), endosulfan (35 EC) and thiodicarb (75 WP) were slightly toxic. Imidacloprid (17.80% SL) and triazophos (40 EC) were moderately toxic. Profenofos (50 EC), indoxacarb (14.5% EC) and methyl demeton were highly toxic. Results of the present study suggested that except profenofos, indoxacarb and methyl demeton, the rest of the insecticides tested can be safely used along with the entomopathogenic fungi *B. bassiana*.

Usha *et al.* (2014) studied the compatibility of *B. bassiana* with insecticides, fungicides and botanical, at three concentrations (0.1X, 0.5X and 1X) on the recommended dose by food poison technique.

Chlorpyrifos is highly detrimental to all the isolates even at low concentrations. Isolate B55 was compatible with imidacloprid even at higher concentration (1X).

Shakir *et al.* (2015) studied the relative efficacy of ecofriendly formulation of *B. bassiana* ( $1.3 \times 10^6$  conidia/ml), potassium silicate (50 mg/lit) and imidacloprid (75 ml/acre) for sustainable management of *Cnaphalocrocis medinalis*. Mortality was maximum (61.91%) with combined application of potassium silicate, *B. bassiana* and imidacloprid after 20<sup>th</sup> day of application.

Faraji *et al.* (2016) reported that the compatibility of *B. bassiana* with insecticides. The compatible insecticide with *B. bassiana* at three concentrations, found to be spinosad. Abamectin, imidachloprid and deltametrin, however at half MC (mean conc.) and MC were compatible with *B. bassiana*.

Abidin *et al.* (2017) reported that the insecticides showing the highest vegetative growth on *B. basiana* at 7 DAI was imidacloprid 0.5 x DF, while at 14 DAI was imidacloprid 0.5 x DF and chlorphyrifos 2 x DF, respectively. The highest conidial production of the fungi was triggered by imidacloprid 0.5 x DF. Based on compatibility calculation, imidacloprid 0.5 x DF worked with *B. bassiana*.

## Chapter III

### MATERIALS AND METHODS

The present investigation on “Cultural and Morphological Characterizations of *Beauveria bassiana*” was conducted during 2018-2019 in the Laboratory of Plant Pathology Section and Department of Agricultural Entomology, College of Agriculture, Nagpur. In this experiment, various material were used and methods adopted during the entire course of investigation are described here.

#### 3.1 Material

##### 3.1.1 Source of culture

The pure culture of fungus *Beauveria bassiana* was isolated from naturally infected larvae of lepidopteran pests on different field conditions from different places.

##### 3.1.2 Test insect

*Spodoptera litura* was used as test insect. The larvae were collected from cotton, soybean and castor crops from Agronomy and Entomology field of Agriculture College, Nagpur and they were reared in the laboratory on artificial diet. The F1 generation larvae of second instar were used for studies.

##### 3.1.3 Glassware and chemicals used

During the entire course of investigation the petriplates, test tubes, conical flasks of different capacity, pipette, measuring cylinder and plastic containers were used in the laboratory. Plastic tray, plastic containers, thread, cotton, mating chamber, brush, polythene bags, forceps etc. were used for collection and rearing of *S. litura* larvae. Phase contrast microscope and Neubauer haemocytometer were used for counting the spore/ml of the fungal suspension. Hand atomizer and Hamilton pipette were used for application of fungal spore suspension and culture filtrate of *B. bassiana* for pathogenicity studies against laboratory reared second instar larvae *S. litura*.

### 3.1.4 Instruments used

The analytical and other scientific instruments were used from section for various studies in the present investigation. The name and purpose of instruments used in present investigation are presented in table.

#### Name of instruments and their uses

Sr.No.	Name of instrument	Purpose of use
1.	Laminar air flow	For inoculation of culture under aseptic condition.
2.	Autoclave	For sterilization of media, water etc.
3.	Hot air oven	For sterilization of glassware.
4.	Micro applicator	For application of toxins in micro quantities.
5.	Micro pipettes of variable volumes	For measuring chemicals
6.	Haemocytometer	For counting the spore/ml of fungal suspension.
7.	Electronic balance	For weighing chemicals
8.	Refrigerator	To store and maintain the cultures

### 3.2 Methods

#### 3.2.1 Isolation of *B. bassiana* from infected larvae

#### 3.2.2 Petri dish culture

The infected larvae of *Spodoptera litura* were collected from soybean field. The collected cadavers were surface sterilized with 0.1% mercuric chloride for five seconds and then rinsed three times with sterile distilled water to remove the traces of mercuric chloride. The surface sterilized specimen was cut into small bits in a sterile petridish and a bit of infected tissue was transferred to a sterile petridish containing potato dextrose agar media and kept in BOD at 25°C temperature and 90±5% relative humidity. After development of mycelial mat and sporulation, the fungus was under taken for initial

identification. Identification of the selected isolates was again confirmed on the basis of morphological studies (Debnath, 2015).

### **3.2.3 Slide culture method**

A small piece of sterilized potato dextrose agar media was placed in the sterilized slide and the media was inoculated with the test fungal species in two diagonal corners and covered with cover slip. The slides were then kept separately in petridishes, which were then sealed with parafilm M tape. The petridishes were kept in BOD at 25°C temperature and 90±5% relative humidity. After seven days the slides were removed from the BOD and each of the cover slips were placed individually on fresh slides each with a drop of cotton blue indicator. The stained fungal growth on the cover slip was examined under microscope for identification of the test fungal species (Debnath, 2015).

#### **Test fungal species**

White muscardine fungus

Kingdom : Mycota

Division : Eumycota

Sub-division : Deuteromycotina

Class : Deuteromycetes

Order : Moniliales

Family : Moniliaceae

Genus : *Beauveria*

Species : *bassiana*

Binomial nomenclature: *Beauveria bassiana* (Balsamo) Vuillemin.

### **3.2.4 Sterilization of glassware, media, distilled water and other materials**

The glassware and plastic containers were sterilized by keeping in cleaning solution (60 g of potassium dichromate and 100 ml concentrated sulphuric acid mixed in 1000 ml water) for 24 hrs followed by washing with tap water and air dried in shade. After drying, they were sterilized in hot air oven at 181°C temperature for 2 hrs. Different media and distilled water were sterilized in autoclave at 1.04 kg/cm<sup>2</sup> pressure at 121°C temperature for 15 minutes to avoid contamination. A laminar flow chamber was disinfected by 0.1% mercuric chloride solution and also by keeping the laminar flow unit in working condition for about 30 minutes before inoculation.

### **3.2.5 Purification and maintenance of test isolate**

The isolate was purified by single spore method. A dilute spore suspension of culture was prepared in sterilized test tube taking sterile distilled water. A loopful of suspension made was mixed with 15 ml of 2% melted SDA (cooled to 45°C temperature) in test tube. This medium with load of spores was then poured into previously sterilized petriplates. After solidification, the petriplates were incubated at 25°C. After 96-120 hrs, the spores started germinating giving rise to small colonies. The isolated colonies were well marked and then lifted aseptically with sterile inoculating needle and transferred to sabouraud's dextrose agar slants. As soon as the fungus had made desired growth at room temperature, it was further multiplied in more tubes for further studies. The culture was stored in refrigerator and subcultured at subsequent intervals as and when required.

### **3.2.6 Micrometry**

The morphological measurements of mycelium and conidia were taken at high power (45X, 100X) using the microscope.

### 3.2.7 Preparations of culture media

The cultural studies were carried out using the various media i.e. SDA (sabouraud dextrose agar), and PDA (potato dextrose agar), However, the culture was maintained on SDA medium and the same medium was used for further studies. All the media were prepared according to their standard composition. For broth medium, same composition but without agar were used. In each petriplate approximately 20 ml warm medium was poured in previously sterilized petriplates under aseptic condition by using laminar flow and allowed to solidify.

#### Different solid media and their chemical composition

Sr.No.	Name of the media	Chemical composition
1	Sabouraud Dextrose Agar (SDA)	Dextrose- 40.00 g Peptone- 10.00 g Agar- 20.00 g Distilled water- 1000 ml
2	Potato Dextrose Agar (PDA)	Peeled potato-200.00 g Dextrose- 20.00 g Agar- 20.00 g Distilled water- 1000 ml

### 3.2.8 Method of inoculation

For inoculating both solid and liquid media as per requirement with equal amount of inoculation, a sterile cork borer of 5 mm diameter was used and discs were transferred with the help of sterile inoculating needle in an inverted position.

### 3.2.9 Measurement of growth and sporulation

Linear growth of the fungus colony on solid media was recorded by measuring the colony diameter along with the two marked direction at right angles to each other passing through the centre of the colony

including the original inoculum disc of 5 mm diameter and average diameter of colony was recorded.

For recording sporulation, a visual method of comparison to mention the density of sporulation was used as under.

### **3.2.10 Maintenance of test insect culture:**

Field collected larvae of *S. litura* were taken as the initial culture. Castor crop was raised in the net house of Plant Pathology Section, College of Agriculture, Nagpur to serve as source for leaf material for culturing of *S. litura*. Care was taken to avoid any insecticidal usage in the crop to prevent mortality of *S. litura* culture. Rearing jars of 20×15 cm size were used for maintenance of culture. Jars and leaves were disinfected with 10 per cent formaldehyde before usage. The larvae were maintained in those jars by daily cleaning and changing. The mature larvae were transferred to fresh oviposition jars for pupation and adult emergence. The oviposition jars were provided with cotton swabs dipped in 10 per cent sugar solution, folded papers for resting and also to facilitate oviposition by the mated female. Egg masses laid on the papers were collected and were placed in rearing jars provided with fresh castor leaves. The newly hatched larvae were allowed to feed on fresh leaves and then transferred to jars for further rearing and pupation, thereby maintaining cultures for experimental purposes (Rajnikanth 2007 and Fatima *et al.* 2017).

### **3.2.11 Preparation of spore suspension**

The conidia for the bioassay tests were harvested from the two week old cultures of *B. bassiana* by washing the surface of the plates with 75-100 ml of sterile distilled water containing 0.02 per cent tween-20 (Rombach *et al.* 1986 and Fatima *et al.* 2017). Spore suspensions of different concentrations ranging from  $10^5$  to  $10^9$  spores per ml were standardized after assessing the number of spores in the suspension with an improved Neubour haemocytometer by adopting standard protocols.

### 3.2.12 Spores per unit area

A circular disc of 10 mm diameter was cut from two weeks old uniformly grown culture plates and was placed in a test tube containing 10 ml of distilled water. The spores present in the disc were allowed to disperse uniformly in water by rotating the test tube on a vertex for one minute. Proper care was taken to avoid spillage of the suspension while rotation. The suspension was serially diluted and spores were counted with the help of Neubauer haemocytometer under a microscope at 40x magnification and number of spores present per ml was calculated using the below mentioned formula (Aneja 1996 and Fatima *et al.* 2017). No. of spores per ml = Total No. of spores in 5 randomly selected squares of haemocytometer  $\times 5 \times 10^4$ . The reading thus obtained was computed to 10 ml to determine the number of spores per unit area of 10 mm diameter disc.

### 3.2.13 Pathogenicity studies

To test the pathogenicity of *B. bassiana* against *S. litura* simultaneously five different concentrations were evaluated. The test concentrations taken up for the study include (Fatima *et al.* 2017).  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  and  $10^9$  spores per ml and control was maintained.

The second instar larvae of *S. litura* were taken separately in sterile petriplates lined with filter paper and were directly sprayed with 2 ml spore suspension using hand atomizer. For each concentration, three replications were maintained with 10 larvae in each replication. Untreated control was maintained simultaneously by spraying with only 0.02 per cent tween-20 in sterile distilled water. After air drying, the treated larvae were placed in petriplates that were carefully reared by providing fresh castor leaves daily to *S. litura*. The experiment was conducted at room temperature of 25°C and relative humidity of 95 per cent. The efficacy of *B. bassiana* in terms of pathogenicity to *S. litura* was evaluated and data were recorded on per cent larval mortality (from date of inoculation to final instar). The larval mortality on incubation for each subsequent day up to 10<sup>th</sup> day after treatment were

recorded. From these observations, per cent cumulative larval mortality after 3, 5, 7 and 10<sup>th</sup> days was calculated.

### **3.2.13 Evaluation and characterization of *B. bassiana* on the basis of physiological and morphological parameters**

Effects of nutrition, pH, temperature and humidity were assessed on the basis of radial growth. Following methodology was adopted for assessment of these growth parameters. Petriplates containing SDA medium were prepared by evenly spreading 0.5 ml of the inoculum as prepared above, over the agar surface. Agar plugs were cut after six days incubation at 25°C using a sterile 5 mm diameter cork borer. The control and treated plates were inoculated with these agar plugs in the centre. The mycelial growth was assessed periodically by measuring the colony diameter at right angles on each plate. For calculations, all the observations were recorded on 10<sup>th</sup> day of inoculation (Kotwal *et al.* 2006).

#### **The following parameters were evaluated**

##### **A. Media**

Radial growth of different isolates of *B. bassiana* was estimated on different culture media. Fungal growth was assessed as colony diameter.

##### **B. pH**

To study the effect of pH on growth of different isolates of *B. bassiana*, four pH levels (6.0 to 7.5) were tested. The pH of culture medium was adjusted by adding 1 N HCl and 1 N NaOH with the help of indicator paper prior to autoclaving. Fungal growth was assessed in the form of radial growth.

##### **C. Temperature**

For studying the effect of temperature on growth of *B. bassiana* isolates, four levels of temperatures (20, 25, 30, and 35°C) were

tested. The petriplates containing the inoculated media were incubated at respective temperatures in an incubator. The fungal growth was assessed on the basis of colony diameter.

#### **D. Effect of relative humidity**

The inoculated petriplates in three replications were exposed to 80, 85, 90, and 95 per cent relative humidity levels in desiccators of uniform size. The humidity levels in desiccators were maintained by using concentrated sulphuric acid + water as per method suggested by Soloman (1951). The quantity of sulphuric acid used to obtain different humidity levels was as follows:

Relative humidity ( % )	Conc. sulphuric acid (ml/100 ml solution)
80	26.79
85	22.88
90	17.91
95	11.02

The observation on colony diameter were recorded after ten days of incubation while sporulation was recorded on 10<sup>th</sup> day.

#### **3.2.16 Compatibility studies of *B. bassiana* with insecticides**

##### **Insecticides used for experiments**

Common name	Trade name & formulation	Active ingredient	Normal conc. (%)
Tracer	Spinosad	45 SC	0.018
Confider	Imidachlopid	200 SL	0.0045
Dursban	Chloropyriphos	20 EC	0.05
Avaunt	Indoxacarb	14.5 SC	0.0145

The compatibility of four commonly used insecticides viz. spinosad, imidachlopid, chloropyriphos and indoxacard on variety of field crops were tested against *B. bassiana* by adopting poison food

technique (Rajanikantha *et al.* 2010). The insecticides were tested at recommended dose concentrations. For each treatment 100 ml sabouraud dextrose agar medium was taken in 250 ml conical flask and autoclaved at 1.04 Kg/cm<sup>2</sup> for 20 minutes. The specified concentration of insecticide was added at lukewarm temperature and mixed thoroughly by shaking the flask. The poisoned medium was poured into three petriplates and allowed to solidify. Test fungus culture was cut into 5 mm disc from the periphery of 10 day old pure culture with sterilized cork borer and transferred to the centre of each of the plates containing poisoned medium. Controls were maintained by placing fungal disc in medium without insecticides. All the plates were incubated at 25° C. The entire procedure was carried out under aseptic conditions. The diameter of fungal colony was measured seven days after inoculation and per cent inhibition over the control was calculated.

## Chapter IV

### RESULTS AND DISCUSSION

The research study on “CULTURAL AND MORPHOLOGICAL CHARACTERIZATIONS OF *Beauveria bassiana*” was undertaken in the Plant Pathology Section, College of Agriculture, Nagpur during the year 2018-19. The results are presented in tables, plates and figures are depicted in each head in this chapter.

#### 4.1 Isolation of *Beauveria bassiana*

**Table 1: Isolates of *Beauveria bassiana***

Sr.No.	Isolates	Source	Scientific name of insects	Order
1	Bb1	Blister beetle	<i>Mylabris pustulata</i>	Coleoptera
2	Bb2	Larvae of american bollworm	<i>Helicoverpa armigera</i>	Lepidoptera
3	Bb3	Pupa of tobacco leaf eating catterpillar	<i>Spodoptera litura</i>	Lepidoptera
4	Bb4	Larvae of paddy stem borer	<i>Scirpophaga incertulas</i>	Lepidoptera
5	Bb5	Bhendi fruit borer	<i>Earias insulana</i>	Lepidoptera
6	Bb6	White grub	<i>Holotrichia serrata</i>	Colioptera
7	Bb7	Corn ear worm	<i>Helicoverpa armigera</i>	Lepidoptera
8	Bb8	Sesamum gall fly	<i>Asphondylia sesami</i>	Diptera
9	Bb9	Caster semilooper	<i>Achaea janata</i>	Lepidoptera
10	Bb10	Brinjal stem borer	<i>Euzophera perticella.</i>	Lepidoptera
11	Bb11	Market product		
12	Bb12	Market product	-	-
13	Bb13	Market product	-	-
14	Bb14	Market product	-	-
15	Bb15	Market product	-	-
16	Bb16	Market product	-	-

Intensive and repeated visit were takes place for the occurrence of the insects infected by entomopathogenic fungi *Beauveria bassiana* in the field of College of Agriculture, Nagpur as well as different tahsils of Nagpur district. Sixteen different isolates of *Beauveria bassiana* were collected during the cropping season of 2018-2019 (table 1). Among them ten isolates were collected from the naturally infected insects. And cultures were isolated from market cultures. The occurrence of *Beauveria bassiana* was noticed on blister beetle, american bollworm, tobacco leaf eating caterpillar, paddy stem borer, bhendi fruit borer, white grub, corn ear worm, sesamum gall fly, caster semilooper and brinjal stem borer.

The collected cadavers were surface sterilized with 0.1% mercuric chloride for five seconds and then rinsed three times with sterile distilled water to remove the traces of mercuric chloride. The surface sterilized specimen was cut into small bits in a sterile petridish and a bit of infected tissue was transferred to a sterile petridish containing potato dextrose agar media and kept in BOD at 25°C temperature and 90±5 per cent relative humidity.

Infection of *B. bassiana* in larvae *S. litura* was characterised by symptoms like fungus with a colony of white hyphae growing on the integument of larvae. The phenotypic character of the colony growth was apical. This growth was caused by the isolates which were reared in the same PDA media. The growth type of colony *B. bassiana* was apical, where it grew in all direction. The observation on colony of isolate from the larvae *S. litura* is in line with Afandhi *et al.* (2012), where the colony *B. bassiana* was opaquely white. The phenotypic characters in colony of isolate were qualified by round shape, yellowish white colour, curved surface, and intact edge. The isolate *B. bassiana* from larvae *S. litura* revealed the white colony with hyphae resembling floss in cotton, which characterizes class of hyphomycetes. This was consonance with Shnmugam *et al.* (2017) The *B. bassiana* isolates at first appeared as white colour, later becoming yellowish and powdery

type. Spore structured were characterized viz., spherical or oval and non septate.

#### 4.2 Pathogenicity of fungus

**Table 2: Pathogenicity of different isolates of *Beauveria bassiana* on 2<sup>nd</sup> instar larvae of *Spodoptera litura* at 10 days after treatment**

Sr. No.	Isolate ( $10^7$ CFU ml <sup>-1</sup> )	Per cent mortality
1	Bb1	83.33
2	Bb2	78.33
3	Bb3	92.33
4	Bb4	88.33
5	Bb5	87.00
6	Bb6	80.00
7	Bb7	82.66
8	Bb8	76.00
9	Bb9	79.66
10	Bb10	80.66
11	Bb11	75.33
12	Bb12	86.66
13	Bb13	89.00
14	Bb14	85.00
15	Bb15	78.33
16	Bb16	82.00

The data obtained in the experiment carried out for studying pathogenicity of *B. bassiana* against second instar larvae of *S. litura* indicated that, significant increase in per cent mortality when compared to untreated was recorded at  $10^7$  spores per ml was in the range of 75.33 to 92.33 per cent with inoculation of different isolates. Highest larval mortality of 92.33 per cent was noticed with Bb3 isolate where concentration of *B. bassiana* was  $10^7$  spores per ml which was significantly different from untreated control (0.0 per cent), followed by 89.00 per cent (Bb13). Least larval mortality of 75.33 per cent was noticed by Bb11 isolate (table 2).

Pathogenesis of disease development in *S. litura* due to *B. bassiana* was observed. At first 24 hrs, the larvae showed no changes,

feeding normally and responding to external stimuli. At incubation period ranging from 2 to 8 days larvae were sluggish and ceased to feed on third/fourth day after treatment then, after body become slightly bent, tough and mummified. Initial growth of fungus was noticed on the seventh days and on eight day whole body covered with white mycelia then it turned into yellowish white on the insect body (Kotwal *et al.* 2006).

Among the sixteen isolates, effective isolate of *B. bassiana*, isolate Bb3 was more virulent (92.33 per cent mortality) than the other fifteen isolates. This potential virulent isolate (Bb3) was used for the check out the different growth parameters such as growth on different media, temperature, humidity and pH.

#### 4.2.1 Effect of spore suspension

**Table 3. Mean cumulative per cent mortality of 2<sup>nd</sup> instar larvae of *Spodoptera litura* with *Bauveria bassiana* at different concentration**

Conc. of spore/ml	Per cent cumulative mortality of 2 <sup>nd</sup> instar larvae of <i>S. litura</i> days after treatment (DAT)*			
	3 Days**	5 Days***	7 Days***	10 Days***
10 <sup>9</sup>	10.00 (3.16)	28.33 (32.15)	45.00 (42.13)	71.66 (57.79)
10 <sup>8</sup>	11.66 (3.41)	40.00 (39.23)	66.66 (54.73)	81.66 (64.64)
10 <sup>7</sup>	18.00 (4.24)	71.66 (57.87)	78.33 (62.25)	92.33 (73.92)
10 <sup>6</sup>	3.33 (1.82)	16.66 (24.08)	26.66 (31.04)	36.66 (37.26)
10 <sup>5</sup>	0.00 (00)	10.00 (18.43)	20.00 (26.56)	31.66 (34.24)
Control	0.00 (00)	0.00 (00)	0.00 (00)	0.00 (00)
SE(m)±	1.42	2.04	2.04	1.89
CD (P=0.01)	5.84	8.36	8.36	7.76

\*Average of three replications,

\*\* Figures in parenthesis are square root mean transformed value,

\*\*\* Figures in parenthesis are arcsin transformed value.



Eggs mass



2<sup>nd</sup> instar larvae



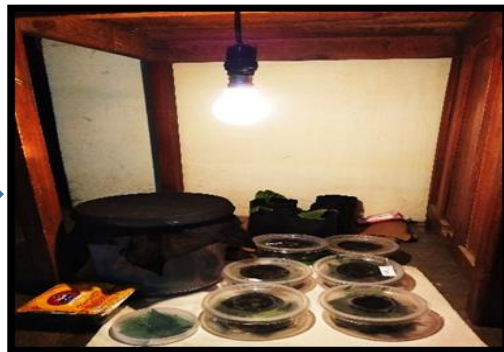
Pupation



4<sup>th</sup> instar larvae



Adult



Temperature maintain

Plate 1: Rearing of *Spodoptera litura*



Plate 2: Pathogenicity studies on 2<sup>nd</sup> instar larvae of *Spodoptera litura* by *Beauveria bassiana*



Healthy larvae of *S. litura*    Larvae infected by *B. bassiana*    Growth of *B. bassiana* on larvae

Plate 3: Effect of *B. bassiana* on the growth and development of *S. litura* larvae

The test concentrations taken up for the study include  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$  and  $10^9$  spores per ml and control was maintained. The second instar larvae of *S. litura* were taken separately in sterile petriplates were directly sprayed with 2 ml spore suspension were maintained with 10 larvae in each replication. Providing fresh castor leaves daily to *S. litura*. The experiment was conducted at room temperature of 25°C and relative humidity of 95 per cent.

The effect of different spore concentrations of *B. bassiana* on the second instar of *S. litura* was evaluated and were presented in the (table 3). Perusal of the data obtained in the experiment carried out for studying pathogenicity of *B. bassiana* against *S. litura* indicated that, significant increase in per cent mortality when compared to untreated (0.0 per cent) was recorded at  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^6$  and  $10^5$  spores per ml was 92.33, 81.66, 71.66, 36.66 and 31.66 per cent respectively (plate 2). Highest larval mortality of 92.33 per cent was noticed in the treatment where concentration of *B. bassiana* was  $10^7$  spores per ml which was significantly different from untreated control (0.0 per cent), followed by 81.66 per cent at  $10^8$  spores per ml concentration. Least larval mortality of 31.66 per cent was noticed at a test concentration of  $10^5$  spores per ml. Mortality of 36.66 and 71.66 per cent was observed when *B. bassiana* was used at  $10^6$  and  $10^9$  spores per ml concentration, respectively.

This was evidenced by the fact that  $1 \times 10^7$  spores per ml concentration of *B. bassiana* in general was found promising in terms of increased per cent larval mortality, reduced pupation and reduced per cent adult emergence of both *S. litura* and *P. xylostella*. Fatima *et al.* (2017). The results obtained in the earlier investigations carried out by Sivasankaran *et al.* (1990) are in accordance with the present findings. In the above mentioned study it was clearly mentioned that spore load of  $1 \times 10^7$  spores per ml recorded higher larval mortality while  $1 \times 10^5$  spores per ml recorded the least mortality. This was consonance with Moorthi *et al.* (2011) reported that the isolates of *B.*

*bassiana* were evaluated against *S. litura* using leaf spray method. Mortality of 66.67, 73.33 and 80.0 per cent with respect to the isolates Bb2, Bb9 and Bb10, respectively. The isolate Bb10 was the most virulent with potential for the management of *S. litura*.

#### 4.3 Cultural studies of *B. bassiana*

**Table 4. Different media tested for growth and sporulation of effective isolate of *Beauveria bassiana***

Sr. No.	Particulars of medium	Colony diameter after 10 days (mm)	Sporulation after 10 days	Remark
1	Sabouraud's Dextrose Agar (SDA) medium	41.50	++++	Abundant sporulation
2	Potato Dextrose Agar (PDA) medium	36.50	+++	Good sporulation

The growth and sporulation of virulent isolate of *Beauveria bassiana* (Bb3) was studied on two different solid media such as SDA and PDA on 10<sup>th</sup> days after incubation (plate 4). Observation were recorded and result were presented in (table 4). The highest radial growth of *Beauveria bassiana* was observed in SDA media (41.50 mm) as abundant sporulation and the least growth was observed in PDA media (36.50 mm) as good sporulation

This was in consonance with Deb *et al.* (2017) reported that the maximum colony diameter of 45.33 mm was observed in SDA followed by SDB (43.00 mm). This was in conformity with Senthamizhlesvan *et al.* (2010) who reported that SDA media were found to be suitable for radial growth and sporulation of *Beauveria bassiana*. According to Shanmugam *et al.* (2017) maximum growth *B. bassiana* has supported by SDA medium by recording the mycelial growth of 85.3 mm.

#### 4.4 Morphological characters of *B. bassiana*

Microscopic observations of conidia show that the different isolates cultured on SDA medium do not differ in shape. The hyaline,

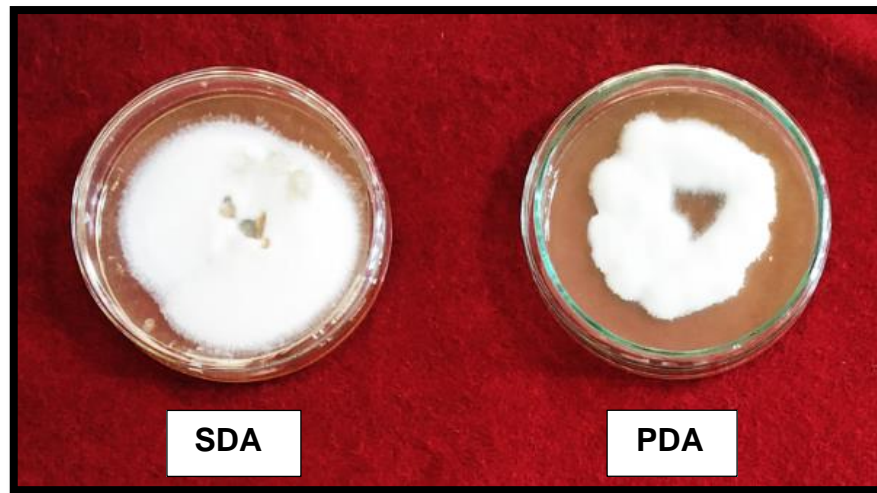
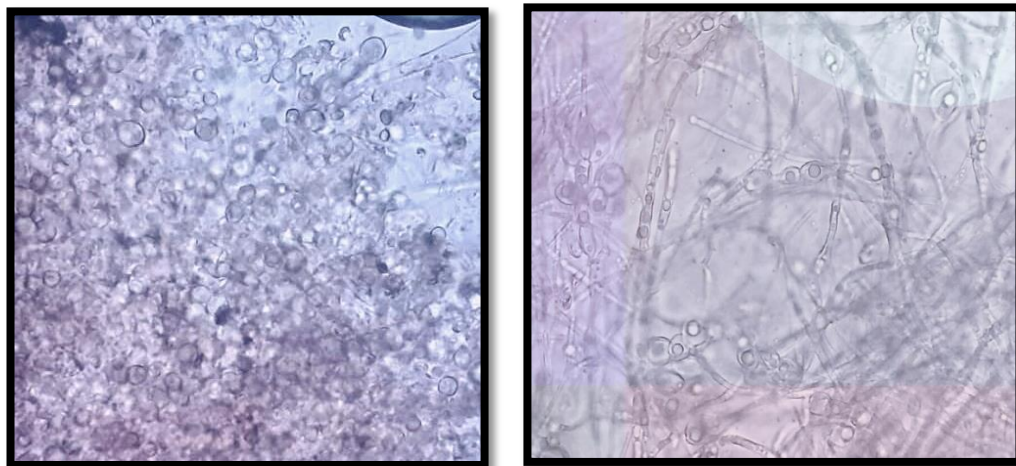


Plate 4: Different media tested for growth and sporulation of effective isolate of *Beauveria bassiana*



(A)

(B)

Plate 5: (A) Conidia of *B. bassiana* and (B) germinated conidia with 45x magnification

smooth walled conidia were globose to sub-globose in shape in all the 16 isolates studied. The size of conidia varied from 1.80 - 2.50  $\mu\text{m}$  x 1.70 2.40  $\mu\text{m}$  between different isolates. Variation was however also observed in size of conidia within the isolates.

The detailed morphological studies of effective isolate (Bb3) showed that the fungus grew well on SDA. Mycelium on this medium, appeared white, dense and powdery. The colonies appeared raised in the center and developed radial furrows. The culture was turned yellowish white and powdery type. Spore structure was globose to sub-globose (plate 5).

#### 4.5 Effect of temperature

**Table 5. Effect of different temperature regimes tested for growth and sporulation of *Beauveria bassiana***

Sr. No.	Temp.(°C)	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	20	35.50	+++	Good sporulation
2	25	41.50	++++	Abundant sporulation
3	30	38.50	+++	Good sporulation
4	35	32.00	++	Fair sporulation
SE(m)±		0.80		
CD (P=0.01)		3.52		

\*Average of three replications

The levels of temperatures (20, 25, 30, and 35°C) were tested. The petriplates containing the inoculated media were incubated at respective temperatures in an incubator. The fungal growth was assessed on the basis of colony diameter.

In general, *Beauveria bassiana* grew at a wide temperature range from 20°C to 35°C. The radial growth and sporulation were affected by temperature observation recorded and result were



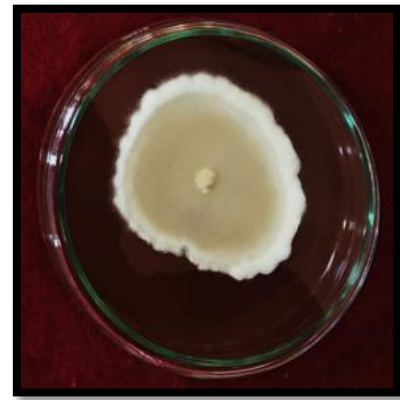
20° C



25° C

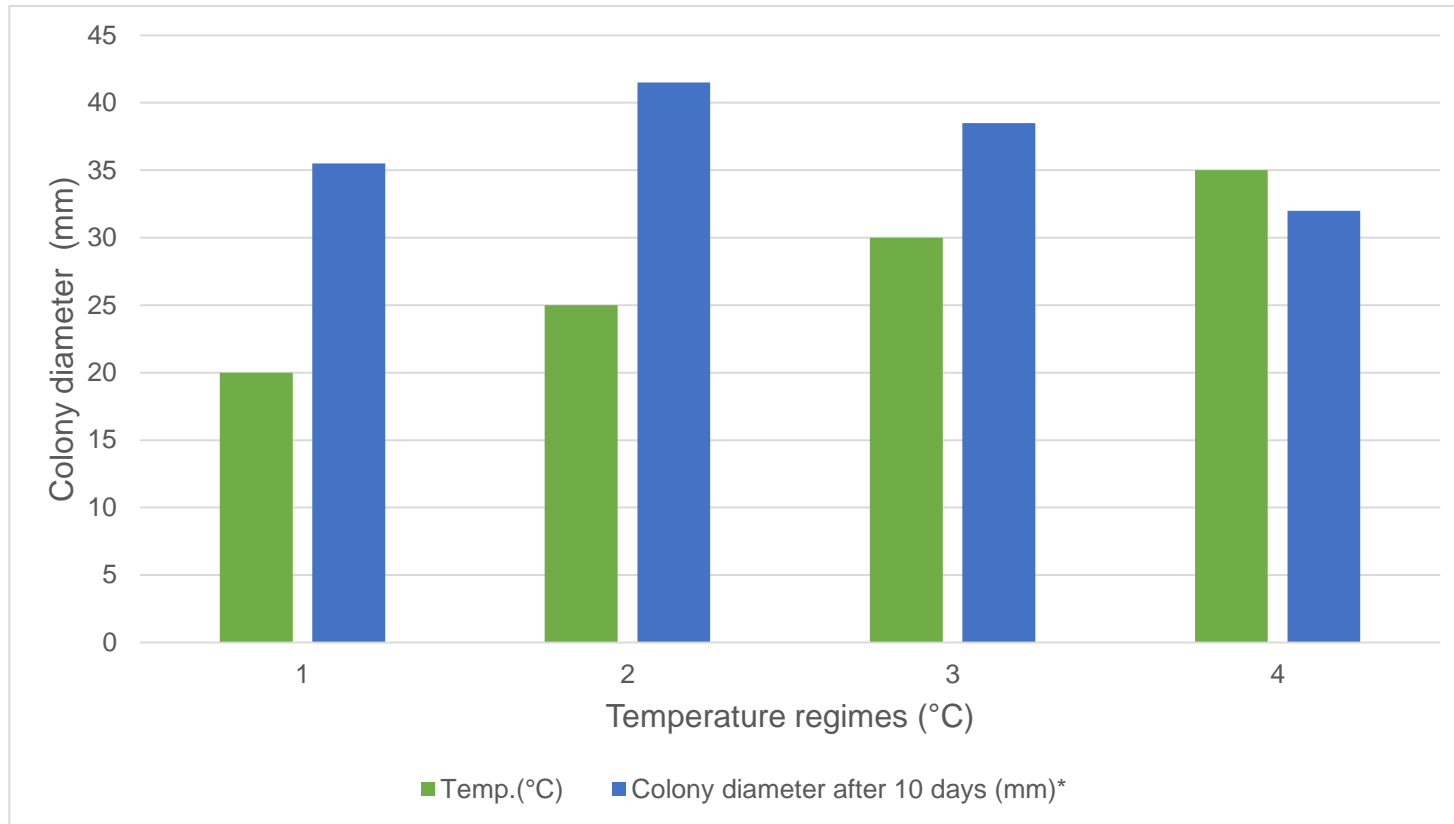


30° C



35° C

**Plate 6: Effect of different temperature regimes tested for growth and sporulation of *Beauveria bassiana***



**Fig1: Effect of different temperature regimes on growth and sporulation of *Beauveria bassiana***

presented in (table 5). The maximum radial growth 41.50 mm and abundant sporulation was recorded at temperature 25° C. The least radial growth 32.00 mm and fair sporulation was at temperature 35° C (plate 6).

This was in consonance with Ahmad *et al.* (2016) studied on the growth rate, spore production and spore germination of two different isolates of the fungus *B. bassiana*. The optimum temperature was 25°C without significant differences. Ying and Feng (2006) studied that the conidia produced on media sabouraud dextrose medium at 15-30°C. The culture conditions were optimized as 25°C. This was in line with Hallsworth *et al.* (1996) *Beauveria bassiana* were studied under the temperature (5 to 35°C) were determined. The growth was optimal between 20°C and 35°C depending on the isolate.

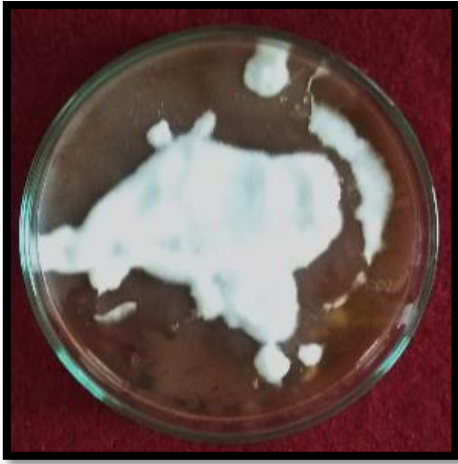
#### 4.6 Effect of humidity levels

**Table 6. Effect of different humidity levels tested for growth and sporulation of *Beauveria bassiana***

Sr. No.	Relative humidity levels %	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	80	31.38	+++	Good sporulation
2	85	38.83	++++	Abundant sporulation
3	90	40.00	++++	Abundant sporulation
4	95	41.66	++++	Abundant sporulation
SE(m)±		0.47		
CD (P=0.01)		2.08		

\*Average of three replications

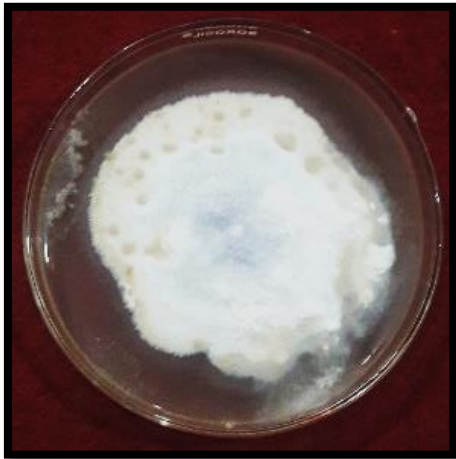
The inoculated petriplates were exposed to 80, 85, 90, and 95 per cent relative humidity levels in desiccators of uniform size. The humidity levels in desiccators were maintained by using concentrated sulphuric acid + water.



80% RH



85% RH

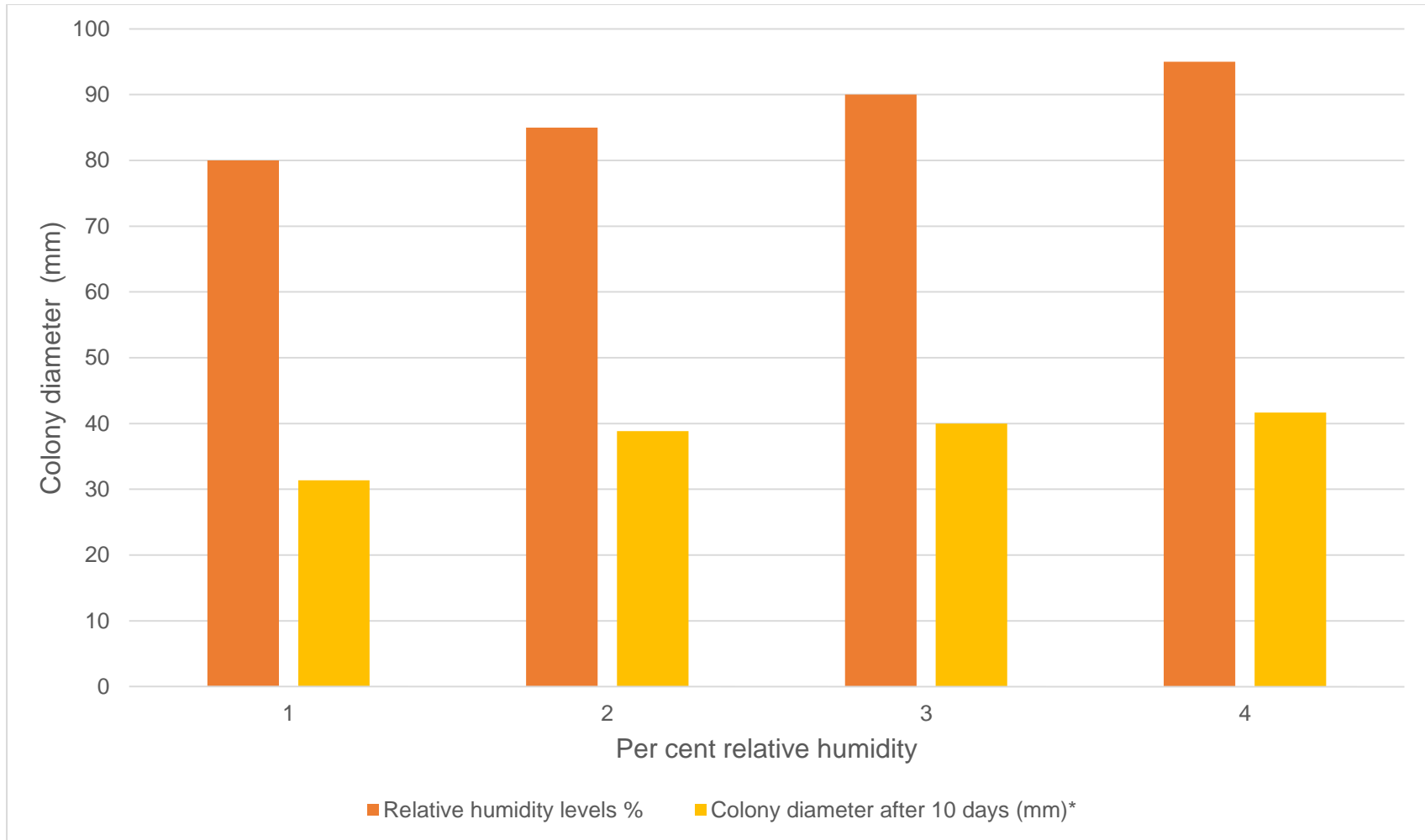


90% RH



95% RH

**Plate 7: Effect of different humidity levels tested for growth and sporulation of *Beauveria bassiana***



**Fig 2: Effect of different humidity levels on growth and sporulation of *Beauveria bassiana***

At 95% relative humidity, highest radial growth of 41.66 mm was recorded till 10<sup>th</sup> day of incubation with abundant sporulation, result were presented in (table 6). Lowest radial growth of 31.38 mm and god sporulation was observed 10<sup>th</sup> day of incubation (plate 7).

Shipp *et al.* (2003) investigated the influence of different humidity levels on per cent infection by *Beauveria bassiana* on greenhouse insect. In petridish trials, a humidity of 97.5 per cent relative humidity resulted in significantly higher percentage of infection (60-88.8 per cent). This was consonance with Mishra *et al.* (2015) reported during assay on adult housefly, 100 per cent mortality was achieved at relative humidity, 90 per cent and 100 per cent. Lethal time, LT<sub>50</sub> was 2.9 days at 100 per cent relative humidity.

#### 4.7 Effect of pH levels

**Table 7. Effect of different pH levels tested for growth and sporulation of *Beauveria bassiana***

Sr. No.	pH	Colony diameter after 10 days (mm)*	Sporulation after 10 days	Remark
1	6.0	41.15	++++	Abundant sporulation
2	6.5	39.66	++++	Abundant sporulation
3	7.0	36.33	++	Fair sporulation
4	7.5	33.55	+	Moderate sporulation
SE(m)±		1.25		
CD (P=0.01)		5.52		

\*Average of three replications

The pH levels (6.0 to 7.5) were tested by adding 1 N HCl and 1 N NaOH with the help of indicator paper prior to autoclaving. Fungal growth was assessed in the form of radial growth in mm.

The effect of pH of medium on radial growth and sporulation was assessed using SDA as the basal medium and the results are presented (table 7). The highest radial growth of 41.15 mm was



**pH 6.0**



**pH 6.5**

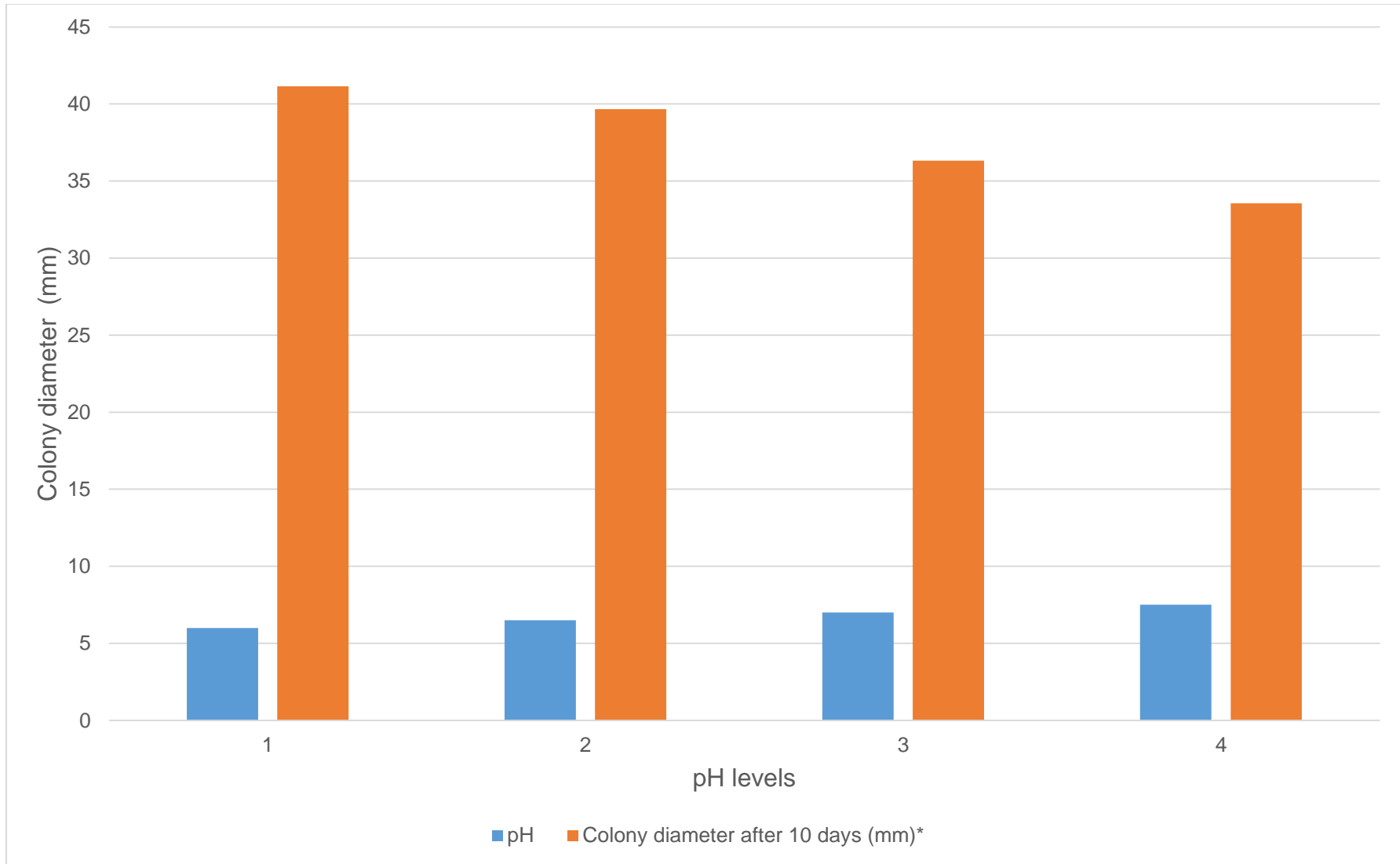


**pH 7.0**



**pH 7.5**

**Plate 8: Effect of different pH levels tested for growth and sporulation of of *Beauveria bassiana***



**Fig 3: Effect of different pH levels on growth and sporulation of *Beauveria bassiana***

achieved after 10<sup>th</sup> days at pH of 6.0 with abundant sporulation. The lowest radial growth of 33.55 mm and sporulation were observed at pH 7.5 (plate 8).

According to Pham, *et al.* (2009) the optimal initial pH for the spore production of *B. bassiana* using potato dextrose broth was 5.2. Similarly Karthikeyan *et al.* (2008) studied optimum pH and growth pattern of *Beauveria bassiana*. The pH of 6.0-8.0 was found to be the most suitable for growth and spore formation. This was accordance with Ying and Feng (2006) studied that conidia produced on media sabouraud dextrose medium at pH 4.0-8.0, the culture conditions were optimized as pH 5.0-6.0.

#### 4.8 Compatibility of *B. bassiana* with selective insecticides

**Table 8. Effect of selective insecticides on radial mycelial growth of *Beauveria bassiana***

Sr. No.	Treatment	Conc. (%)	Radial mycelial growth after 7 days in (mm)*	Per cent growth inhibition
1	Imidacloprid 200 SL	0.0045	29	27.25
2	Spinosad 45 SC	0.018	24	40
3	Chloropyriphos 20 EC	0.05	9.12	77.20
4	Indoxacarb 14.5 SC	0.0145	15.66	60.85
5	Control	-	40	-
SE(m)±			0.59	
CD (P=0.01)			2.52	

\*Average of three replications

The effect of insecticides were tested at recommended dose concentrations on radial growth of *B. bassiana*. For each treatment 100 ml autoclaved sabouraud dextrose agar medium was taken in 250 ml conical flask. The specified concentrations of insecticide was added into three petriplates and allowed to solidify. Test fungus culture was cut in to 5 mm disc from the periphery of 10 day old pure culture with

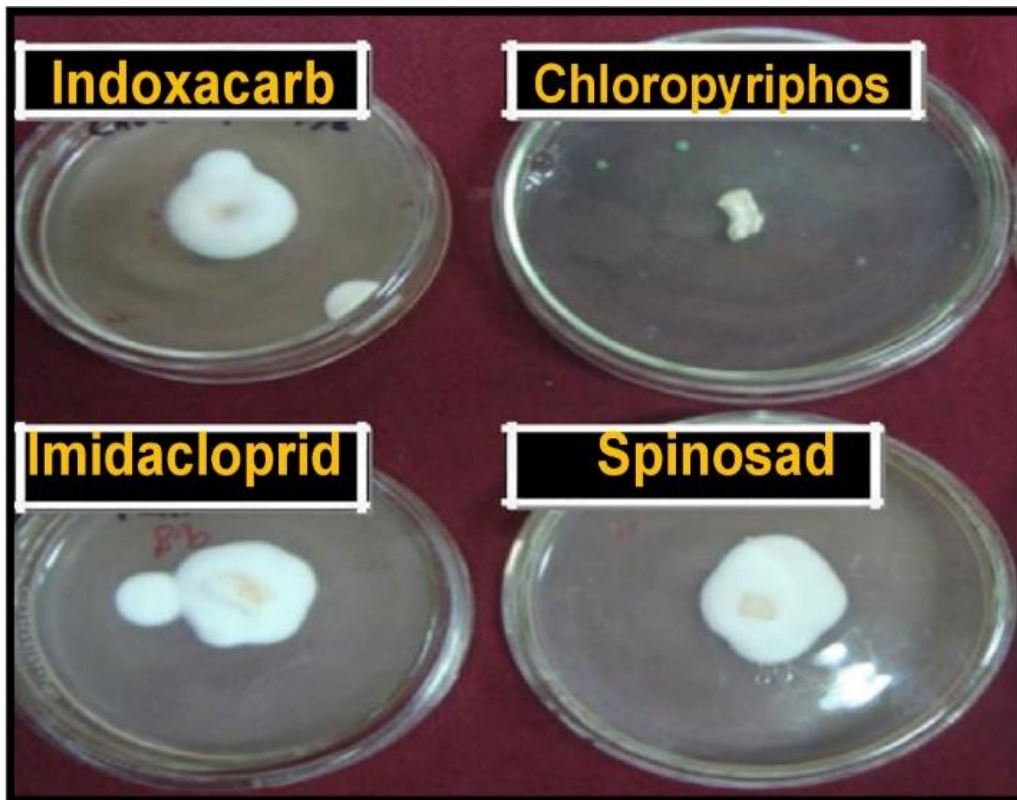


Plate 9: Effect of selective insecticides on radial mycelial growth of *Beauveria bassiana*

sterilized cork borer and transferred to the centre of each of the plates containing poisoned medium. The controls plates were maintained by placing fungal disc in medium without insecticides. All the plates were incubated at 25° C.

The isolate of *B. bassiana* were found compatible with the insecticide imidacloprid when compared to other insecticides spinosad, chlorpyrifos and indoxacarb by recording 27.25 per cent of least inhibition of growth. Chlorpyrifos was found to be highly incompatible with *B. bassiana* and exhibited high inhibition of growth with 77.20 per cent (table 8) and (plate 9).

The results of the present study suggest imidacloprid can be used with *B. bassiana* in pest management. This combination would give an added advantage where the insecticide pathogen mixtures introduce multiple mortality factors against the target pes. The insecticide making insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens. It also delay the chances of expression of resistance to new insecticides.

The results concur with the findings of Rajanikanth *et al.* (2010) reported that the *B. bassiana* was compatible with imidacloprid. Chlorpyrifos was found to be incompatible with all the strains. According to Abidin *et al.* (2017) reported that the highest conidial production of the fungi was triggered by imidacloprid 0.5 x DF. Based on compatibility calculation, imidacloprid 0.5 x DF worked with *B. bassiana* (BI: 67.77). This was similar to Alizadeh *et al.* (2007) studied the compatibility of *B. bassiana* with imidaclopride, flufenoxuron, teflubenzuron + phuzalon, endosulfan and amitraz. The compatible formulation with *B. bassiana* (isolate DEBI008) was imidacloprid.

## Chapter V

### SUMMARY AND CONCLUSIONS

Experiment were carried out on “Cultural and morphological characterizations of *Beauveria bassiana*” the results obtained are summarized herein.

*Beauveria bassiana* was present in the nature. Out of 10 diseased larvae collected from the field, all larvae were selected for isolation of the entomopathogenic fungi. These fungal cultures were developed and finally identified as *Beauveria bassiana*. The mummified bodies of lepidopteran pest larvae with symptoms of fungal infection were collected from the field conditions from different places. After surface sterilization, collected cadavers were placed individually in sterilized petridishes on sterilized water soaked tissue papers in the BOD at 25°C temperature and 85±5 per cent relative humidity for development of mycelial growth on the cadavers. The cadavers showing whitish mycelial growth, which is the symptom of infection by *B. bassiana*, were selected for isolation of the fungus.

The pathogen *B. bassiana* isolates were developed from selected cadavers in petridish containing potato dextrose agar media. The culture so obtained identified was at Plant Pathology Section, College of Agriculture, Nagpur.

The detailed morphological studies of effective isolate (Bb3) show that the fungus grew well on SDA (sabouraud dextrose agar). The mycelium on this medium, appeared white, dense and powdery. The colonies appeared raised in the center and developed radial furrows. The culture was turned yellowish white and powdery type. Spore structures was globose to sub-globose.

Two different semi synthetic media namely sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were evaluated for their suitability to support the mycelial growth and spore production of the

entomopathogenic fungus *B. bassiana* in the laboratory. The colony diameter and sporulation was measured after 10<sup>th</sup> day of inoculation, better growth of *B. bassiana* was observed on SDA medium (41.50 mm). The rapid radial growth of *Beauveria bassiana* in SDA medium was also consistent with virulence and this can be used to biological control pests in the country and the region.

Significantly higher hyphal growth was however, seen in media with pH values between 6.0 and 6.5 and temperature range of 20 to 30°C and relative humidity range 90 per cent to 95 per cent. On the basis of these results, pH value 6.0, temperature of 25°C and 95 per cent relative humidity was considered as optimum for growth and sporulation of *B. bassiana*. In conclusion, this study assessed the effect of various parameters temperature regimes, relative humidity and pH levels on the radial growth and sporulation of *B. bassiana* in SDA medium and maximize their performance in biological control of integrated pest management (IPM) practices.

In terms of larval mortality, 10<sup>7</sup> spores per ml was found to be most promising concentration, recording highest mortality in case of *S. litura* followed by 10<sup>8</sup> spores per ml, least larval mortality was observed at 10<sup>5</sup> spores per ml. The symptoms of *Spodoptera litura* larvae infected by *Beauveria bassiana* was decreasing movement and eating activity. Result obtained for treatment of the second instar larvae of *S. litura* treated with *B. bassiana* for eco-friendly management of the insect pest population. This fungus also be included successfully in integrated pest management (IPM).

Compatibility of commonly used insecticides imidacloprid, spinosad, chlorpyrifos and indoxacarb with *B. bassiana* was tested by poison food technique *in vitro*. Imidacloprid were non-toxic to *B. bassiana*, as no significant reduction in radial growth was noticed. The insecticide chlorpyrifos recorded 77.20 per cent inhibition of growth and was toxic to *B. bassiana*. The results of the present study suggest imidacloprid can be used with *B. bassiana* in pest management. This

combination would give an added advantage where the insecticide pathogen mixtures introduce multiple mortality factors against the target pest. The insecticide making insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens. It also delay the chances of expression of resistance to new insecticides. Isolate Bb3 proved to be more promising for development as mycopesticides and considered as an integral part of plant protection has to meet the requirement of compatibility with insecticides recommended for pest management. The application of entomopathogenic fungi for insect control is increasing largely because of, greater environmental awareness and food safety concerns. The failure of conventional chemicals due to an increasing number of insecticide resistant species. Entomopathogenic fungi provides us significant and selective insect control.

## Chapter VI

### LITERATURE CITED

- Abidin, A. F., N. Ekowati and N. I. Ratnaningtyas, 2017. Insecticide compatibility to the entomopathogenic fungi *Beauveria Bassiana* and *Metarhizium Anisopliae*. Scripta Biologica., **4**(4): 273-279.
- Afandhi, A., S. R. C. Syamsidi, S. M. Mimbar and B. Wirotmodjo, 2012. Isolation and phenotypic characterization of morphology in fungus *B. bassiana* (Balsamo) Vuill. colony naturally from leaf surface, soil and insect as host in tomato plantation. Agrivita. **34**(3): 303-310.
- Ahmad, M. A., I. Ghazal and L. H. Raja, 2016. Laboratory evaluation of the temperature and several media on the radial growth, conidia production and germination of the fungus *Beauveria Bassiana* (Bals.) Vuil. SSRG Int. J. Agri. Env. Sci., **3**(6): 35-41.
- Alalil, S., V. Mereghetti, F. Faoro, S. Bocchi, F. A. Azmeh and M. Montagnal, 2019. Thermotolerant isolates of *Beauveria bassiana* as potential control agent of insect pest in subtropical climates. J. pone.22: 31-39.
- Alizadeh, A., M. A. Samih, M. Khezri and R. S. Riseh, 2007. Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. Int. J. Agri. Biol., **9**(1): 31-34.
- Amutha, A., J. G. Banu, T. Surulivelu and N. Gopalakrishnan, 2010. Effect of commonly used insecticides on the growth of white muscardine fungus, *Beauveria bassiana* under laboratory conditions. J. Biopesticide., **3**(1): 143-146.
- Anderson, T. E. and D. W. Roberts, 1983. Compatibility of *B. bassiana* isolate with insecticides formulations used in colorado potato beetle (coleopteran: chrysomelidae) control. J. Econ. Entomol., **76**: 1437-1441.
- Aneja, K. R. 1996. Experiment in microbiology, plant pathology and tissue culture. Wishwaprakasham, New Delhi. 31.
- Castrillo, L. A., L. S. Bauer, H. Liu, M. H. Griggs and J. D. Vandenberg, 2010. Characterization of *Beauveria bassiana* isolates associated with *Agrilus planipennis* populations in Michigan. Bio. control. **54**: 135-140.
- Deb, L., R. Thangaswamy and M. Hajong, 2017. Growth of *Beauveria bassiana* in different solid media. Trends Biosci., **10**(23): 4815-4817.

- Debnath, M. 2015. Mass multiplication and bio-efficacy of *Beauveria bassiana* in controlling *Helicoverpa armigera*. PhD. (Agril. Entomology) Thesis (unpub.) Institute of Agriculture Visva-Bharati Sriniketan, West Bengal, 1-8.
- Diane, J. H., B. H. Kirkland and M. W. Lewis, 2007. Surface characteristics of the entomopathogenic fungus *Beauveria bassiana*. Microbiol., **153**: 3448-3457.
- Faraji, S., A. D. Shadmehri and A. Mehrvar, 2016. Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with some pesticides. J. Entomological Society Iran. **36**(2): 137-146.
- Fargues, J. M., S. Goettel, N. Smits, A. Ouedraogo and M. Rougier, 1997. Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. Mycologia., **89**: 383-392.
- Fatima, T., K. D. Reddy, M. G. Rafi, T. Umamaheswari and J. Sumalatha, 2017. Evaluation of pathogenicity of *B. Bassiana* against major lepidopteran pests, *Spodoptera Litura* and *Plutella Xylostella* in cabbage. Int. J., **12**(1): 959-963.
- Gangwar G. P., 2013. Evaluation of different substrates for mass multiplication of *Beauveria Bassiana* (Balsamo) Vuill. Agric. Sci. Digest., **33**(4): 321-323.
- Godonou, I., B. James, C. Atcha-Ahowe, S. Vodouhe, C. Kooyman, A. Ahanche and S. Korie, 2009. Potential of *Beauveria bassiana* and *Metarhizium anisopliae* isolates from benin to control *Plutella xylostella* L. Crop Protection., **28**: 220-224.
- Gupta, S .D and B. Kumar, 2014. Bio efficacy of *Beauveria bassiana* (Balsamo) against third instar larvae of *Spodoptera litura* (Far.). Int. J. Pl. Sci., **9**(1): 97-100.
- Hallsworth, J. E. and N. Magan, 1996. Culture age, temperature, and p<sup>H</sup> affect the polyol and trehalose contents of fungal propagules. American Society Microbiol., **62**(7): 2435-2442..
- Hassanloui, R. T., A. K. Pakdel, M. Goettel and J. Mozaffari, 2006. Variation in virulence of *Beauveria bassiana* isolates and its relatedness to some morphological characteristics. Bicontrol. Sci. Tech., **16**(5): 525-534.
- Indriyanti, D. R., S. Mahmuda and M. Slamet, 2017. Effect of *Beauveria bassiana* doses on *Spodoptera litura* mortality. Int. J. Sci. Tech. Res., **6**(9): 206-210.
- Ismail, M., W. Wakill, N. H. Bashir, W. Hassan and U. W. Muhammad, 2017. Entomocidal effect of entomopathogenic fungus

*Beauveria bassiana* and new chemistry insecticides against *Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera) under controlled conditions. Int. J. Agri. Inn. Res., **5**(6): 2319-1473.

Karthikeyan, A., V. Shanthi and A. Nagasathya, 2008. Effect of different media and pH on the growth of *Beauveria bassiana* and its parasitism on leaf eating caterpillars. Res. J. Agri. Bio. Sci., **4**(2): 117-119.

Kaur, S., H. P. Kaur, K. Kaur and A. Kaur, 2011. Effect of different concentrations of *Beauveria bassiana* on development and reproductive potential of *Spodoptera litura* (Fabricius). J. Biopesticides. **4**(2): 161-168.

Kim, J. S., M. Skinner, S. Gouli and B. L. Parker, 2011. Generating thermotolerant colonies by pairing *Beauveria bassiana* isolates. FEMS Microbiol. Lett., **324**: 165-172.

Kotwal, S., R. L. Parate, S. S. Mane, and V. V. Deshmukh, 2012. Influence of nutrition and different physical parameters on the growth and sporulation of *Metarhizium anisopliae*. Int. J. Sci., Environ. Tech., **5**: 479-484.

Kulu, I. P., A. L. Abadi, A. Afandhi and Nooraidawati, 2015. Morphological and molecular identification of *Beauveria bassiana* as entomopathogen agent from central Kalimantan Peatland, Indonesia. Int. J. Chem. Tech. Res., **8**(4): 2079-2084.

Kumar, R. and A. Kumar, 2017. Growth rate in media and pathogenicity of two species of entomopathogens found in Fiji. J. South Pacific Agri., **20**: 44-52.

Mabelle, J. C. R., M. G. M. Blanco, J. J. H. Escareno, L. J. G. Wong and C. F. S. Coronado, 2011. Study of *Beauveria bassiana* growth, blastospore yield, desiccation tolerance, viability and toxic activity using different liquid media. African J. Biotechnol., **10**(30): 5736-5742.

Malrvannan, S., P. D. Murali, S. P. Shanthkumar, V. R. Prabavathy and S. Nair, 2010. Laboratory evaluation of the entomopathogenic, *Beauveria bassiana* against the tobacco caterpillar, *Spodoptera litura* fabricius. J. Biopesticides. **3**(1): 126-131.

Marcos, R. F. and P. S. Wraight, 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Sci. Dir. Biol. Control. **43**: 237-256.

Mishra, S., P. Kumar and A. Malik, 2015. Effect of temperature and humidity on pathogenicity of native *Beauveria bassiana* isolate against *Musca domestica* L. J. Parasit Dis., **39**(4): 697-704.

- Moorthi, P. V., C. Balasubramanian and T. Kubendran, 2011. Efficacy of local isolates of *Beauveria bassiana* against *Spodoptera litura* (F.). J. Biol. Control. **25**(1): 22-25.
- Nadeau, M. P., G. B. Dunphy and J. L. Boisvert, 1996. Development of *Erynia conica* on the cuticle of the adult black flies *Simulium rostratum* and *Simulium decorum*. J. Invertebrate Pathol., **68**: 50-58.
- Padmavathi, J., K. U. Devi and C. U. M. Rao, 2003. The optimum and tolerance pH range is correlated to colonial morphology in isolates of the entomopathogenic fungus *Beauveria bassiana* – a potential biopesticide. World J. Microbial. Biotech., **19**: 469-477.
- Padmini, P. P. C. and V. Padmaja, 2010. Impact of different relative humidifies on *in vitro* growth and sporulation of entomopathogenic fungal isolates of *Beauveria* sp. Int. J. Pharmaceutical. Biol. Archives. **1**(4): 355-359.
- Pham, T. A., J. J. Kim, S. G. Kim and K. Kim, 2009. Production of blastospore of entomopathogenic *Beauveria bassiana* in a submerged batch culture. Mycobiol., **37**(3): 218-224.
- Prasad, A., N. Syed and S. Purohit, 2010. *Beauveria Bassiana* (Balsamo) Vuill. successful biopesticide against key pest *Helicoverpa Armigera* (Hubner). Int. J. Pharma. Bio. Sci., **1**(2): 1-7.
- Quintela, E. D. and C.W. McCoy, 1998. Synergistic effect of imidaclopride and two entomopathogenic fungi on the behavior and survival of larvae of the *Diaprepes abbreviates* in soil. J. Econ. Entomol., **91**: 110-122.
- Quintela, E.D and McCoy, C.W. 1997. Effect of imidacloprid on development locomotory response and survival of first instars of *Diaprepes abbreniatus*. J. Economic. Entomol., **90**: 988-995.
- Rajanikanth, P., G. V. Subbaratnam and S. J. Rahaman, 2010. Compatibility of insecticides with *Beauveria bassiana* (Balsamo) Vuillemin for use against *Spodoptera litura* Fabricius. J. Bio. Control. **24**(3): 238-243.
- Rajnikanth, P. 2007. Evaluation of strains of *Beauveria bassiana* Vuillemin to certain production parameters and virulence against *Spodoptera litura* Fabricus. Ph. D Thesis.(unpub) Acharay N.G Ranga Agricultural University, Hyderabad, India.
- Revathi, N., G. Ravikumar, M. Kalaiselvi, D. Gomathi and C. Uma, 2011. Pathogenicity of three entomopathogenic fungi against *Helicoverpa armigera*. J. Plant Pathol. Microbiol., **2**(4): 1-4.

- Rombach, M.C., R. M. Aguda, B. M. Shephard, and D. W. Roberts, 1986. Infection of rice brown plant hopper, *Nilaparvata lugens* by field application of entomopathogenic hyphomycetes. *Enviro. Entomol.*, 15: 1070-1073.
- Senthamizhselvan, P., J. Alice, R. P. Sujeetha and C. Jeyalakshmi, 2010. Suitable medium for fungal production growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. *J. Biopesticides*. 3(2): 466-469.
- Shakir, H. U., M. Saeed, N. A. Anjum, A Farid, I. A. Khan, M. Liaquat and T. Badshah, 2015. Combined effect of entomopathogenic fungus *Beauveria bassiana*, imidacloprid and potassium silicate against *Cnaphalocrocis medinalis* Guenée in rice crop. *J. Entomol. Zool Studies*. 3(4): 173-177.
- Shanmugam V. and P. Seethapathy, 2017. Isolation and characterization of white muscardine fungi *Beauveria bassiana* (Bals.) Vuill. A causative of mulberry silkworm. *J. Entomol. Zoo. Studies*. 5(3): 512-515.
- Shipp, J. L., Y. Zhang, D. W. A. Hunt and G. Ferguson, 2003. Greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests, *Environ. Entomol.*, 32(5): 1154-1163.
- Singh, R., K. D. Verma, J. Singh, R. Nigam, M. Singh and A. Kumar, 2017. Multiplication of entomopathogenic fungus *Beauveria bassiana* (Balsamo) on solid and liquid media. *J. Pharmacognosy. Phytochemistry*. 345-351.
- Sivasankaran, P., S. Easwaramoorthy and H. David, 1990. Pathogenicity and host range of *Beauveria bassiana*, a fungal pathogen of *Chilo infuscatellus* Snellen. *J. Biol. Control*. 4(1): 48-51.
- Soloman, M. E., 1951. Control of humidity with potassium hydroxide, sulphuric acid or other solution, *Bull. Ento. Res.*, 42: 543-554.
- Swathi, P., P. N. G. Visalakshy and S. B. Das, 2017. Potentiality of *Beauveria bassiana* strains against *Helicoverpa armigera* through laboratory bioassay. *J. Ento. Zoo. Studies*. 5(3): 463-667
- Ummidi, V. R. S., U. Josyula and P. Vadlamani, 2013. Germination rates of *Beauveria bassiana* and *Metarhizium anisopliae* its possible correlation with virulence against *Spodoptera litura* larvae. *Int. J. Adv. Res.*, 2(1): 625-630.
- Usha, J., M. N. Babu and V. Padmaja, 2014. Detection of compatibility of entomopathogenic fungus *Beauveria bassiana* (bals.) Vuill.

with pesticides, fungicides and botanicals. Int. J. Plant Ani. Env. Sci., **4**(2): 613-624.

Vijayavani, S., K. R. K. Reddy and G. B. V. N. Murthy, 2009. Pathogenicity of *Beauveria bassiana* strains on *Spodoptera litura* (Fab.). J. Biopesticides. **2**(2): 205-207.

Wagner, B. L., and L. C. Lewis, 2000. Colonization of corn, zea mays, by the entomopathogenic fungus *Beauveria bassiana*. American Society Microbial., **66**(8): 3468-3473.

Wang, J and C. Zheng, 2011. Characterization of a newly discovered *Beauveria bassiana* isolate to *Frankliniella occidentalis* Perganda, a non-native invasive species in china. Microbiol. Res., **167**: 116-120.

Ying, S. H. and M. G. Feng, 2006. Medium components and culture conditions affect the thermotolerance of aerial conidia of fungal biocontrol agent *Beauveria bassiana*. Letters in Applied Microbiol., **43**: 331-335.

Younas, A., W. Wakil, Z. Khan, M. Shaabanb and S. M. Prager, 2016. The efficacy of *Beauveria bassiana*, jasmonic acid and chlorantraniliprole on larval populations of *Helicoverpa armigera* in chickpea crop ecosystems. Pest Manage. Sci., **73**: 418-424.

## VITA

1. **Name of Student** : Wargane Vilas Sukhadeo
2. **Date of Birth** : 23/09/1995
3. **Name of the College** : College of Agriculture, Nagpur
4. **Residential Address** : At- Sawangi, Tq Post- Jaffrabad; Dist-  
Jalna  
Pin code- 431208.  
Mo.No. 8805165461.  
Email- vilaswargane33@gmail.com

5. **Academic qualifications** :

Sr. No.	Name of degree awarded	Year in which obtained	Division / Class	Name of awarding University/Board	Subjects
1.	B.Sc. (Agri)	2017	First	Dr.PDKV, Akola	Agriculture
2.	H.S.C.	2013	First	Amravati	Science
3.	S.S.C.	2011	First	Aurangabad	General

6. **Field of interest** : Research in Agriculture

**Place:** Nagpur

**Date:** / /2019

**Signature of Student**