PREPARATION AND EVALUATION OF FORMULATIONS OF ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (BALSAMO) VUILLEMIN AGAINST LEPIDOPTERAN INSECT PESTS

Thesis

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By

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CERTIFICATE

This is to certify that the thesis entitled **"PREPARATION AND EVALUATION OF FORMULATIONS OF ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (BALSAMO) VUILLEMIN AGAINST LEPIDOPTERAN INSECT PESTS"** submitted in partial fulfillment of the requirements for the degree of **Master of Science in Agriculture** with major in **Entomology,** of the College of Post-Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Mr. Rajnish Rai, Id. No. 52524,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Pantnagar
July, 2019

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CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Mr. Rajnish Rai, Id. No. 52524,** a candidate for the degree of **Master of Science in Agriculture** with major in **Entomology**, agree that the thesis entitled **"PREPARATION AND EVALUATION OF FORMULATIONS OF ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (BALSAMO) VUILLEMIN AGAINST LEPIDOPTERAN INSECT PESTS"** may be submitted in partial fulfillment of the requirements for the degree.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Per cent
@	at the rate of
B. bassiana	*Beauveria bassiana*
B.O.D.	Biological Oxygen Demand
CD	Critical Difference
CD (p=0.05)	Critical Difference at 5 percent level
CFU	Colony Forming Units
cm	centi meter
CPD	Critical Point Drier
CV	Coefficient of Variation
DMRT	Duncan's Multiple Range Test
EPF	Entomopathogenic fungi
et al.	and others
Fig	Figure
g	gram
G. mellonella	*Galleria mellonella*
g^{-1}	Per gram
HAT	Hours after treatment
HCHO	Formaldehyde
hr	Hour
i.e.	that is
IPM	Integrated Pest Management
L. A. F. C.	Laminar air flow chamber
l^{-1}	Per liters
mg	milli gram
ml^{-1}	Per milli liters
mm	milli meter
mm	milli meter
^{o}	Degree
^{o}C	degree celcius

^{o}E	degree east
^{o}N	degree north
pH	Power of hydrogen ion concentration
psi	per square inch
RH	Relative Humidity
S. litura	*Spodoptera litura*
SEM	Scanning Electron Microscopy
Sem	Standard error of mean
UV	Ultraviolet radiation
viz.	namely
µ	micron
µl	micro litter
µm	micro meter

Introduction

Chapter 1 INTRODUCTION

Among the different segments of Indian economy, Agriculture assumes a noteworthy job, as 54.60 per cent of the population is occupied with agriculture and allied activities and it contributes about 17.41 per cent to the nation's Gross Value Added for the year 2016-17, (at current Maximum Support Price) **(Anonymous, 2018).** The utilization of high-yielding, dwarf and semi-dwarf varieties of crops, and expanded utilization of water system and agrochemicals favored the development of crop pests. This has brought about increment in the force of a few pests, numerous minor nuisances expecting the status of major and a few new pests issue have shown up in specific locales **(Dhaliwal *et al.,* 2010).** Yield efficiency is in danger because of the rate of pests, particularly weeds, pathogens and animal pests **(Oerke, 2006).** The harm brought about by insects pest is one among the most significant components for lessening the productivity of crop plants **(Pimentel, 1976).** Insects being most conspicuous invertebrates, cause an annual economic losses of 15.7 per cent to the Agricultural crops **(Dhaliwal *et al.*, 2015)**.

Tobacco caterpillar, *Spodoptera litura* Fabricius (Order-Lepidoptera, Family-Noctuidae) is an important agricultural and horticultural crops pest. It is sporadic and polyphagous in nature, causing serious foliar damage and considered as one of the foremost coercion to the present intensive agriculture and altering cropping patterns worldwide, next only to *Helicoverpa armigera* Hubner. All over the world, it spoils more than 389 species of economic cultivated crop plants belonging to 109 families **(Lin *et al.,* 2019; Shankara Murthy *et al.,* 2006)** of which 40 genera are cultivated in India **(Basu, 1981; Muthukrishnan *et al.,* 2005)**. The host range of this pest is cabbage, cauliflower, radish, tomato, turnips, Lucerne, berseem, soybean, groundnut, maize, sunflower, potato, cowpeas, gram, cotton, castor and carrot and such more **(Lohar *et al.,* 2007)**. The outburst of this pest normally occurs with a good rainfall after a long dry spell **(Chelliah, 1985)**. *S. litura* can cause 10 to 30 per cent economic losses based on different crop phase and its invasion level in the field **(Cheng *et al.,* 2017)**. Because of their capability to impose severe economic injury to the crops, the pest has been subjected to heavy dose of insecticide applications. The wide spread and

blind use of synthetic insecticides has led to the expansion of resistance in insects. The level of resistance to various insecticides constitutes a serious risk to crop protection **(Rao and Dhingra, 2000)**.

Grater wax moth larvae, *Galleria mellonella* L. causes heavy damage to wax combs in storage of apiaries which leads to financial suffering every year. Thus, leads to a great economic losses reaching upto 60 to 70 per cent beekeepers in developing countries. Besides damaging wax combs, larval feeding also destroying the wooden parts and frames in Beehive. adult moth and Larvae both perform as a vector for a disease like foulbrood **(Swamy *et al.,* 2009).**

The synthetic, broad-spectrum insecticide has been acceptable and possible counsel for the managing of these insect-pests. However, the extensive use of these synthsetic chemicals pesticides became a menace to human health, natural flora, fauna,and even to the atmosphere **(Mahmoud *et al*., 2014).**Thus, in the place of synthetic chemicals, there is an urgent need to develop eco-friendly approaches, which improves the soil health and suppress the insect-pests' population. The only possible solution to this must be bioinsecticides. Bioinsecticides are the novel-insecticide products with a biological origin, which are based on entomopathogenic bacteria, viruses, nematodes, and fungi.The supreme property of these biological agents is their low persistence in the atmosphere and effectiveness in small quantities. Even they are safer to human and animal compared to synthetic insecticides pesticides. With proven potentials, these microbial agents bacteria, virus,nematodes, and fungi have got recognition as potential candidates for suppression of various insect pests but the exploitation of these microbial insecticides is meager and the contribution is negligible in the total crop protection interventions.Among different microbial agent, entomopathogenic fungi (EPF) taint there host by penetrating the insect exoskeleton **(De Faria and Wraight, 2007)**.

Basically, the EPF refer to those microorganisms that are able of infecting insects-pests, using them as hosts to complete their life cycle **(Delgado and Murcia, 2011)**. They are a group of phylogenetically miscellaneous, eukaryotic, heterotrophic, unicellular or multicellular (filaments) microorganisms which reproduce via sexual or asexual spores, or in cooperation and have chitinized cells and are generally non-mobile **(Badii and Abreu, 2006).** There are approx 1000 or more known species of

EPF grouped in the Neocallimastigomycota, Basidiomycota, Microsporidia, Chytridiomycota, Blastocladiomycota, Glomeromycota, and Ascomycota phyla. These phyla are known to infect and kill the insect-pests **(Kirk *et al*., 2001, 2008).** Fungi under these phyla can cause monolithic insect mortality under congenial abiotic conditions. These are versed with great breeding capacity. Normally, they can be found innature where they cause a phenomenal biological hazard to insect pests' population **(Evans, 1986; Mccoy *et al*., 1988).**

Most of the EPF have been isolated either from the dead larvae (cadaver) or from soil **(Meyling and Eilenberg, 2007).** The successful use of EPF as mycoinsecticide depends not only on its high efficacy against insect-pests but also on its non-pathogenic behavior towards non-target insect-pests **(Thungrabeab and Tongma, 2007).** For the commercial use of these mycoinsecticide, mass production is done on grains or synthetic media. As plethora of mass production technologies of EPF already exists in the market, solid and liquid fermentation techniques have been extensively utilized for the formulation of aerial conidia from the infectious bodies of the fungal pathogen **(Skinner *et al*., 2012).** The commercial productions of mycoinsecticides requirean input of economically per good quality inoculums as substrate and novel mass production technology for the maximum conidial production **(Burges and Hussey, 1981)**.

Many commercial formulations of EPF have been developed for crop insect-pest management. Among the 171 products of EPF developed, products based on *Metarhizium anisopliae* and *Beauveria bassiana* represent 33.92% of total products, and *Beauveria brongniartii* and *Isaria fumosorosea* products represented 5.81% and 4.10% respectively **(Moorhouse *et al*., 1992; De Faria and Wraight, 2007).**

Thus, this present investigation take interest in the preparation of EPF formulations and the generosity of giving an array of involvement in the endeavor aspect of formulations and their studies. Hence, the present study is aiming at the following objectives:

1) Mass multiplication of *Beauveria bassiana* on grains and synthetic media.
2) Preparation of various formulations of *Beauveria bassiana.*
3) Bioassay of the prepared formulation against insect pests.

Review of Literature

Chapter 2 REVIEW OF LITERATURE

Disastrous havoc about the synthetic chemical insecticides has resulted catastrophic atmosphere. Thus, it has been leading researchers to an enormous emphasis on alternative strategies for insect pests' population control which would be sustainable and safer to the eco-environmental organisms and nature. There is a global revival of interest in the use of insect entomopathogenic fungi (EPF) as the biological control agents. It is an important gain in the development of biological control agents and manufacturing of potential agents for present and future with the intervention of the most recent biotechnological techniques.

EPFs are phylogenetically diverse-heterotrophic and unicellular or multicellular (filaments) microorganisms. They commonly reproduce *via*. sexual or asexual spores, or both ways. EPF on insect acts as a facultative or obligate parasite with a capability of high sporulation and high survival rate on insect, using them as host for completion of life cycle **(Mora *et al*., 2017).**

2.1 History and systematic positioning of *Beauveria bassiana*

EPF, *B.bassiana* was the first disease causing micro-organisms which naturally infects Silkworm i.e. *Bombyx mori* L. (Order-Lepidoptera, Family-Bombycidae) and was reported in the year 1835 by Agostino Maria Bassi **(Glare and Milner, 1991).** Being a ubiquitous fungus having wider host ranges it occurs naturally and exist worldwide **(Feng *et al*., 1994).**

In recent years, about 90 genera and approximately above 700 species (sp.) were considered as insect infecting fungi that represents about the entire major classes of fungi. However, there is only about 20 EPF sp. which have been outstandingly studied for their use against insect pests of the agricultural crops. Most of them stimulate natural epizootics on insect pests' population. The EPFs have been observed to cause severe mortality in insect pest populations. Plethora of EPF sp. have been investigated for their potential use as an alternative resource to synthetic chemical pesticides, to control or as a part of the IPM programme in agricultural field **(Hajek and Leger, 1994; Moorhouse *et al*., 1992 and Hemshree, 2013)** and up to till date, over 750 fungi species are known to infect insect-pests and mites **(Ramanujam *et al*., 2014).**

B. bassiana is isolated from over more than 700 sp. of insect-pests from the 9 different order of insects and infestation occur most frequently in the order of Orthoptera, Hemiptera, Diptera, Lepidopteran, Coleopteran hosts **(Moore and Prior, 1996).** During infestation it produces conidia which about ≤3.5 µm smaller in diameter, spherical in shape, white in colour and it forms singly on denticles (**Humber, 1997).** Transportation of dry and single spore basically occurs with the aid of air **(Roberts and Humber, 1981).**

Old literature describes *B. bassiana* as to be an inmate to the sub-division of Deuteromycotina, class Hyphomycetes, in the order Moniliales. Hypomycetes is characterized by their naked asexual conidial spores which are free on the mycelia. *B.bassiana* is also known as White muscardine fungus **(Feng *et al*., 1994).** But the recent classification (Table 1) defined in the Annual Reviews of Entomology, which the major division is Ascomycota, Class-Sordariomycetes, Order-Hypocreales, and the family is Clavicipitaceae (**Roy *et al.,* 2006**). The studies documented on evolution and phylogenetic basis, showed dramatic changes due to *Incertae sedis* (uncertain position) of Deuteromycetes. Thus, the problem of uncertainness of the position was taken down and Deuteromycetes got affixed with respective phylogentic group examined by **Kirk *et al.* (2001, 2008)** on the basis of phylogenic evolution.

Table 1: Hierarchical positioning of various entomopathogenic genuses (Source: Roy *et al.,* 2005)

Division	**Class**	**Order**	**Family**	**Genus**
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	*Beauveria*[a], *Cordyceps, Cordycepioideus, Lecanicillium*[a], *Metarhizium*[a], *Nomuraea.*
[a]*Beauveria* and [a]*Metarhizium*: anamorphic Clavicipitaceae with teleomorphic connections to Cordyceps; Lecanicillium: anamorphic Clavicipitaceae with teleomorphic connections to **Torrubiella**.				

2.2 Morphological characterization of *B. bassiana*

The criterions which are used to classify *Beauveria* sp. are based on morphological characteristics. The characterization is on the basis of conidiphores which are consisting of whorls and dense clusters of the sympodial which are having globose and short conidiogenous cell are flask shaped with a narrow zig-zag appearance on terminal extension and conidia are single celled **(Samson *et al*., 1988).**

Morphologically *B. bassiana* was studied by **Larone (2002)** and he described that the surface of this fungal colony is generally white and fluffy to powder, hyphae of *B. bassiana* is tubular, narrow, septate, fragile filaments with a width of 1.70 to 2.80 µm. The flask shaped conidiogenous cells are narrow and zig-zag and the conidia are single celled with ovate or globose in shape and usually it is germinated at each bend point of zig-zag rachis or on a geniculate. The diameter of conidial size ranges from 2-4 µm.

Aneja (2004), Barnett and Hunter (1999) described the colony is fluffy and white, fungal hyphae cylindrical 3.50 µm width septate, hyaline, conidiophores cell single or branched, arising from vegetative cells globose or ovate to flask shaped (3-5x3-7 µm) with well developed zig-zag rachis up to 20.00 µm long and 1-1.50 µm width, conidia were born at thread like apex of the phialide on a series of zig-zag branch lets, more or less comparable to a cyme, conidia globose (1-4 µm) to oval (1.5-5x1.0-3.0 µm), hyaline and smooth.

Slavimira *et al*. (2010) reported that *B. bassiana* exhibited the following morphological parameters:

Table 2: Conidia size of *B. bassiana* that isolated obtained from the bark beetles

Fungal isolate	*B. bassiana*					
	433	**434**	**501**	**502**	**503**	**559**
Length (minimum - maximum) µm	1.04-1.91	0.95-1.91	0.95-1.56	0.86-1.72	1.30-1.99	1.56-2.17
Width (minimum - maximum) µm	1.13-1.56	0.95-1.65	0.78-1.56	0.43-1.72	0.69-1.65	1.30-1.82
Average size (Length x Width) µm	1.34 x 1.32	1.28 x 1.25	0.28 x 1.27	1.16 x 1.02	1.63 x 1.11	1.87 x 1.60

The isolates of *B. bassiana* colony growth on SDAY media showed the following results (14 days after cultivation at 23±1^{o}C):

Table 3: Fungal colony size on SDAY of *B. bassiana*

Fungal isolate	*B. bassiana*					
	433	**434**	**501**	**502**	**503**	**559**
Diameter (mm)	21.3	28.7	20.3	18.3	20.7	21.0
Height (mm)	3	4	2	2	2	2

2.3 Mode of action

In general, fungal pathogenesis in insects occurs *via* a series of systematic events *viz.* spore attachment, germination, penetration, growth, proliferation within the body of the host, interaction with insect defense mechanism and lastly re-emergence on the cadavers **(Nadeau *et al*., 1996; Thomas *et al*., 1996).**

The conidia, a basic infective unit of mitosporic fungus when lands on a suitable host it starts developing germ tubes or infection pegs and forms appressoria. These structures secrete a complex of enzymes *viz*., proteases, chitinases, and lipases which are capable of hydrolyzing corresponding cuticular constituents; *viz*., chitin, protein and lipids **(Leger *et al*., 1992).** Protease is one of the most important and earliest enzymes involved in the host invasion. High protease activity was observed during four to six days of culture incubation **(Dhar and Kaur, 2010).**

Thus, germ tube invades haemocoel and fat bodies. The invading vegetative hyphae consume the contents of haemolymph. On exhaustion of the haemolymph content, the host insect becomes dilapidated and the fungus starts to sporulate after death of the host. Aerial conidia sporulate from infected or mummified cadavers and get disseminated by wind. Splash of rain has also been accounted for spreading of the conidia but only for short distances **(Ambethgar, 1991).**

2.4 Bioassay of *B. bassiana*

Lacey and Brooks (1997) reported that pathogenicity means the genetically determined ability of an aeitiological agent to the cause disease.

Zayed (2003) studied the virulence of two *B. bassiana* indigenous isolates on the last instar larvae of *G. mellonella* L. by larval dip method. The LC_{50} values of Bb-1 were 8.2 x 10^{10}, 2.3 x 10^{8} and 2.2 x 10^{7}spores/ml at 4, 6 and 8 days after exposure, respectively. However, the LC_{50} values of Bb-2 were 19 x10^{7}, 3.3 x10^{6} and 10^{6}spores/ml, at the same exposure periods. At conidial concentration of 2.5 x10^{6}, Bb-1 isolate showed LT_{50} of 10 days, whereas for Bb-2, the LT_{50} was 6.4 days.

Hussein *et al.* (2012) studied the efficacy of different isolates of *B. bassiana* against the fourth instar larvae of *G. mellonella* by topically application method. The results revealed that the isolates BbaAUMC-3076 and BbaAUMC-3263 caused 100% mortality at concentrations of 5.50×10^{6} conidia/ml and 5.86×10^{5} conidia/ml, respectively. The LC_{50} values were 1.43×10^{3} and 1.04×10^{5} for BbaAUMC-3263 and BbaAUMC-3076, respectively. However, in their study, 100% mortality was observed at 96 HAT when third instar larvae of *G. mellonella* were atomized with 1000×10^{5} concentration and at 120 HAT at a concentration of 700×10^{5} in case of sunflower oil as well as in conidial suspension.

Malarvannan *et al.* (2010) conducted pathogenicity test of *B. bassiana* against *S. litura* at different concentration i.e. 2.40×10^{7}, 2.40×10^{6}, 2.40×10^{5}, 2.40×10^{4} conidia/ml. The least pupation (43.33%) was observed in larvae treated with the highest spores' concentration (2.40×10^{7}) of *B. bassiana*. A sequential follow up from this assay was done on the resultant pupae and adults. The healthy moth emergence was least in (2.40×10^{4}) spore concentration of the treatment, while the fecundity was completely arrested in the highest concentration (2.40×10^{7}).

Moorthi *et al.* (2011) studied the pathogenicity of 3 local isolates of EPF, *B. bassiana* (Bb-02, Bb-09 and Bb-10) against third instar larvae of *S. litura* using leaf spray method. Mortality of 66.67%, 73.33%, and 80.00% with respect to the isolates Bb-02, Bb-09 and Bb-10, was obtained four days after treatment. LC_{50} of the isolates was 2.10×10^{6}, 3.60×10^{7} and 1.20×10^{7} conidia/ml for Bb-02, Bb-09 and Bb-10, respectively.

Joseph *et al.* (2010) studied pathogenicity of *B. bassiana* against *S. litura* larvae, as well as its impact on the larval gut micro-flora. In the contaminated food bioassay, a spore density of 1.0×10^{9} spore/ml caused 100% larval mortality while LC_{50}

value was found to be $0.5x10^6$ spore ml^{-1}. The heterotrophic bacterial population and the generic composition in the digestive tract of the larvae treated with the Entomopathogenic Fungi were analyzed. Nine species of bacterial genera, *Proteus* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp., *Escherischia* sp., *Salmonella* sp., *Bacillus* sp., were identified in the digestive tract. The ingestion of fungal spores eliminated three genera of bacteria in the digestive tract.

Vijayavani *et al.* (2009) reported pathogenicity of two isolates of *B. bassiana* (SBT#11and SBT#16) against *S. litura* larvae which caused 100% mortality under laboratory condition. SBT#11 was more virulent with a LT_{50} of 5.10 and 6.00 days in laboratory for SBT#11 and SBT#16 respectively.

Marti *et al.* (2005) reported pathogenicity of *B. bassiana* on *Triatoma infestans* adults at 1.0×10^7 conidia/ml concentration and obtained mortality rate of 100% at 15 days post infection.

Carneiro *et al.* (2008) reported 24 isolates cultured in Potato Dextrose Agar (PDA) showed morphological homogeneity, bioassays revealed a wide variation in their pathogenicity against the fall army worm, *S. frugiperda* Smith, where the larval mortality ranged from 0-100%. Only 12 isolates out of the 24 isolates were infectious to the fall army worm and among them CNPMS-71, CNPMS-72, CNPMS-73 and CNPMS-591 were able to kill 100%.

Castrillo *et al.* (2008) conducted bioassay of different strains (ARSEF-252, 5813, 6567, 6986, 6987, 6988, 8237, 8238, 8239, 8240, CH 1-5, HN 1-6, ST 1-10, ST 11-16, ATCC-74040 and GHA) of *B. bassiana* against third instar larvae, pupae and adults of shore flies. Results showed that adults were more susceptible to the fungus than the other two stages. Secondary pick up of inoculums from sprayed pupae by adults resulted in 52.00% and 55.00% mortality from *B. bassiana* strains ST-1 and ST-2, respectively. Direct spray application on adults resulted in 91.00% and 94.00% mortality from these two strains, suggesting their potential as biological control agent against shore flies.

Kaur and Padmaja (2008) conducted bioassay test by using second instar larvae of *S. litura* in order to categorize the 24 isolates based on LT_{50} value, 3 isolates

showed least LT_{50} value i.e. 0-130 hours which were categorized as highly virulent. 11 isolates showing a range of LT_{50} value from 130-140 hours were categorized as moderately virulent and remaining 10 isolates showing a range of LT_{50} value from 141-154 hours were categorized as less virulent.

Vimaladevi and Prashanth (2009) reported that a local isolate of the entomopathogenic fungus, *B. bassiana* (ITCC-4513) was formulated as a suspension concentrate using mineral oil as carrier. In Laboratory bioassay against 5-days old *H. armigera* larvae, the formulation had LC_{50} value of 61.22/mg at 3 days after treatment.

Prasad and Syed (2010) used 4 different concentrations (0.10, 0.12, 0.20 and 0.25×108 conidia/ml) of fungus *B. bassiana* against third instar larvae of *H. armigera* Hubner and found 86.70% mortality at highest dose level as against 23.20% in control. Besides causing such heavy mortality, treated third instars expressed various morphological abnormalities such as extensive hairy growth, swollen body and lethargic larvae covered with fungal mycelium.

Prasad *et al.* (2010) used four different concentrations (0.1, 0.125, 0.2 and 0.25×108 conidia/ml) of fungus *B. bassiana* against four instar larvae of *H. armigera*, and found 76.7% mortality with highest dose of 0.25×108 spores/ml.

Tyagi *et al.* (2010) reported the mortality of *H. armigera* in all age group larvae (2, 4, 6, 8, and 12 days old) with 4 dose concentrations (1.0×10^9, 1.0×10^8, 1.0×10^7 and 1.0×10^6) of *B. bassiana* and the mortality achieved in all age group larvae was 87.20, 72.60, 54.40, 46.50 and 40.70%. It revealed that susceptibility of larvae was negatively associated with age of larvae.

2.5 Mass production of *B. bassiana* on grain and synthetic media

The success of biological control of insect pests depends not only on the isolation, characterization, and pathogenicity, but also on the success of the mass production of the microbial agents.

The study conducted by **Zapata *et al.* (2018)** showed that the broken rice was the most productive substrate for conidial production of both fungal genera, with a yield of 4.62×10^7 and 2.22×10^6conidia/g respectively.

Rajanikanth *et al.* (2010) evaluated five substrates viz., sorghum, rice bran, rice husk, press mud and bagasse for mass multiplication of different strains of *B. bassiana* and reported that sorghum yielded highest conidial count for all the strains.

Kaur and Joshi (2014) evaluated rice, rice straw, maize, sorghum, wheat, rice bran and mini potato tubers for mass production of three *B. bassiana* strains. They reported rice as the most suitable substrate for examined fungi as it yielded highest conidial count ($31.8x10^5$cfu/g) and colony forming unit ($30.5x10^5$cfu/g) whereas rice straw recorded minimum conidial count ($12.2x10^5$cfu/g).

Singh *et al.* (2017) tested naturally accessible substrates for mass mu1tiplication of *B. bassiana* EPF in liquid and solid media. Data observed on the seventh day after inoculation and it was found that Sabouraud's dextrose broth (SDB), was the best treatment by bringing down the *B. Bassiana* production up to (48.30) spore/ml in liquid medium. Overall finding for liquid and solid media showed the best media for spore production was SDB (224.59 spores/ml) and which was followed by PDB (193.06 spore/ml).

Chase *et al.* (1986) studied *B. bassiana* and *M. anisopliae* on eight different basal media out of which, the basal media that contain Oatmeal agar (OMA) was most superior to other seven media for isolation of both EPF.

Arora *et al.* (2013) took five media into consideration for the study the colony characteristics, growth and development of the *Cordyceps sinensis* Berk. The media were SDAY, PDA, CDA (Czapek Dox Agar), MEA (Malt Extract Agar) and OMA (Oat Meal Agar).

Shreekant *et al.* (2011) studied *Gliocladium roseum* on four different media *viz.* PDA, Corn Meal Agar (CMA), OMA, and V-8 Juice Agar media. After conducting experiments on growth and development of the fungus, it was observed that V-8 Juice Agar was considered to be the best media followed by CMA, PDA, and OMA media.

Sachin *et al.* (2011) observed that the conidial production of *B. bassiana* was highest on media containing rice, sorghum, jowar husk, SDA and SDB. The least conidial growth was observed in cow urine and sugarcane bagasse. However, among the substrates, the sugarcane bagasse was the cheapest.

2.6 Mycoinsecticide formulation

Formulation is the preparation of product with the use of an active agent and can be applied in the field **(Mathews, 1992; Bateman *et al.,* 1995)**. The uniqueness of myco-insecticide formulation in comparison with chemical insecticide is the presence of living organism which does not get dissolved, requires being stored under favorable conditions, and some ultra-violet protecting agents too. These myco-formulations are uniformly suspended because of the additives used to make the formulations. Not only this but, these additives alter the viscosity and eliminate spores to become airborne **(Moore and Caudwell, 1997)**. Formulating a myco-insecticidal product is an intricate process as the standards has to be quite on point as the viability of the spore is paramount check and the stability of the product should be similar to the chemical product formulations. Certain prerequisites before formulating a myco-insecticide has to be taken into consideration like the solvent as the base of the formulation, the spore in the product should not become hydrophilic and lipophilic, even a quick setup to get a photo-protectant for the product is must **(Stock, 1996; Moore and Caudwell, 1997)**.

To a myco-formulation, viable spores are basic infective unit to cause an effective obtrusion into the body of the host. Therefore, mode of action is validated by its effectiveness and avid involvement of salient factors addressing perfect performance becomes notable before developing a desired right formulation. These salient factors would be: insect-host relationship, desired insect onto action, vulnerable stages of insect, comportment of insect, the possible ways of spore contacting the target site, and the possible ways to deliver spray technology **(Stock, 1996; Evans, 1997)**. Once these factors coincide with the technology of formulation, the only one thing is left to work out, and that would be, the ways to operate it on a large scale. And this will enable to apply the product without getting involved in preparatory steps **(Georgis and Dunlop, 1994)**. **Tanda and Kaya (1993)** gave a list of ingredients to formulate a myco-insecticide and have talked about testing of the ingredients to check their side effects on the pathogens, the plants, and the non-target organisms.

Various formulation of myco-insecticide can be formulated like liquids, wettable powder, dusts, granules, oils, and baits **(Bateman, 1995; Tanda and Kaya,**

1993). **Johnsons and Goettel (1993)** used various formulations of the same strain to show that formulation could affect the efficacy of the fungus in the field.

Formulation can be of many types, one of which is oil based formulation. Oils have been the earliest insecticides used as repellents. At present in agriculture, oils are used as carrier of chemical insecticides and in pure form as insecticides for crops like citrus and apple in the U.S.A **(Chapman, 1967)**. The first oil based mycoinsecticide was developed by Prior **(Prior *et al*., 1988).** To prepare an oil based formulation, dry spores are used and which are kept in dry atmosphere. The spore should not be clumped or wet to formulate, during harvesting, a good formulation **(Bateman, 1995).** Usually oils are used for preparation of formulation because of the reasons that they are compatible with spores, more suitable for ultra low volume application, able to stick to the water-repellent insect cuticle, allow germination in a dry atmosphere, protect spores from the UV radiation, themselves can be insecticidal, can prolong the shelf life to over 30 months, have a better spreading coefficient, appropriate for application where the water storage, transport and formulation pose problems **(Prior *et al*., 1988; Hajek, 1993; Stathers *et al*., 1993; Bateman, 1995; Moore and Caudwell, 1997**).

It was **Ummidi and Vadlamani (2014)** who used eight vegetable oils in-vitro and studied vegetative growth, germination rate, and conidiogenesis of conidia of *M. anisopliae* and *B. bassiana*. Thus, the use of various oils showed different results. The compatibility results of Almond and Gingelly (sesame) oil has been tested with *M. anisopliae* and *B. bassiana.* And it was found that at concentration 1%, 2%, and 3% both the oils were remarkably stunning with the effects. On the contrary, toxic nature of Mustard and Eucalyptus oils was found to be at concentration of 3%. But Sunflower, Olive, and castor oils exhibited congruous property with *M. anisopliae* and showed toxic trait to *B. bassiana.* But in contrast, at 1% concentration of Olive oil and Castor oils did not show toxic property. Hence, the result showed that the samples which showed toxicity affected more to conidiogenesis rather than germination. Their research enabled the paths for examination of tested oils for formulation to document the toxicity as toxic and moderately toxic.

However, oils have become the new trend in formulation industry of EPF for their shelf-life. Formulations basically impart special roles in delivering of

entomopathogenic microbes to the desired ecosystem. In a formulation, microbes are typically the technical active ingredient which gets dispersed in oil **(Faria and Wraight, 2007).**

Luz and Batagin (2005) did some research on in-vitro development of *B. bassiana*. The conidia were immersed in a concentration of 6% of seven non-ionic (MP-6400, MP-600, Renex-60, Renex-95, Span-80, Tween-20 and Tween-80), three anionic surfactants (DOS-75, Hostapaval BVQ-9 and Surfax-220), and 11 vegetable oils (linseed, soybean, groundnut, rapeseed, thistle, sunflower, olive, sesame, corn, castor). The effect of oils on *Triatoma infestans* and the behavior of its nymphs to settle and the properties of oil-water for formulation of the fungus was tested and determined against this vector.

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The present study **"PREPARATION AND EVALUATION OF FORMULATION OF ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* (BALSAMO) VUILLEMIN AGAINST LEPIDOPTERAN INSECT PESTS"** was undertaken during 2017-19 in the Insect Pathology Laboratory, Department of Entomology of College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, (U.S.Nagar) Uttarakhand, this place was located in Tarai region at the lower hills of Himalayas (Shivalik range) at 29˚N latitude, 79.3˚E longitude and at an altitude of 243.83 m above mean sea level.

3.1 Optimizing the aseptic conditions during the experimentation

In order to forbid the contamination of *B.bassiana* culture from other microbes such as opportunistic saprophytic fungi and bacteria, aseptic culture conditions were maintained in the laboratory. The laminar air flow chamber (L. A. F. C.) was sterilized with potassium per magnet and formaldehyde. After this, the L. A. F. C. was cleansed with spirit and then on the UV-rays for 15 to 20 minutes before every fungi inoculation, it reduced the chances of contamination. Experimental room was neatly cleaned every alternate day and was fumigated with 4 per cent formaldehyde solution (HCHO).

The glassware used in the experiment was washed thoroughly with laboline solutions (laboratory reagent). Once the glassware got dried they were kept in the autoclave at 121^0C for at least 30 minutes by using saturated steam, under at least 15 psi of pressure or in hot air oven at 180^0C for 20 minutes, for the hot air sterilization.

Glassware: Required glassware that were used in whole experiment – test tubes, petriplates (90 mm diameter), blue cap bottles i.e. borosil bottles (250, 500 ml), measuring cylinders (5, 10, 50, and 100 ml), conical flasks (50,100, 250, 500, and 1000 ml), beakers (50, 100, 250, 500, and 1000 ml) etc. The glassware having with standard measurement was used during the whole experiment.

3.2 Collection, isolation, identification and maintenance of local isolate of entomopathogenic fungi (EPF)

The diseased samples were collected from Crop Research Centre (C.R.C.) and Avenue Plantation, Pantnagar during autumn and winter season. The samples of

cadaver were then subjected to series of washing with sodium hypochlorite solution and a series of distilled water. Then aseptic inoculation protocol was followed. After achieving pure culture of the local isolate, identification of the fungus was done and the final process of maintenance of culture was done by doing sub-culturing of the isolate. For Identification various slides were prepared with lacto-phenol and methyl blue. The examination was done under microscope (Olympus Cx33) for the morphological characters **(Plate1).**

3.3 Mass multiplication of *B. bassiana* thorough grain and synthetic based media

The maintained fungal culture used to get mass produce in different synthetic and grain media in the Insect Pathology laboratory, Department of Entomology of College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar) Uttarakhand.

3.3.1 On solid grain media

The grains for mass multiplication of *B. bassiana* used were rice, sorghum, and maize. The grains crushed into half and were soaked in distilled water (1:1) for 24 hour. In case rice, soaking was done for three to four hours. After soaking, de-straining was done for grain and extra moisture was removed by using filter paper. Finally, these grains were filled in autoclavable polythene bag (10"x6"). Later, the bag was plugged with sterilized non-absorbent cotton plugs and then these bags were autoclaved at 121^{o}C for at least for 30 minutes (15 psi). Once these bags were autoclaved, they were set to cool under room temperature.

After the cooling of bags, these bags were brought into the L. A. F. C. for inoculation and were inoculated with 0.7 cm or 7 mm diameter disc of fungal isolate, which was 15 to 18 days old. At least two to four discs were inoculated in each bag. Once bags were inoculated, they were placed in B. O. D. incubator for growth and development at 25±2^{o}C with 90±5% relative humidity (R. H. %). The bag was shaken once at 3 days interval for the uniform growth of mycelia.

3.3.2 On synthetic media

For selection of the best media, both solid media, like Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar+1%Yeast (SDAY), Sabouraud's Maltose Agar+1%

Yeast (SMAY), V-8 Juice Agar (V-8), Oatmeal Agar (OMA) media and broth/liquid media, like Potato Dextrose Broth (PDB), Sabouraud's Dextrose+1%Yeast (SDY) Broth, Sabouraud's Maltose+1%Yeast (SMY) Broth, V-8 Juice Broth (V-8B), Oatmeal Broth (OMB) were prepared with the standard protocol. All the ingredients of the different media were prepared in sterilized conditions with distilled water. The media were then autoclaved at 15 psi for 20 minutes at 121^0 C. The different media were then poured under L. A. F. C. in sterilized petri-plates (90 mm of diameter) for the comparative study to find the suitable media and finally kept at different temperature to find out best abiotic conditions.

3.3.2.1 Ingredients for solid media

S. no.	Media	Composition	Quantity
1.	Potato Dextrose Agar media (PDA)	Peeled and sliced Potatoes	250 g
		Dextrose	20 g
		Agar	20 g
		Distilled water	1000 ml
2.	Sabouraud's Dextrose Agar+1%Yeast (SDAY)	Peptone	10 g
		Dextrose	40 g
		Agar	15 g
		Yeast	2 g
		Distilled water	1000 ml
3.	Sabouraud's Maltose Agar+1%Yeast (SMAY)	Maltose	40 g
		Agar	15 g
		Yeast	15 g
		Distilled water	1000 ml
4.	Oatmeal Agar (OMA)	Oatmeal	60 g
		Agar	12.5 g
		Distilled water	1000 ml
5.	V-8 Juice Agar(V-8)	V-8 juice (100 ml)	8.3g
		L-Asparagine	10g
		Yeast extract	2g
		Calcium carbonate	2g
		Glucose	2g
		Agar	20g
		Distilled water	1000 ml
		Final pH (at 25^{o}C)	5.7±0.2

3.3.2.2 Ingredients for liquid media

S. No.	Media	Composition	Quantity
1.	Potato Dextrose Broth (PDB)	Peeled and sliced Potatoes	250 g
		Dextrose	20 g
		Distilled Water	1000 ml
2.	Sabouraud's Dextrose+1%Yeast (SDYB) Broth	Peptone	10 g
		Dextrose	40 g
		Yeast	2 g
		Distilled Water	1000 ml
3.	Sabouraud's Maltose+1%Yeast (SMYB) Broth	Maltose	40 g
		Yeast	15 g
		Distilled Water	1000 ml
4.	Oatmeal Broth (OMB)	Oatmeal	60 g
		Distilled water	1000 ml
5.	V-8 Juice Broth(V-8B)	V-8 juice(100 ml)	8.3g
		L-Asparagine	10g
		Yeast extract	2g
		Calcium carbonate	2g
		Glucose	2g
		Distilled water	1000 ml
		Final pH (at 25^{o}C)	5.7±0.2

3.3.3 Study on different factors affecting growth and sporulation on synthetic media

The temperature and relative humidity majorly affects growth, germination, and virulence and survival biology of the aetiological agent. Hence, the fungal culture was tested at different temperature and relative humidity along with constant dark and light hours.

Five different solid synthetic media were taken as 5 treatments and 5 replications, and in liquid/broth media, 5 treatments and 3 replications are used. All these five media were studied under different temperature and relative humidity (RH%) *viz.* 17^{o}C and 48% RH, 21^{o} C and 60% RH, 27^{o} C and 76% RH, and 31^{o} C and 90% RH

in BOD and these were provided with 12:12 hours light and dark condition (12 hours light, 12 hour dark).

Studies were conducted under the following parameter:

3.3.3.1 Radial growth

The synthetic media was exposed to various temperatures viz. 17 ^{o}C, 21 ^{o}C, 27 ^{o}C and 31^{o}C**,** and were studied every day for the growth and development parameter of the fungal colony of *B. bassiana* by following formula:

$$\text{Growth per day} = \frac{\text{Horizontal growth + Vertical growth}}{2}$$

Horizontal and vertical growths were taken in centimeter (cm) in case of solid media.

3.3.3.2 Conidial count

On 15th day of growth when fungal plates were fully colonized by *B. bassiana*, a 0.7cm or 7 mm bit was taken with the help of flame sterilized cork borer and finally was placed in 15 ml vial containing 10 ml distilled water. Each vial had one bit. These vials were subjected to vortex mixture for 5 minutes. This suspension was then strained and the final turbid suspension was used for conidial count by dying the spores with cotton blue and lactophenol. The examinations of total number of spores were calculated with the help of Neubauer Haemocytometer and spore viability was evaluated **(Alves, 1998)**. For germination test of spore, a suspension of 10^7 spores in 100 µl was made and it was poured evenly on the media. Final step was to incubate it at room temperature for 24 hours. Determination of viable spores per unit volume was calculated by multiplying total count with germination percentage.

3.3.3.3 Mycelium biomass weight and spore

For attaining and expressing mycelium biomass weight, all the above mentioned liquid/broth media were prepared and studied for 15 days. After 15 days the broth was filtered with Whatman filter (paper grade 1). The harvested liquid media mycelium and spore were weighed to analyze and observation of wet weight was recorded. Then these wet mycelium growth was dried in room temperature. After four days dry weight of mycelium growth was again recorded.

3.5 Preparation of different formulations

3.5.1 Grain based formulation

After observing the full sporulation on grain media (20 days after inoculation), the solid grains along with spore mass of *B. bassiana* was dried (at 25°C for 4 days) and grinded with a mixer grinder. It was sieved (500 mesh/inch sieve) for removing coarse material. The material was separated into two halves after adding 0.05 per cent of tween-80. Prepared formulations were packed in polythene bag and store at different temperature to check the viability of conidia. The following are the formulations made by using different grain media:

1. Rice grain formulation
2. Sorghum grain formulation
3. Maize grain formulation

3.5.2 Preparation of conidial suspension and sunflower oil formulation

The culture plates of 15 days old of *B. bassiana* were taken to prepare stock suspension. With the help of measuring cylinder 10ml tap water was poured on to the culture plates and about two drops of 0.05% Tween 80 was added. Then the culture plate surface adhered with mycelia growth was scrapped gently with sterilised inoculation loop **(Romback, 1989)**. Finally shake the suspension and filter this suspension with muslin cloth.

For the preparation of oil based formulation, pure spore dust (approximately 0.5g form the solid synthetic media) was obtained. It was added to sunflower oil (1 ml) and about two drops of 0.05% Tween 80 was added. After a thorough shake 50 ml of tap water was added until it gets homogenized in vortex mixture and later, this oil suspension was filtered thorough muslin cloth.

Conidial count was taken with the help of Neubauer Haemocytometer. In the end, the both conidial suspensions and sunflower oil formulation were diluted through serial dilution to the concentrations $1000x10^5$, $700x10^5$, $500x10^5$, $250x10^5$, $125 x10^5$and $75x10^5$ conidia/ml. In control only water and two drops of 0.05% Tween 80 was added.

3.5.2.1 Viability of the spores

To set the fungus colony forming units/ml (C.F.U), after 1ml of stock suspension or sunflower oil formulation was taken and suspended in 9 ml distilled water for the serial dilution. Prepared serial dilution was plated at 1ml per plate on PDA media. The plate was gently rotated for uniform spreading of spore suspension and incubated at $25^0C \pm 2^0C$ and R.H 70 %. Each treatment had three replications. The C. F. U. count was recorded on 7^{th} day after plating.

3.6 Maintenance of culture of test insects

3.6.1 Greater Wax moth (*G. mellonella*)

Wax frames of honeybee hives were subjected to infestation by the larvae of *G. mellonella*. And these frames were brought from the stored room of Apiary to the laboratory. These larvae were reared to maintain the culture in the BOD incubator at temperature 27.2^0 C. The population of the wax larvae was fed with wax and this way they were being mass produced. 3^{rd} instar larvae of wax moth were used for bioassay against *B. bassiana* developed conidial suspension and sunflower oil formulation.

3.6.2 Tobacco Caterpillar (*S. litura*)

The running culture of Tobacco Caterpillar, *S. litura* was maintained in the laboratory by standard rearing technique following **Sabry and Khedr (2014)** and **Thodsare and Srivastava (2014)**. Egg masses were collected from the fields of different crops and wildly grown castor plants, at Norman E. Borlaug Crop Research Centre (NEBCRC), Pantnagar. Egg masses were kept in a petri dish (9cm diameter) wrinkled with a wet filter paper to keep away from dried conditions. Upon hatching, neonate larvae were transferred to plastic tubs (35cm diameter, height approx 15cm, also wrinkled with wet filter paper) on a fresh tender castor leaf having a water soaked cotton swab wrapped to petiole so that leaf could remain fresh. Larvae were permitted to grow in tubs, give good sterilized conditions were maintained for the growing larvae. After the larvae of Tobacco Caterpillar attain a specific age groups i.e. 3^{rd} instar larvae, they were used for bioassay against *B. bassiana* developed conidial suspension and sunflower oil formulation.

3.7 Bioassay of prepared stock suspension and sunflower oil formulation of *B. bassiana* by 'larval atomization' method

Experiments for the determination of contact action or toxicity of some myco-insecticides *viz.* stock suspension and sunflower oil formulation of *B. bassiana* were conducted against different larval stages of *S. litura* and *G. mellonella* by larval atomization.

3.7.1 Larval atomization method

To test the contact action or toxicity of myco-insecticides formulation by larval (**Plate 2)** atomization method **(Kumar and Srivastava, 2016**), different dilutions of respective myco-insecticide formulation were prepared in tap water by serial dilution method. Five different concentrations i.e. $1000x10^5$, $700x10^5$, $500x10^5$, $250x10^5$, $125\ x10^5$and $75x10^5$ conidia/ml were used for bioefficacy experiment. The larvae in each treatment were atomized with different dilutions with the help of corning glass hand atomizer (100ml) in a glass petri plate (diameter - 15cm) and given the contact exposure for 30 minutes **(Chilana, 2009; Naorem, 2015; Kumar and Srivastava, 2016).** The control larvae were sprayed with water and two drops of 0.05% Tween 80 was added. Treated larvae were transferred into a corning glass petri dish (diameter - 9cm) wrinkled with the wet filter paper and containing a castor leaf disc for feeding.

3.7.2 Mortality studies

The virulence of *B. bassiana* was studied for the test insect at 12 HAT, 24 HAT, 36 HAT, 48 HAT, 60 HAT, 72 HAT, 84 HAT, 96 HAT, 108 HAT and 120 HAT. Five different concentrations of oil formulation and conidial suspension were used ($1000x10^5$, $700x10^5$, $500x10^5$, $250x10^5$, $125\ x10^5$and $75x10^5$) concentration were sprayed on insects, and leaves with the help of hand atomizer. For each treatment there were 4 replications with 10 insects each replication for all the insects.

3.7.2.1 Mortality observations

Data should be recorded on daily mortalities bases. Mortality occur due to prepared formulation were expressed in terms of as mortality %.

$$\text{Per cent mortality} = \frac{\text{Number of larvae dead}}{\text{Total number of larvae treated}} \times 100$$

Once the per cent mortality was calculated in each replication, then they were calculated as average mortality per cent.

$$\text{Average per cent mortality} = \frac{R1 + R2 + R3}{3}$$

3.8 Statistical analyses

The recorded observations obtained from various experiments, were suitably analyzed by SPSS the statistical probe and the per cent mortality was subjected to **DMRT** analysis.

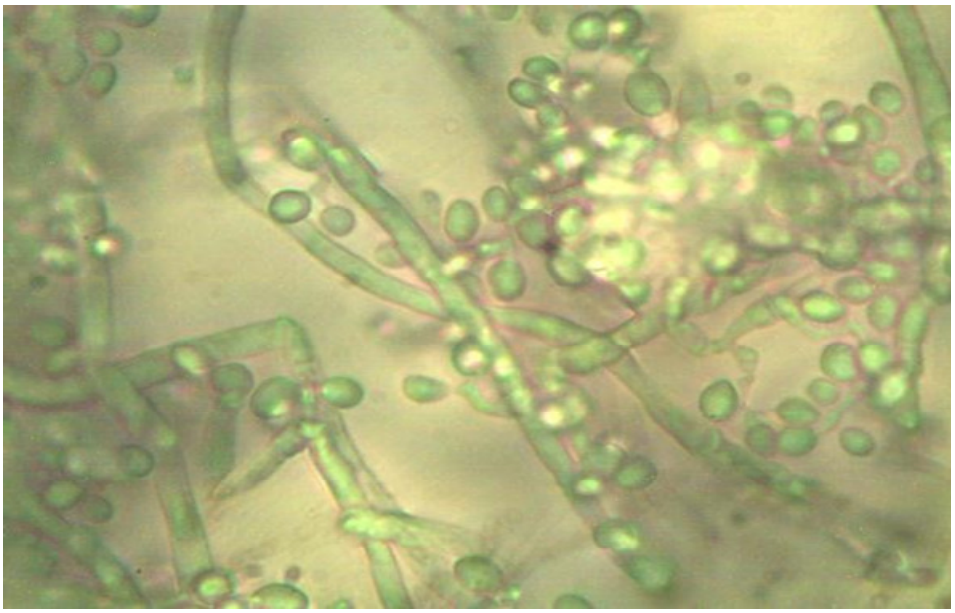

Plate 1: *B.bassiana* mycelium's with conidia at 100X

Plate 2: Corning glass hand atomizer

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

Present study on mass multiplication and formulation of entomopathogenic fungi, ***Beauveria bassianan* (Balsamo) Vuillemin** along with their pathogenicity against lepidopteran insect pest, i.e. Tobacco caterpillar **(*Spodoptera litura*)** and Greater Wax Moth larve **(*Galleria mellonella*)** was conducted in Insect Pathology Laboratory, Department of Entomology, College of Agriculture. All the silent findings are presented below in this chapter.

4.1 Mass multiplication of *B. bassiana* on grains and synthetic media

4.1.1 Mass multiplication of *B. bassiana* on grain substrates

The present experiment on mass multiplication was undertaken on three crushed grain substrates i.e. rice, sorghum and maize. In the whole procedure, the observation was recorded on 20 day after the inoculation.

The experiments on three different crushed grain substrates for mean biomass, conidial count and per cent germination was significantly observed **(Table 4 and Fig. 4.1, 4.2, 4.3 and 4.4)** and concluded. On the basis of highest mean biomass, the best result was in rice (0.62 g), which was followed by sorghum (0.54 g) and the least mean biomass was observed in maize (0.37 g) **(Plate 3)**. When conidia were counted from crushed grain substrates, it was found that the highest conidial count was observed in rice (10.92×10^7 conidia/g) then sorghum (7.35×10^7 conidia/g) and the conidial count least was documented in maize (6.05×10^7 conidia/g). The mean per cent germination was seen highest in rice (86.94per cent) followed by sorghum (77.43per cent). The least per cent germination was observed in maize (72.44per cent).

The best crushed grain substrates on the basis of mean biomass, conidia count, and per cent germination was rice and the second best grain media was sorghum. The least preferable crushed grain substrates were maize because of lowest sporulation. Similar studies were conducted by **Zapata *et al.* (2018)** showed that the broken rice was the most productive substrate for conidial production of both fungal genera, with a yield of 4.62 x 10^7 and 2.22 x 10^6conidia/g respectively. **Kaur and Joshi (2014)** evaluated rice, rice straw, maize, sorghum, wheat, rice bran and mini potato tubers for

mass production of three *B. bassiana* strains. They reported rice as the most suitable substrate for examined fungi as it yielded highest conidial count ($31.8x10^5$cfu/g) and colony forming unit ($30.5x10^5$cfu/g) whereas rice straw recorded minimum conidial count ($12.2x10^5$cfu/g).

Table 4: Studies of media for mean biomass, conidial count, and per cent germination of *B. bassiana*

S.No.	Media	Biomass (g/60g)	Conidial count (10^7 conidia/g)	Per cent germination
1	**Rice**	0.62	10.92	86.94
2	**Sorghum**	0.54	7.35	77.43
3	**Maize**	0.37	6.05	72.44
4	**S.Em.±**	**0.10**	**0.25**	**1.16**
5	**CD (5%)**	**0.32**	**0.75**	**3.52**
6	**CV**	**5.18**	**7.54**	**3.26**

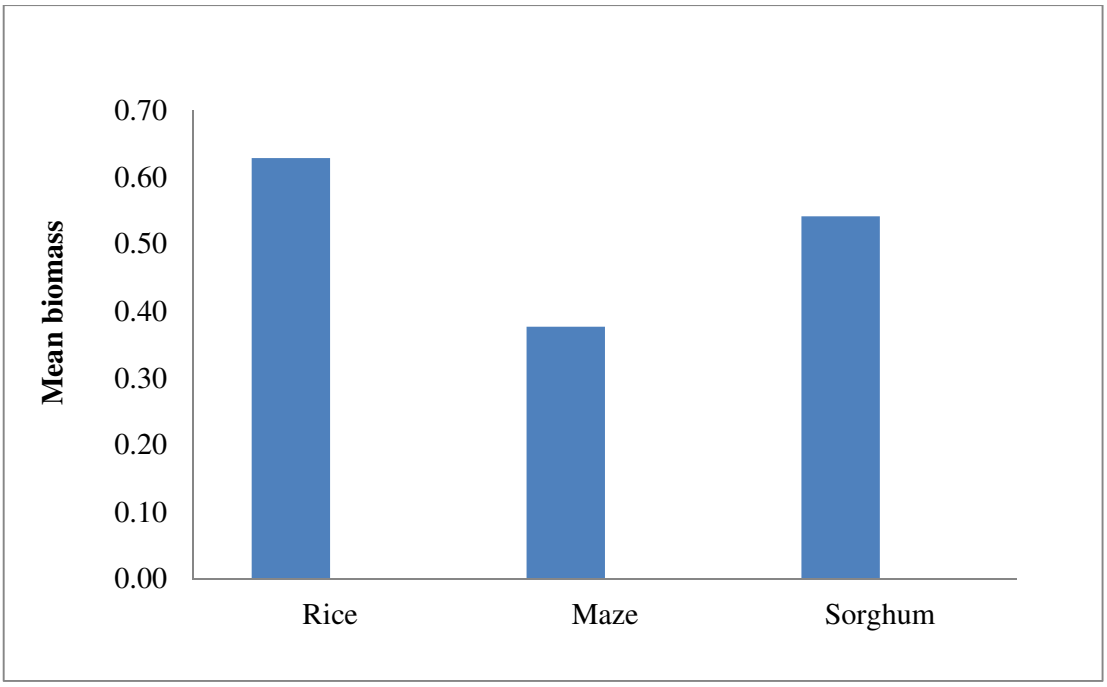

Fig. 4.1: Bar graphs illustrating mean biomass (g/ 60 g) of *B. bassiana* on different grain based media

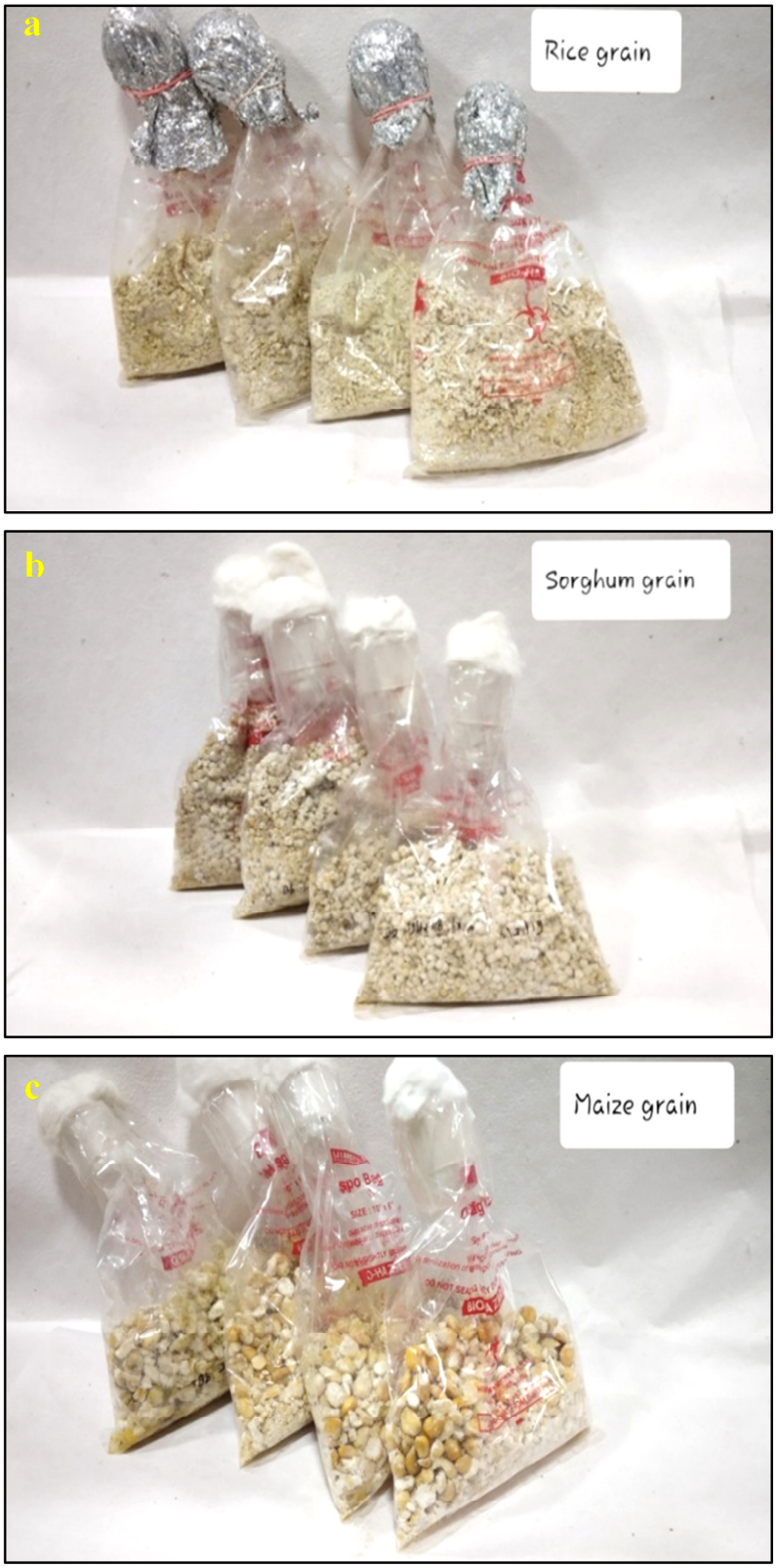

Plate 3: Growth of *B.bassiana* on different crushed grain media a) Rice b) Sorghum c) Maize

Fig. 4.2: Bar graphs illustrating mean conidia count (10^7conidia/g) of *B. bassiana* on different grain based media

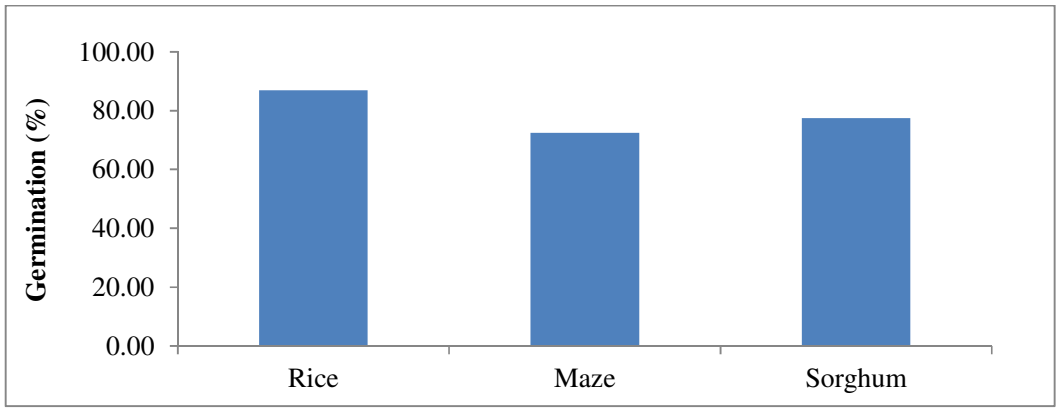

Fig. 4.3: Bar graphs illustrating mean per cent germination of *B. bassiana* on different grain based media

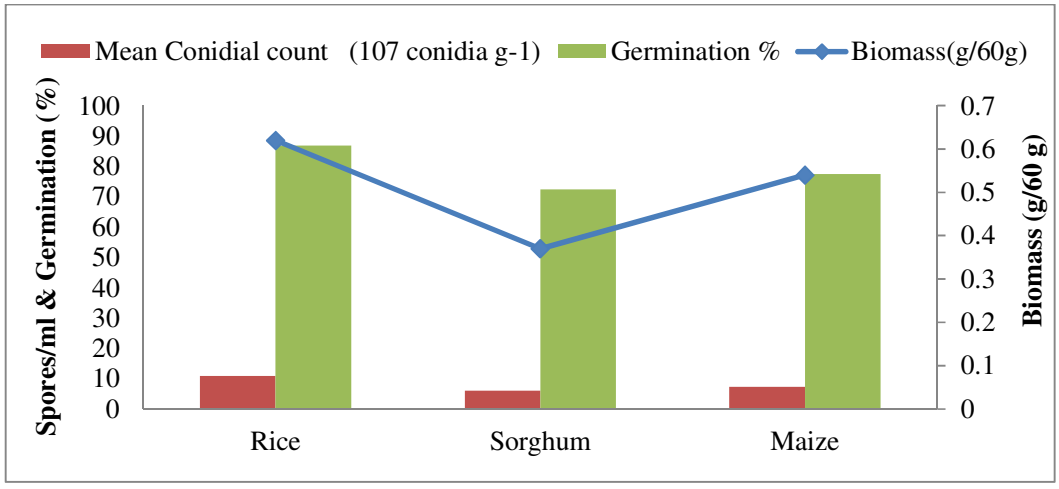

Fig. 4.4: Bar graph illustrating means of biomass, conidial count and per cent germination of *B. bassiana* on different grain based media

4.1.2 Mass multiplication of *B. bassiana* on solid media

The various solid media used in this experiment were Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar+1%Yeast(SDAY), Sabouraud's Maltose Agar+1%Yeast(SMAY), V-8 Juice Agar (V-8) and Oatmeal Agar (OMA). These media were studied at different temperature to understand the optimum requirement for growth and development. The different temperatures in this study were 17^{o}C, 21^{o}C, 27^{o}C, and 31^{o}C. Other extreme temperatures were also studied in preliminary basis like 15^{o}C and 35^{o}C. The temperature in between the extremes showed varied data.

The studies on solid media at 17^{o}C for mean radial growth, conidia count spore/ml, and per cent germination was documented in **(Plate 4)**, **Table 5 and Fig. 4.5, 4.6 and 4.7.** On the basis of mean radial growth, the best result was in OMA (6.88 cm) which was followed by SMAY (6.83 cm), V-8 (6.30 cm), PDA (5.03 cm) and the least mean radial growth was observed for SDAY (3.94 cm). When conidia was counted from solid media at 17^{o}C as spores/ml, it was found that the highest count was observed in OMA (1.92x10^{7}) then V-8 (1.37x10^{7}) followed by SMAY (1.08x10^{7}) and PDA (0.79x10^{7}). The least amount was documented in SDAY (0.59x10^{7}).The mean per cent germination was seen highest in OMA (79.62 per cent) followed by V-8 (76.27 per cent) then SMAY (75.17 per cent) and then PDA (72.65 per cent). The least per cent germination was observed in SDAY (72.60 per cent). The best media on the basis of mean radial growth mean conidia count spore/ml, and mean per cent germination was OMA, and the second best was V-8, followed by SMAY and then PDA. The least preferable media was SDAY because of lowest sporulation and conidial count at 17^{o}C.

The studies on solid media at 21^{o}C for mean growth was documented in **(Plate 5)**, **Table 6 and Fig. 4.8, 4.9 and 4.10.** The highest mean radial growth was obtained in OMA (7.29cm) which was followed by SDAY (6.82 cm), PDA (6.80 cm), V-8 (5.57 cm) and finally the least mean radial growth was obtained in SMAY (3.57 cm). When conidia was counted from solid media at 21^{o}C as spores/ml, it was found that the highest mean conidia count was observed in OMA (11.18x10^{7}) which was followed by V-8 (6.77x10^{7}) then it was PDA (5.38x10^{7}), it was lead by SDAY (3.97 x10^{7}) and the least spores were obtained from SMAY (1.55x10^{7}). The mean per cent germination was seen highest in OMA (82.42 per cent) which was lead by V-8 (79.38 per cent), PDA (79.13 per cent), SDAY (75.56 per cent) and the least mean per cent

germination was examined from SMAY (74.27 per cent). The best media on the basis of mean radial growth mean conidia count/ml, and mean per cent germination was OMA, and the second best was V-8, followed by PDA and then SDAY. The least preferable media was SMAY because of lowest sporulation at 21^{o}C.

Then the media were studied at 27^{o}C for growth and development of test fungus (**Plate 6) (Table 7 and Fig. 4.11, 4.12 and 4.13)**. The highest mean radial growth was obtained in OMA (6.44 cm) which was followed by SDAY (4.98 cm), PDA (4.70 cm), V-8 (4.65 cm) and finally the least mean radial growth was obtained in SMAY (4.43 cm). When conidia was counted from solid media at 27^{o}C as spores /ml, it was found that the highest mean conidia count was observed in OMA ($12.85x10^{7}$) which was followed by V-8 ($1.63x10^{7}$), PDA ($0.85x10^{7}$), and SDAY ($0.50x10^{7}$) and the least spores were obtained from SMAY ($0.31x10^{7}$). Even mean per cent germination was evaluated, the highest per cent germination was obtained in OMA (86.74per cent) followed by SDAY (77.74 per cent), V-8 (75.00 per cent) and PDA (74.42 per cent) and the least mean per cent germination was obtained from SMAY (72.53 per cent). The best media on the basis of mean radial growth, mean conidia count/ml, and mean per cent germination was OMA, and the second best was V-8, followed by PDA and SDAY on the basis of sporulation. The least preferable media was SMAY because of lowest sporulation at 27^{o}C.

Lastly, the studies were conducted at 31^{o}C for mean growth **(Plate 7) (Table 8 and Fig. 4.14, 4.15 and 4.16)**. The highest mean radial growth was obtained in V-8 (4.34 cm) which was followed by OMA (3.52 cm), SMAY (3.23 cm) then it was PDA (3.20 cm) and finally the least mean radial growth was obtained in SDAY (2.89 cm). When conidia was counted from solid media at 31^{o}C as spores/ml, it was found that the highest mean conidia count was observed in OMA ($3.85x10^{7}$) which was followed by V-8 ($2.43x10^{7}$), SMAY ($1.62x10^{7}$), PDA ($1.24x10^{7}$) and the least spores were obtained from SDAY ($0.68x10^{7}$). The mean per cent germination was evaluated with the highest mean per cent germination in OMA (83.72 per cent), then after PDA (80.29 per cent), SDAY (78.69 per cent), V-8 (78.63 per cent) and the lowest mean per cent germination was obtained from SMAY (78.46 per cent). The best media on the basis of mean radial growth, mean conidia count/ml, and mean per cent germination was OMA, and the second best was V-8, followed by SMAY and then PDA. The least preferable media was SDAY because of lowest sporulation at 31^{o}C.

Table 5: Studies of various solid media on mean radial growth, conidial count spore/ ml, and per cent germination of *B. bassiana* at 17°C

Temperature	Media	Mean radial growth (cm)	Mean conidial count spore /ml (10^7 conidia)	Mean per cent germination
17°C	**SDAY**	3.94	0.59	72.60
	SMAY	6.83	1.08	75.17
	PDA	5.03	0.79	72.65
	OMA	6.88	1.92	79.62
	V-8	6.30	1.37	76.27
	S.Em.±	**0.83**	**0.25**	**0.47**
	CD (5%)	**0.24**	**0.75**	**1.41**
	CV	**3.20**	**4.98**	**1.42**

Table 6: Studies of various solid media on mean radial growth, conidial count spore/ ml, and per cent germination of *B. bassiana* at 21°C

Temperature	Media	Mean radial growth (cm)	Mean conidial count spore /ml (10^7 conidia)	Mean per cent germination
21°C	**SDAY**	6.82	3.97	75.56
	SMAY	3.38	1.55	74.27
	PDA	6.80	5.38	79.13
	OMA	7.29	11.18	82.42
	V-8	5.57	6.77	79.38
	S.Em.±	**0.85**	**0.46**	**0.56**
	CD (5%)	**0.25**	**0.13**	**1.65**
	CV	**3.20**	**1.81**	**1.60**

Table 7: Studies of various solid media on mean radial growth, conidial count spore/ ml, and per cent germination of *B. bassiana* at 27°C

Temperature	Media	Mean radial growth (cm)	Mean conidial count spore /ml (10^7 conidia)	Mean per cent germination
27°C	**SDAY**	4.98	4.91	77.74
	SMAY	4.43	2.52	72.53
	PDA	4.70	6.12	74.42
	OMA	6.44	12.85	86.74
	V-8	4.65	8.51	75.00
	S.Em.±	**0.90**	**0.92**	**0.59**
	CD (5%)	**0.26**	**0.27**	**1.74**
	CV	**4.03**	**2.95**	**1.71**

Table 8: Studies of various solid media on mean radial growth, conidial count spore/ ml, and per cent germination of *B. bassiana* at 31°C

Temperature	Media	Mean radial growth (cm)	Mean conidial count spore / ml (10^7 conidia)	Mean per cent germination
31°C	**SDAY**	2.89	0.68	78.69
	SMAY	3.23	1.62	78.46
	PDA	3.20	1.24	80.29
	OMA	3.52	3.85	83.72
	V-8	4.34	2.43	78.63
	S.Em.±	**0.70**	**0.35**	**0.44**
	CD (5%)	**0.20**	**0.10**	**1.30**
	CV	**4.58**	**4.09**	**1.23**

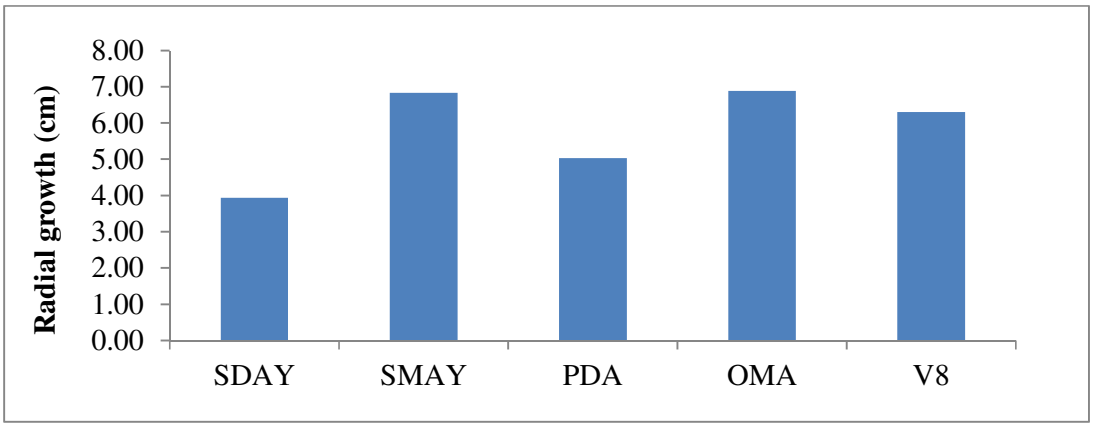

Fig. 4.5: Bar graph illustrating effect of various media on mean radial growth (cm) of *B. bassiana* at 17°C

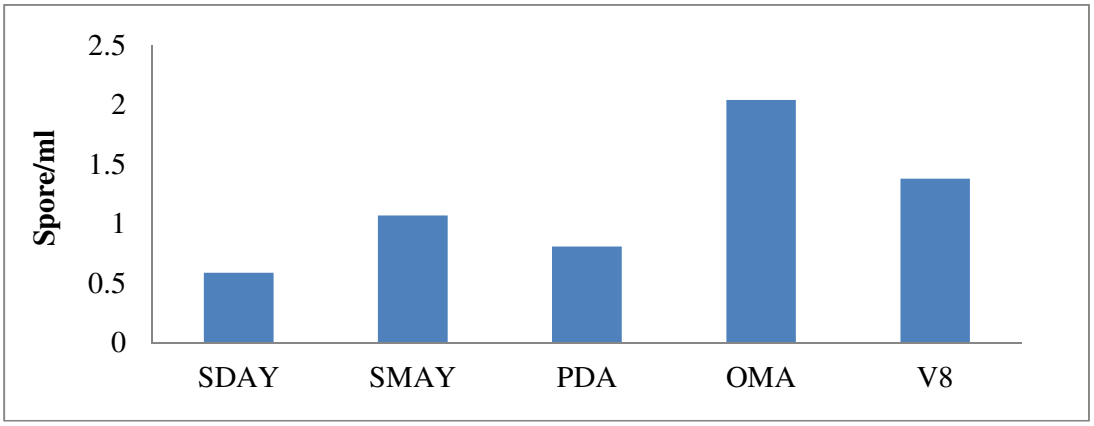

Fig. 4.6: Bar graph illustrating effect of various media on mean conidia count spore/ml (10^7) of *B. bassiana* at 17°C mean

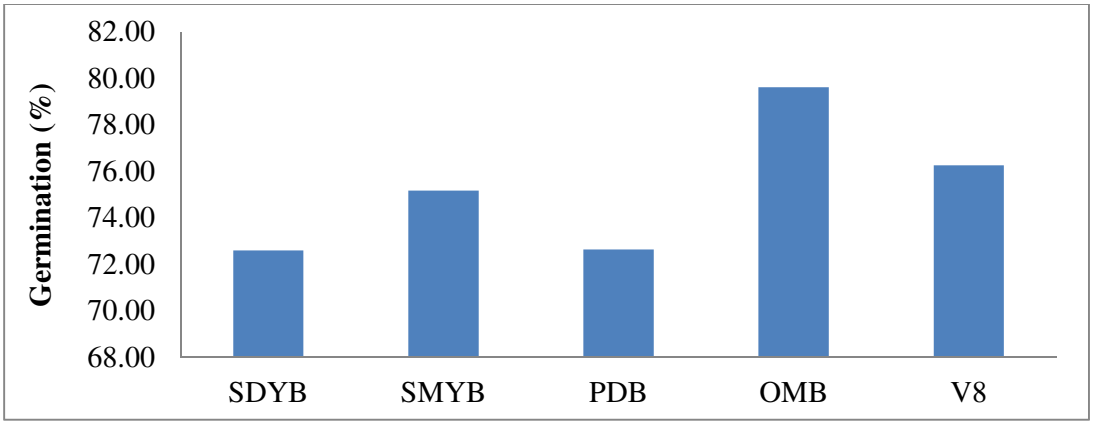

Fig. 4.7: Bar graph illustrating effect of various media on mean per cent germination of *B. bassiana* at 17°C mean

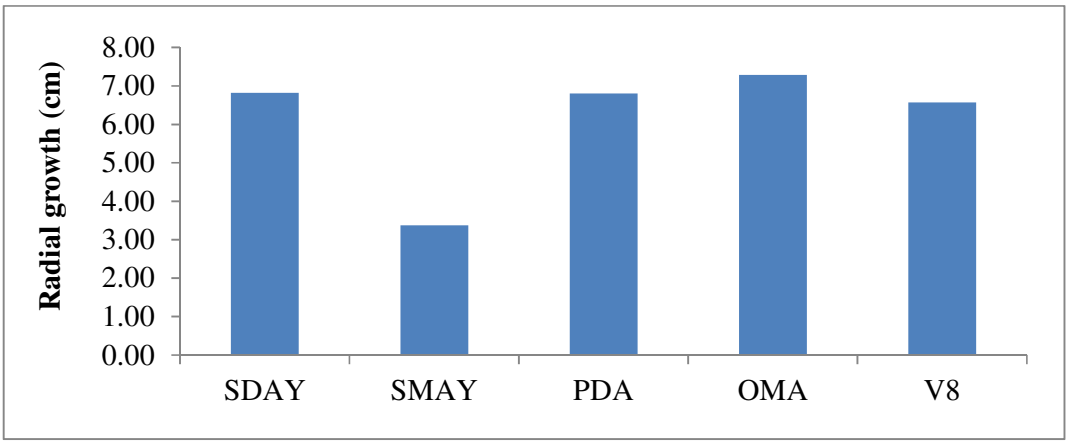

Fig.4.8: Bar graph illustrating effect of various media on mean radial growth (cm) of *B. bassiana* at 21°C

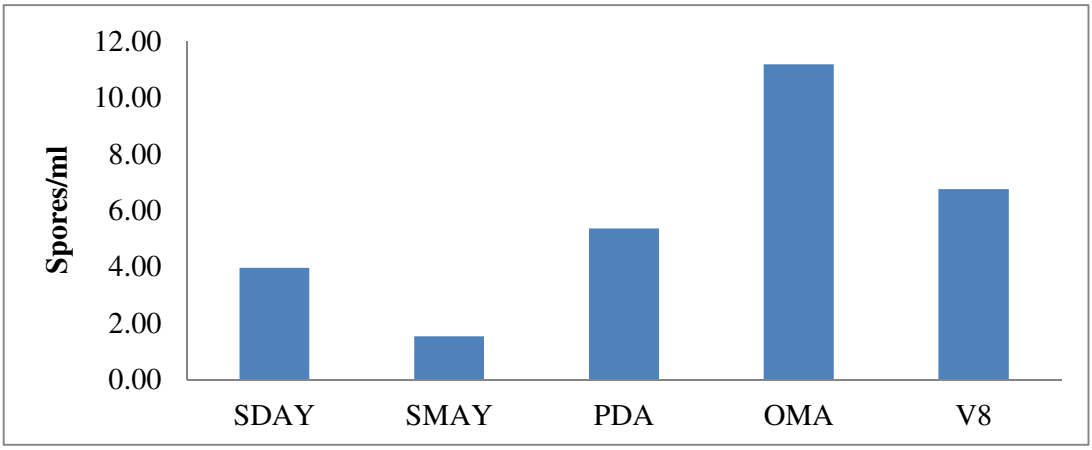

Fig. 4.9: Bar graph illustrating effect of various media on mean conidia count spore/ml (10^7) of *B. bassiana* at 21°C mean

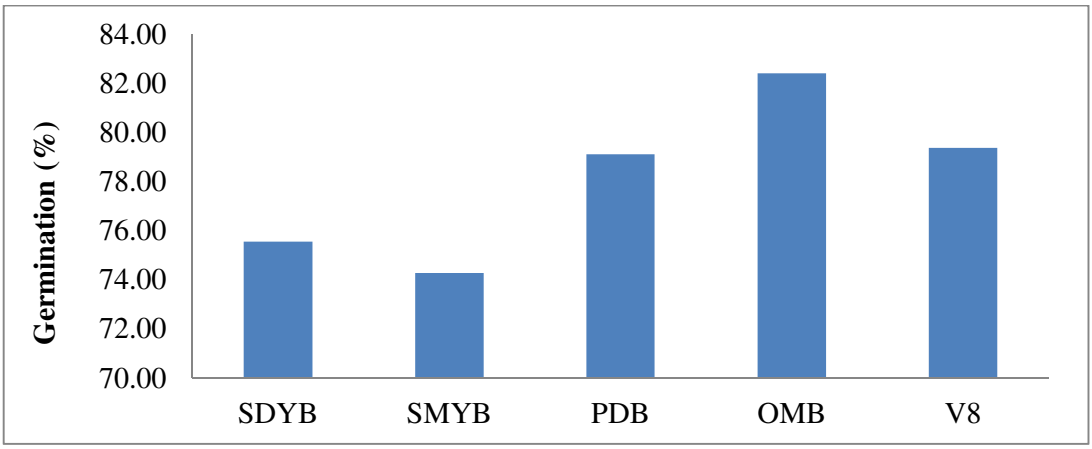

Fig. 4.10: Bar graph illustrating effect of various media on mean per cent germination of *B. bassiana* at 21°C mean

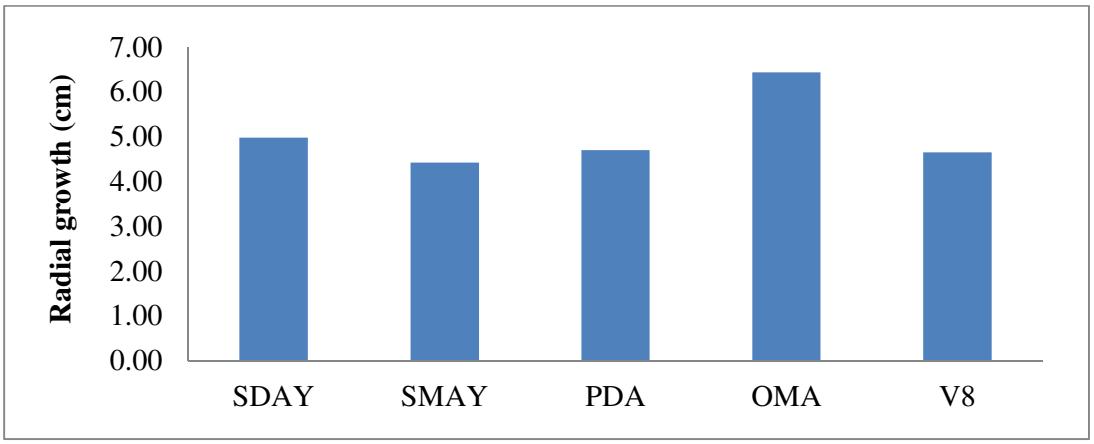

Fig. 4.11: Bar graph illustrating effect of various media on mean radial growth (cm) of *B. bassiana* at 27°C

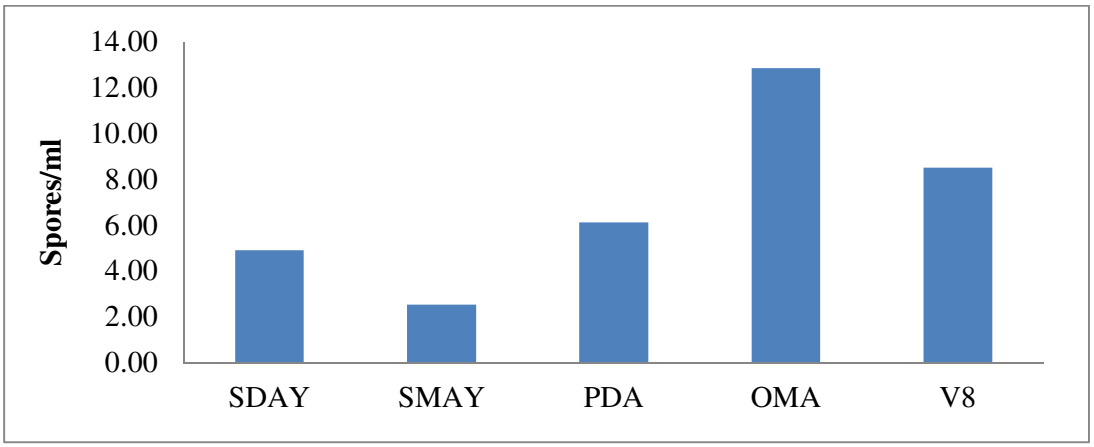

Fig. 4.12: Bar graph illustrating effect of various media on mean conidia count spore/ml (10^7) of *B. bassiana* at 27°C mean

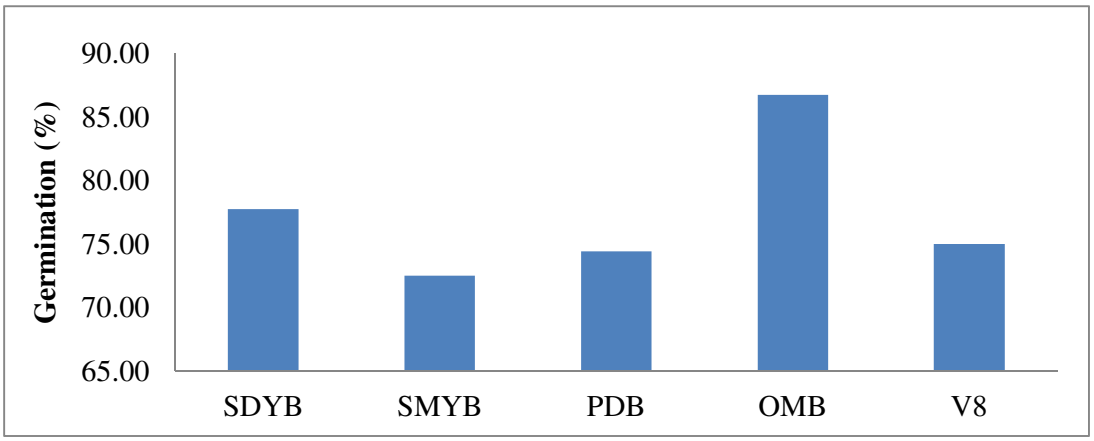

Fig. 4.13: Bar graph illustrating effect of various media on mean per cent germination of *B. bassiana* at 27°C mean

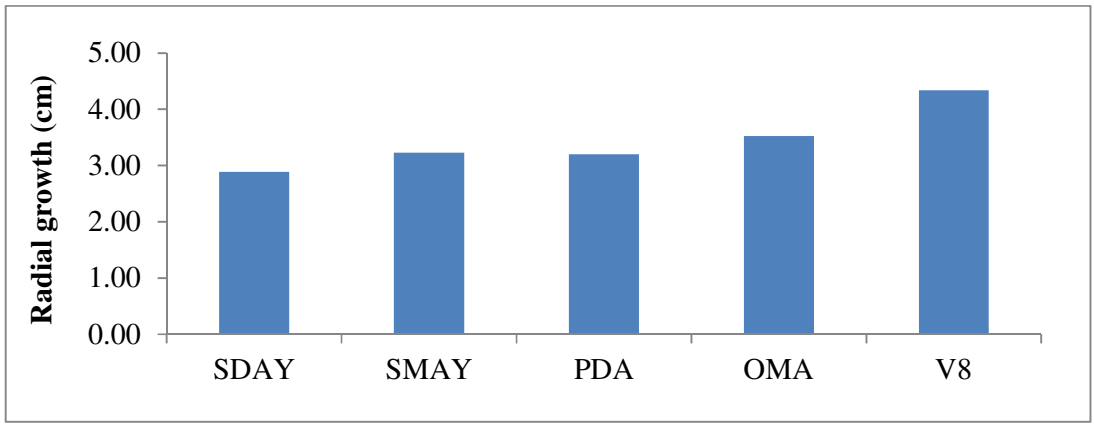

Fig. 4.14: Bar graph illustrating effect of various media on mean radial growth (cm) of *B. bassiana* at 31°C

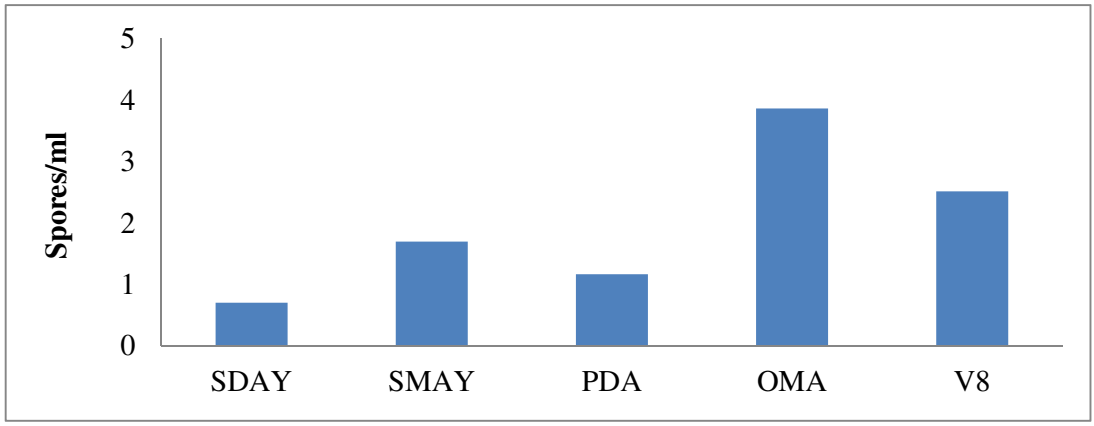

Fig. 4.15: Bar graph illustrating effect of various media on mean conidia count spore/ml (10^7) of *B. bassiana* at 31°C mean

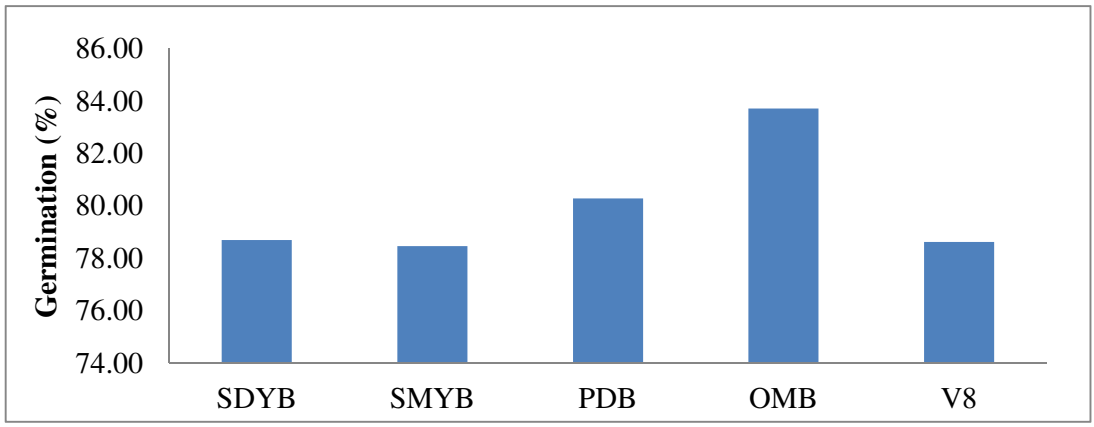

Fig. 4.16: Bar graph illustrating effect of various media on mean per cent germination of *B. bassiana* at 31°C mean

The best media and temperature was documented **(Fig. 4.17, 4.18 and 4.19),** which was identified and ultimately specified from the above mentioned data and experiments were OMA at 21°C. OMA media showed better results in comparison with other four media. It was followed byV-8 then PDA, which was followed by SDAY. The least Fig.ures were obtained from SMAY, keeping all the experiments in consideration these specification were made. The temperature range 21°C-27°C was most suitable for growth and sporulation of *B. bassiana* with maximum spore production.

Similar studies conducted by **Chase *et al.* (1986)** on entomopathogenic fungi *M. anisopliae* and *B. bassiana* on eight different basal media out of which, the basal media that contain Oatmeal agar (OMA) was most superior to other seven media for isolation of both entomopathogenic fungi. **Arora *et al.* (2013)** took five media into consideration for the study the colony characteristics, growth and development of the *Cordyceps sinensis* (Berk.). The media were SDAY (Sabouraud's Dextrose Agar with Yeast extract), PDA (Potato Dextrose Agar), CDA (Czapek Dox Agar), MEA (Malt Extract Agar) and OMA (Oat Meal Agar). **Shreekant *et al.* (2011)** studied *Gliocladium roseum* on four different media *viz.* PDA, Corn Meal Agar (CMA), OMA, and V-8 Juice Agar media. After conducting experiments on growth and development of the fungus, it was observed that V-8 Juice Agar was considered to be the best media followed by CMA, PDA, and OMA media. **Dhar *et al*. (2016)** conducted the study on the growth and development of *B. bassiana* and found that, the optimum temperature for growth and development for most isolates is between the 25˚C and 30˚C. At 40˚C the growth and development of *B. bassiana* came to a full stop, thus making it impossible for the fungus to develop any further. **Fargues *et al.* (1997)** confirmed the effect of temperature on 65 different isolates of *B. bassiana* that the growth was registered in over temperature range between the 8˚C to 35˚C.

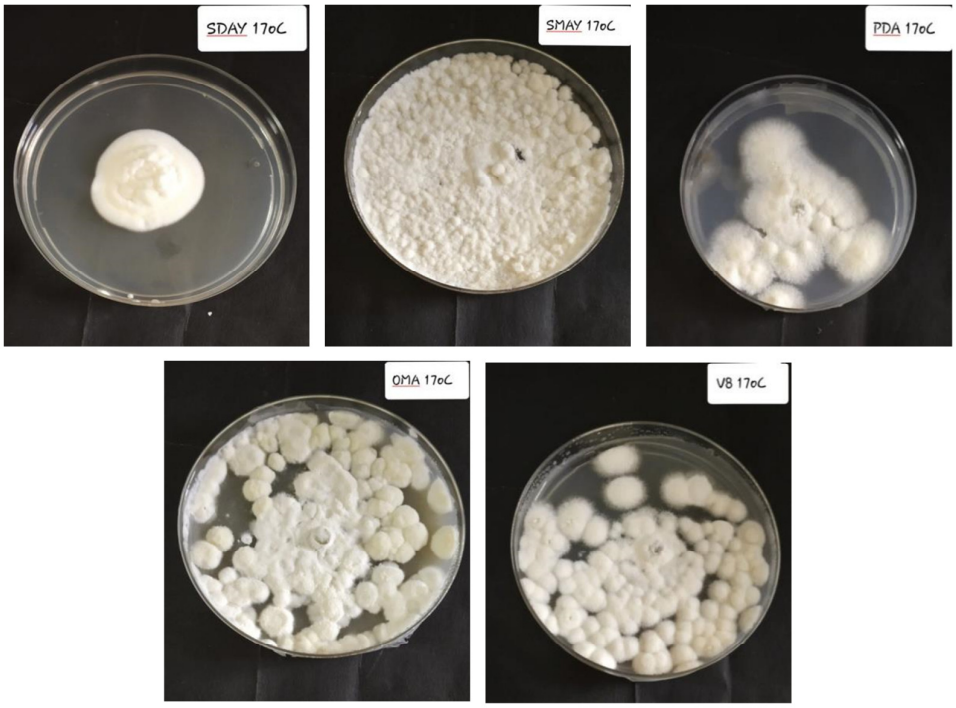

Plate 4: Growth of *B. bassiana* on solid media SDAY, SMAY, PDA, OMA and V-8 at 17°C on 15th day

Plate 5: Growth of *B. bassiana* on solid media SDAY, SMAY, PDA, OMA and V-8 at 21°C on 15th day

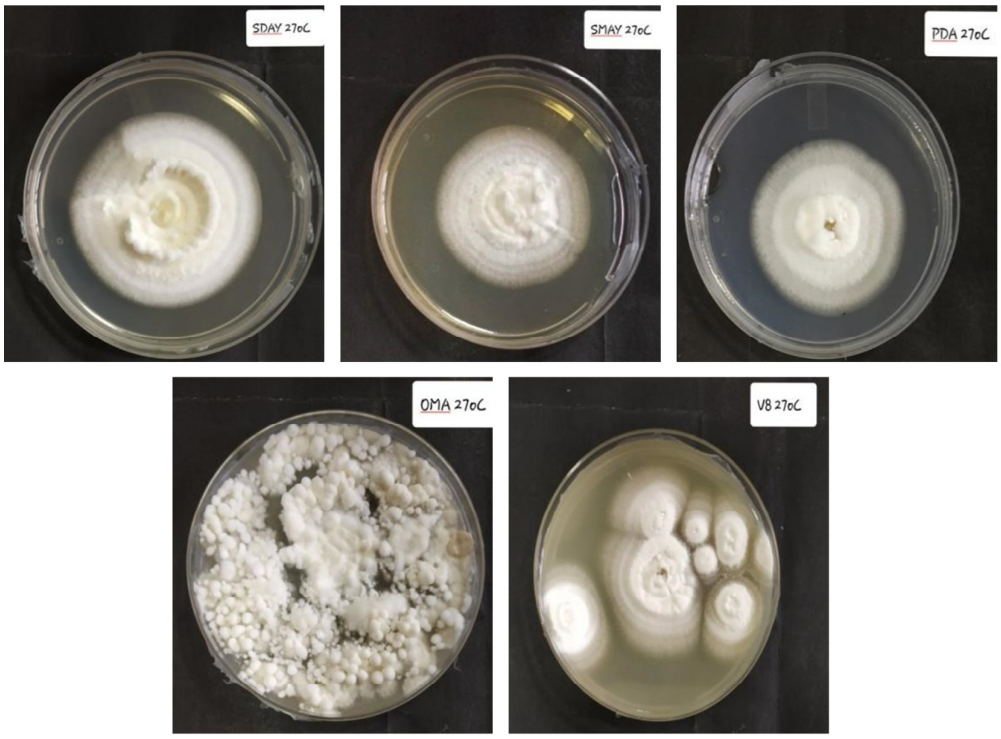

Plate 6: Growth of *B. bassiana* on solid media SDAY, SMAY, PDA, OMA and V-8 at 27°C on 15th day

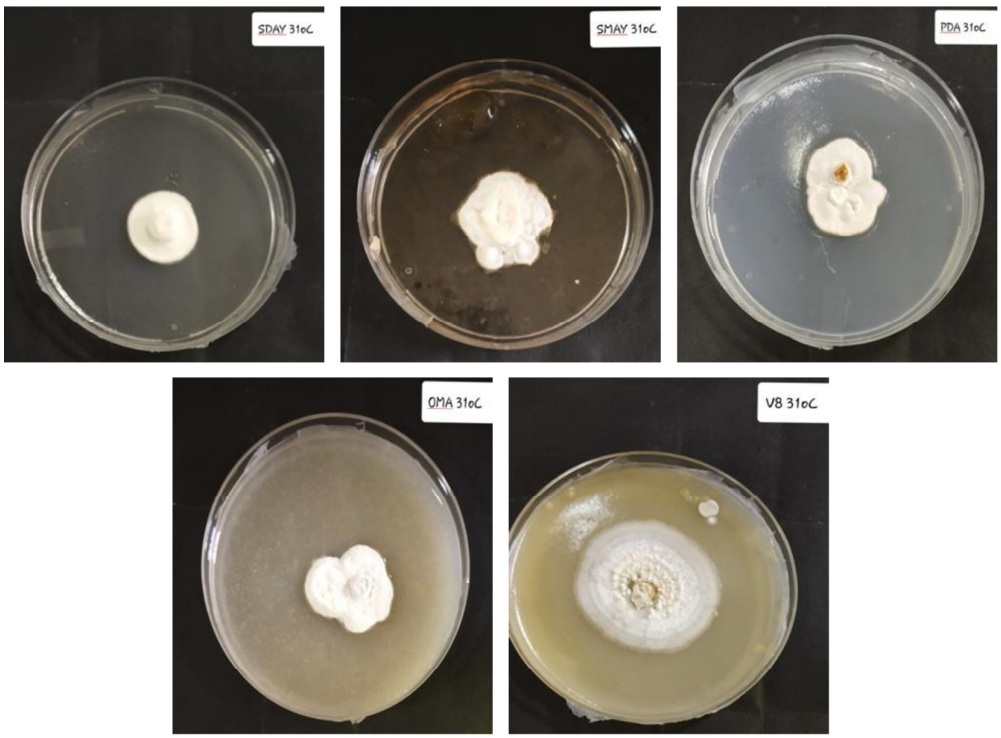

Plate 7: Growth of *B. bassiana* on solid media SDAY, SMAY, PDA, OMA and V-8 at 31°C on 15th day

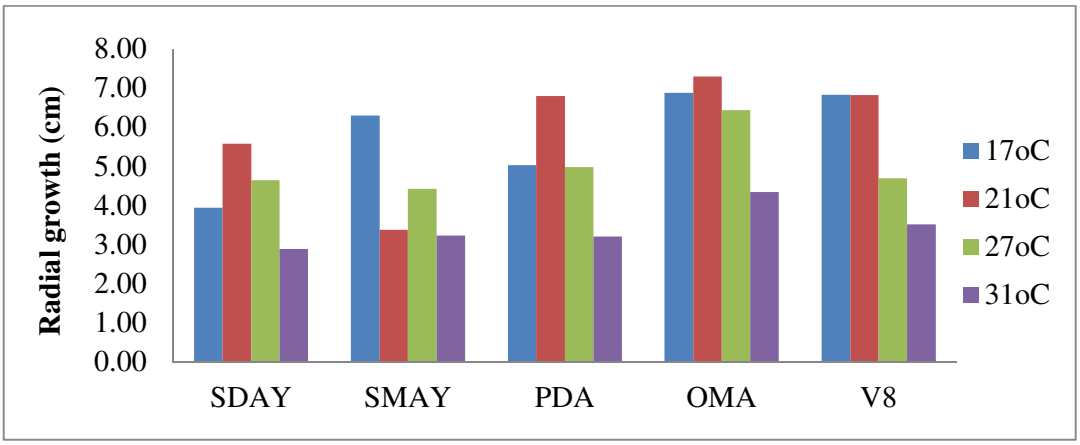

Fig. 4.17: Mean radial growth (cm) at different temperature with response to different media of *B. bassiana*

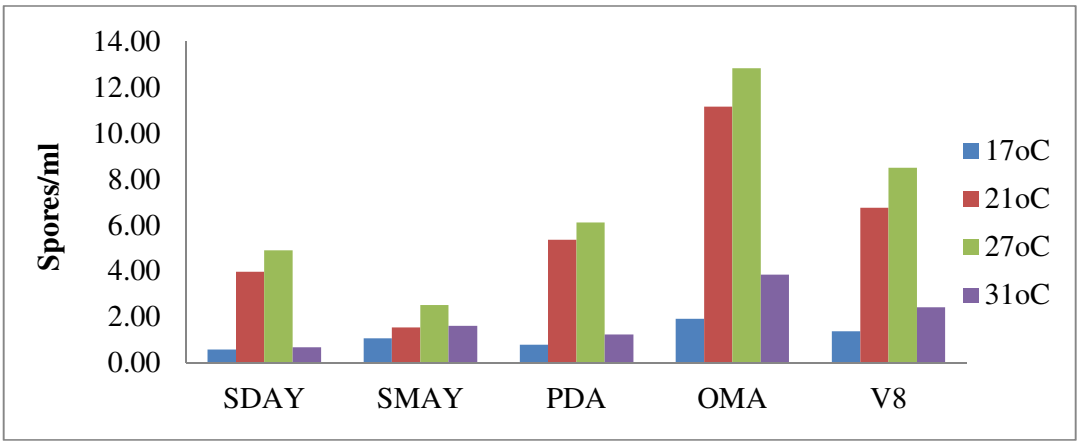

Fig. 4.18: Mean conidia count spore/ml (10^7) at different temperature with response to different media of *B. bassiana*

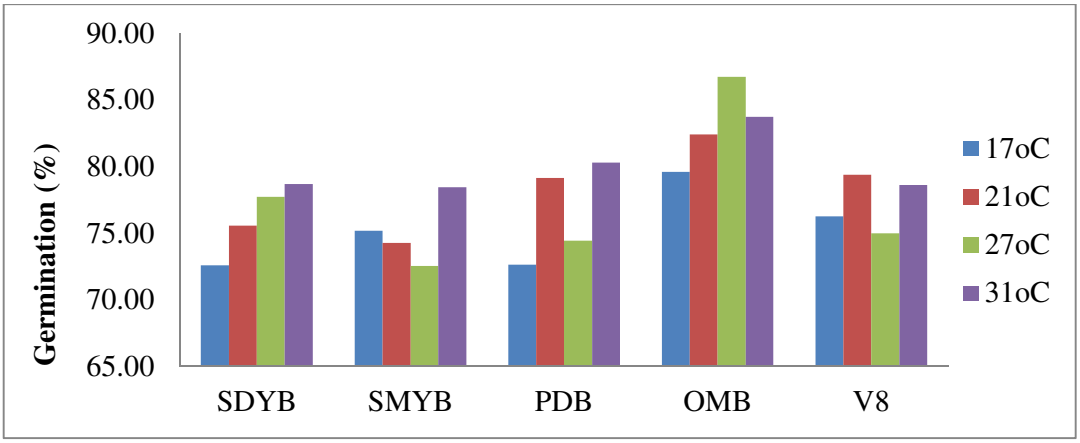

Fig. 4.19: Mean per cent germination at different temperature with response to different media of *B. bassiana*

4.1.3 Mass multiplication of *B. bassiana* on liquid media

The various liquid media/ broth media used in this experiment were SDYB, SMYB, PDB OMB and V-8B. These media were studied at different temperature to understand the growth and development of *B. bassiana*. The different temperatures in this study were 17°C, 21°C, 27°C, and 31°C. Other extreme temperatures were also studied in preliminary basis like 15°C and 35°C. The temperature in between the extremes showed varied data.

The studies of liquid media/ broth on highest biomass (mean wet weight and mean dry weight), and mean conidia/ml at 17°C was documented (**Plate 8) (Table 9 and Fig. 4.20 and 4.21)**. On the basis of highest mean wet weight biomass, the best result was found in OMB (6.89 g) which was followed by SDYB (6.36 g), V-8B (6.24 g), SMYB (5.46 g) and the least mean wet weight biomass was observed for PDB (3.89 g). The highest mean dry weight biomass was seen in OMB (1.98 g) followed by SDYB (1.29 g), V-8B (1.05 g) and SMYB (0.99 g). The least mean dry weight biomass was observed in PDB (0.90 g). When conidia was counted from liquid media/ broth at 17°C as spores/ml, it was found that the highest count was observed in OMB ($2.16x10^7$) then PDB ($2.00x10^7$) followed by SMYB ($1.63x10^7$) and V-8B ($1.27x10^7$). The least amount was documented in SDYB ($0.72x10^7$). The best media on the basis of highest mean wet weight biomass, mean dry weight biomass and mean conidia count/ml was OMB, and the second best was PDB, followed by SMYB and then V-8B on the basis of mean conidial count. The least preferable media was SDYB because of lowest sporulation at 17°C.

The studies on liquid media/ broth at 21°C for biomass (mean wet weight and mean dry weight), and mean conidia/ml was documented (**Plate 9) (Table 10 and Fig. 4.22 and 4.23).** On the basis of highest mean wet weight biomass, the best result was found in OMB (7.14 g) which was followed by SMYB (5.53 g), SDYB (4.47 g), PDB (4.33 g) and the least mean wet weight biomass was observed for V-8B (2.74 g). The highest mean dry weight biomass was seen in SDYB (2.05 g) followed by OMB (1.96 g), SMYB (1.25 g) and V-8B (0.56 g). The least mean dry weight biomass was observed in PDB (0.49 g). When conidia was counted from liquid media/ broth at 21°C as spores/ml, it was found that the highest count was observed in OMB ($13.44x10^7$) then PDB ($10.84x10^7$) followed by V-8B ($8.06x10^7$) and SDYB ($4.80x10^7$). The least amount was documented in SMYB ($1.77x10^7$). The best media on the basis of highest

mean wet weight biomass, mean dry weight biomass and mean conidia count/ml was OMB, and the second best was PDB, followed by V-8B and then SDYB. The least preferable media was SMYB because of lowest sporulation at 21^{o}C.

The studies on liquid media/ broth at 27^{o}C for highest biomass (mean wet weight and mean dry weight), and mean conidia/ml was documented (**Plate 10) (Table 11 and Fig. 4.22 and 4.23)**. On the basis of highest mean wet weight biomass, the best result was in OMA (6.64 g) which was followed by SMYB (4.92 g), SDYB (3.34 g), PDB (1.88 g) and the least mean wet weight biomass was observed for V-8B (1.15 g). The highest mean dry weight biomass was seen in OMB (0.99 g) followed by SDYB (0.73 g), SMYB (0.69 g) and PDB (0.63 g). The least mean dry weight biomass was observed in V-8B (0.49 g). When conidia was counted from liquid media/ broth at 27^{o}C as spores/ml, it was found that the highest count was observed in OMB ($15.38x10^{7}$) then PDB($9.96x10^{7}$) followed by SMYB ($9.76x10^{7}$) and SDYB ($5.56x10^{7}$). The least amount was documented in V-8B ($3.00x10^{7}$). The best media on the basis of highest mean wet weight biomass, mean dry weight biomass and mean conidia count/ml was OMB, and the second best was PDB, followed by SMYB and then SDYB. The least preferable media was V-8B because of lowest sporulation at 27^{o}C.

The studies on liquid media/ broth at 31^{o}C for highest biomass (mean wet weight and mean dry weight), and mean conidia/ml was documented (**Plate 11) (Table 12 and Fig. 4.26 and 4.27)**. On the basis of highest mean wet weight biomass, the best result was in SMYB (2.44 g) which was followed by PDB (0.99 g), OMB (0.95 g), and V-8B (0.80 g) the least mean wet weight biomass was observed for SDYB (0.55 g). The highest mean dry weight biomass was seen in SMYB (0.53 g) followed by OMB (0.47 g), SDYB (0.38 g) and V-8B (0.37 g). The least mean dry weight biomass was observed in PDB (0.29 g). When conidia was counted from liquid media/ broth at 31^{o}C as spores/ml, it was found that the highest count was observed in SDYB ($1.42x10^{7}$) then PDB ($0.89x10^{7}$) followed by OMB ($0.75x10^{7}$) and SMYB ($0.48x10^{7}$). The least amount was documented in V-8B ($0.32x10^{7}$). The best media on the basis of highest mean wet weight biomass, mean dry weight biomass and mean conidia count/ml was OMB, and the second best was PDB, followed by SMYB and then SDYB. The least preferable media was V-8B because of lowest sporulation at 31^{o}C. At 31^{o}C there is not much difference observed among the different media because of low growth and sporulation of fungus.

Table 9: Studies of effect of various liquid media on mean wet weight, dry weight and conidial count of *B. bassiana* at 17°C

Temperature	Media	Mean wet weight (g/100 ml)	Mean dry weight (g)	Mean conidial count spore /ml (10^7 conidia)
17°C	**SDYB**	6.36	1.29	0.72
	SMYB	5.46	0.99	1.63
	PDB	3.89	0.90	2.00
	OMB	6.89	1.98	2.16
	V-8B	6.24	1.05	1.27
	S.Em.±	**0.55**	**0.31**	**0.18**
	CD (5%)	**0.17**	**0.10**	**0.58**
	CV	**1.66**	**4.43**	**2.03**

Table 10: Studies of effect of various liquid media on mean wet weight, dry weight and conidial count of *B. bassiana* at 21°C

Temperature	Media	Mean wet weight (g/100 ml)	Mean dry weight (g)	Mean conidial count spore /ml (10^7 conidia)
21°C	**SDYB**	4.47	2.05	4.80
	SMYB	5.53	1.25	1.77
	PDB	4.33	0.49	10.84
	OMB	7.14	1.96	13.44
	V-8B	2.74	0.56	8.06
	S.Em.±	**0.85**	**0.35**	**0.88**
	CD (5%)	**0.26**	**0.11**	**0.27**
	CV	**3.04**	**4.88**	**1.97**

Table 11: Studies of effect of various liquid media on mean wet weight, dry weight and conidial count of *B. bassiana* at 27°C

Temperature	Media	Mean wet weight (g/100 ml)	Mean dry weight(g)	Mean conidial count spore / ml (10^7 conidia)
27°C	**SDYB**	3.34	0.73	5.56
	SMYB	4.92	0.69	9.76
	PDB	1.88	0.63	9.96
	OMB	6.64	0.99	15.38
	V-8B	1.15	0.49	3.00
	S.Em.±	**0.48**	**0.61**	**0.19**
	CD (5%)	**0.15**	**0.19**	**0.60**
	CV	**2.36**	**1.51**	**0.39**

Table 12: Studies of effect of various liquid media on mean wet weight, dry weight and conidial count of *B. bassiana* at 31°C

Temperature	Media	Mean wet weight (g/100 ml)	Mean dry weight (g)	Mean conidial count spore / ml (10^7 conidia)
31°C	**SDYB**	0.55	0.38	1.42
	SMYB	2.44	0.53	0.48
	PDB	0.99	0.29	0.89
	OMB	0.95	0.47	0.75
	V-8B	0.80	0.37	0.32
	S.Em.±	**0.33**	**0.60**	**0.17**
	CD (5%)	**0.10**	**0.19**	**0.55**
	CV	**5.02**	**2.57**	**4.28**

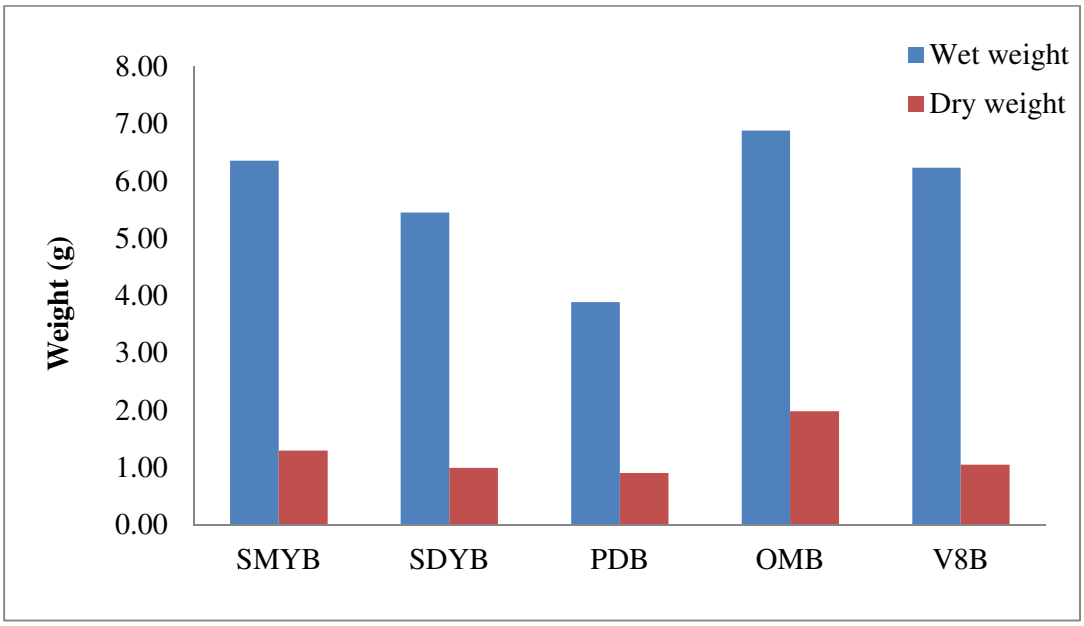

Fig. 4.20: Bar graphs representation of mean wet and dry biomass (g) at 17°C in response to different liquid broth of *B. bassiana*

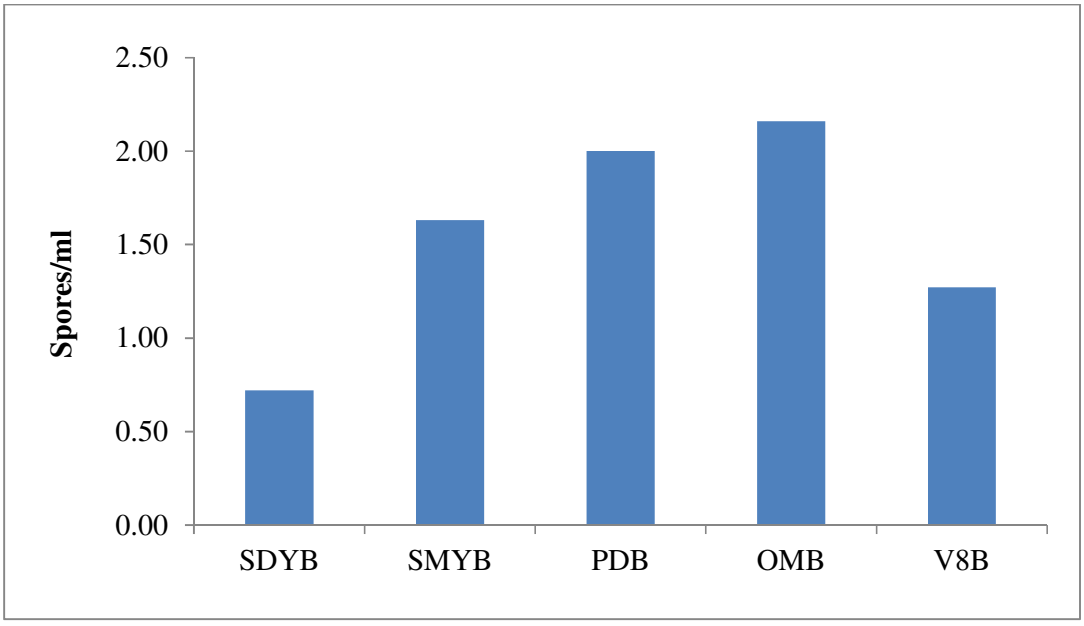

Fig. 4.21: Bar graphs illustrating various media and their respective effect of 17°C temperature on mean conidia count spore/ml (10^7) of *B. bassiana*

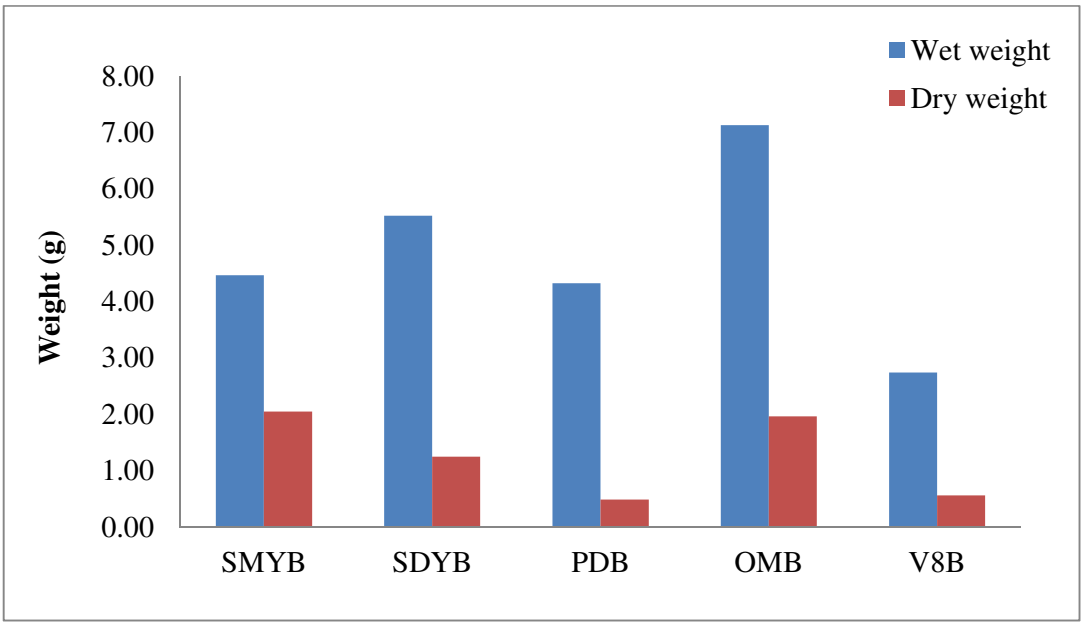

Fig. 4.22: Bar graphs representation of mean wet and dry biomass (g) at 21°C in response to different liquid broth of *B. bassiana*

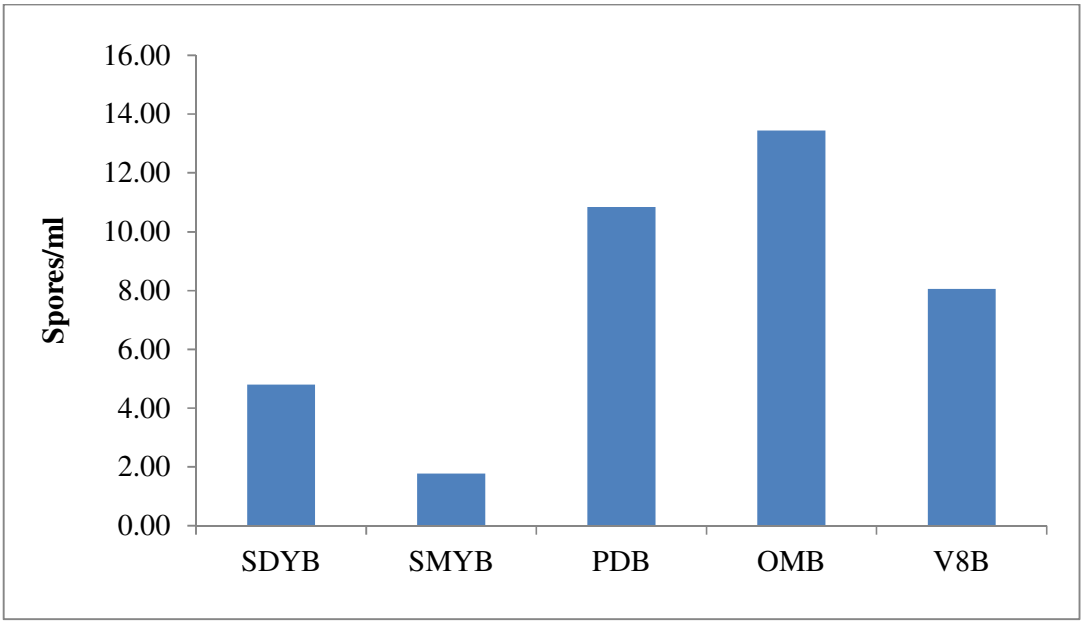

Fig. 4.23: Bar graphs illustrating various media and their respective effect of 21°C temperature on mean conidia count spore/ml (10^7) of *B. bassiana*

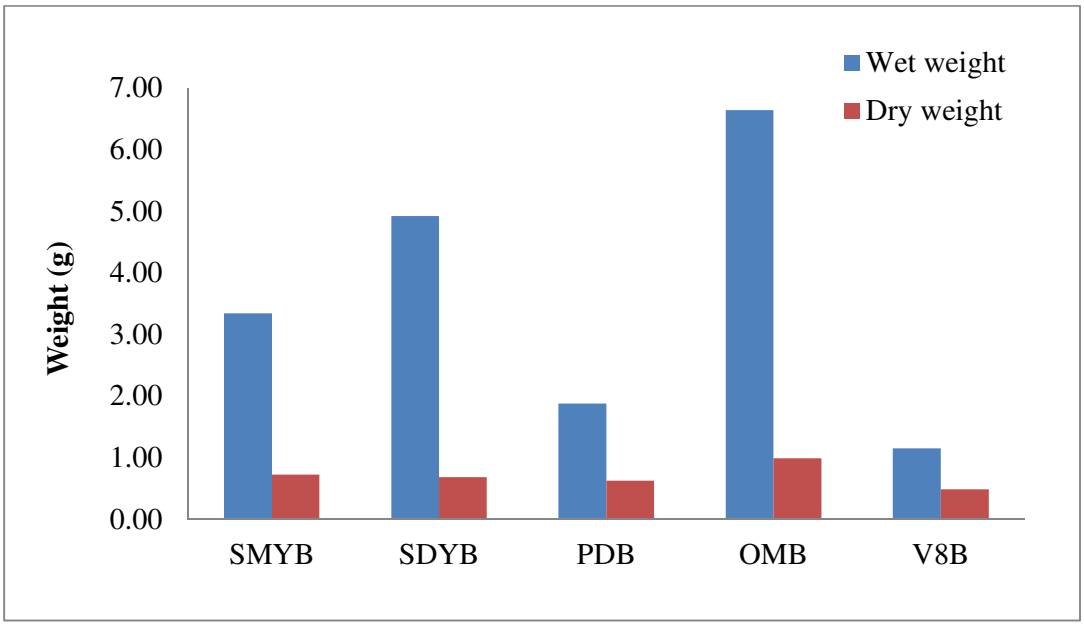

Fig. 4.24: Bar graphs representation of mean wet and dry biomass (g) at 27°C in response to different liquid broth of *B. bassiana*

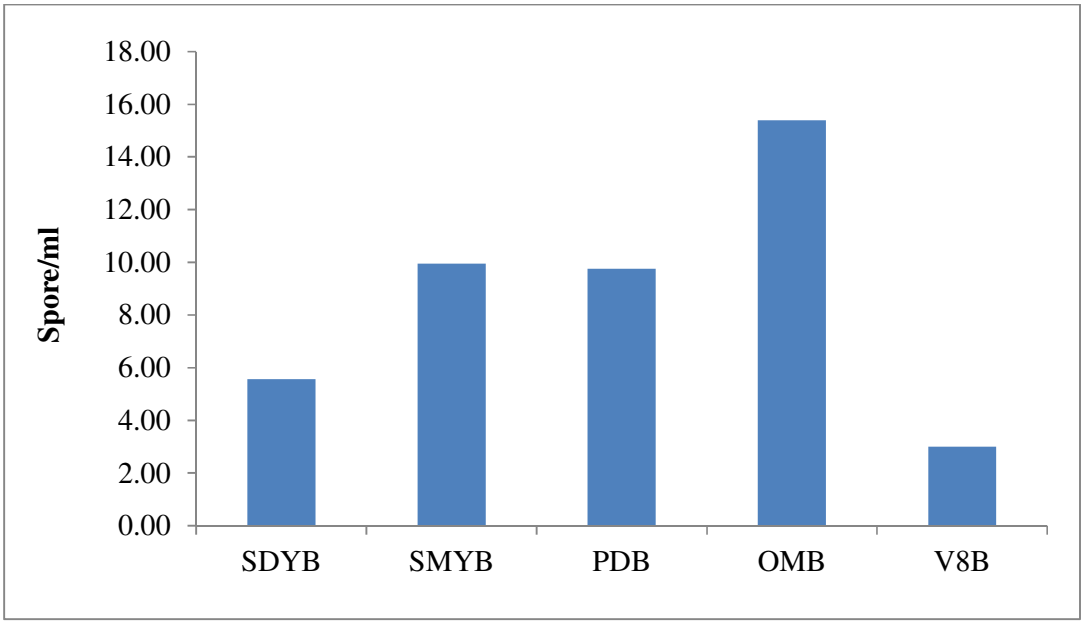

Fig. 4.25: Bar graphs illustrating various media and their respective effect of 27°C temperature on mean conidia count spore/ml (10^7) of *B. bassiana*

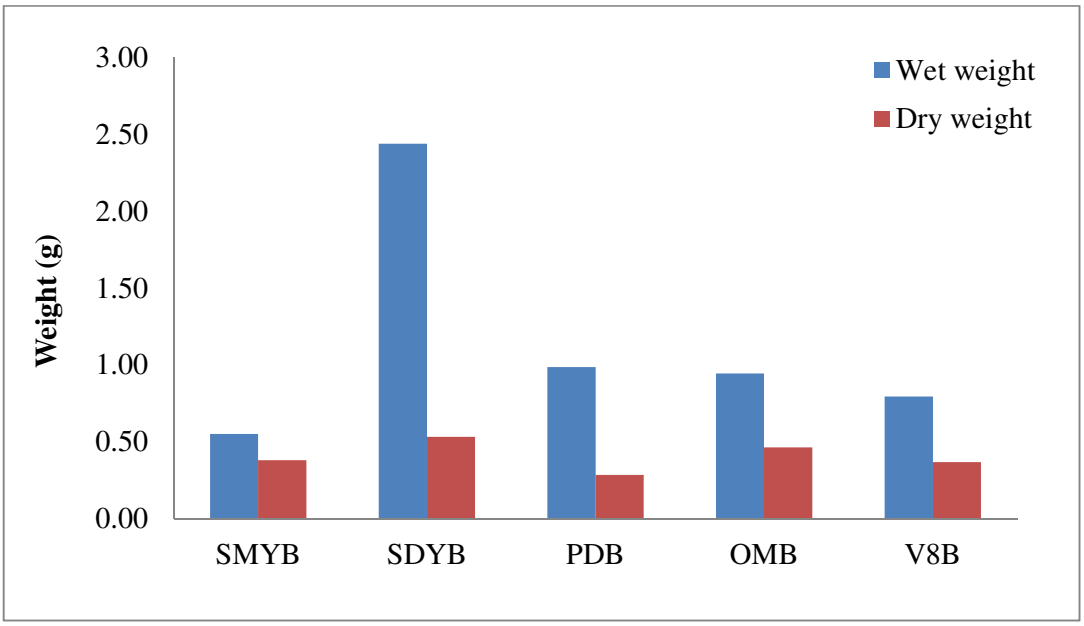

Fig. 4.26: Bar graphs representation of mean wet and dry biomass (g) at 31°C in response to different liquid broth of *B. bassiana*

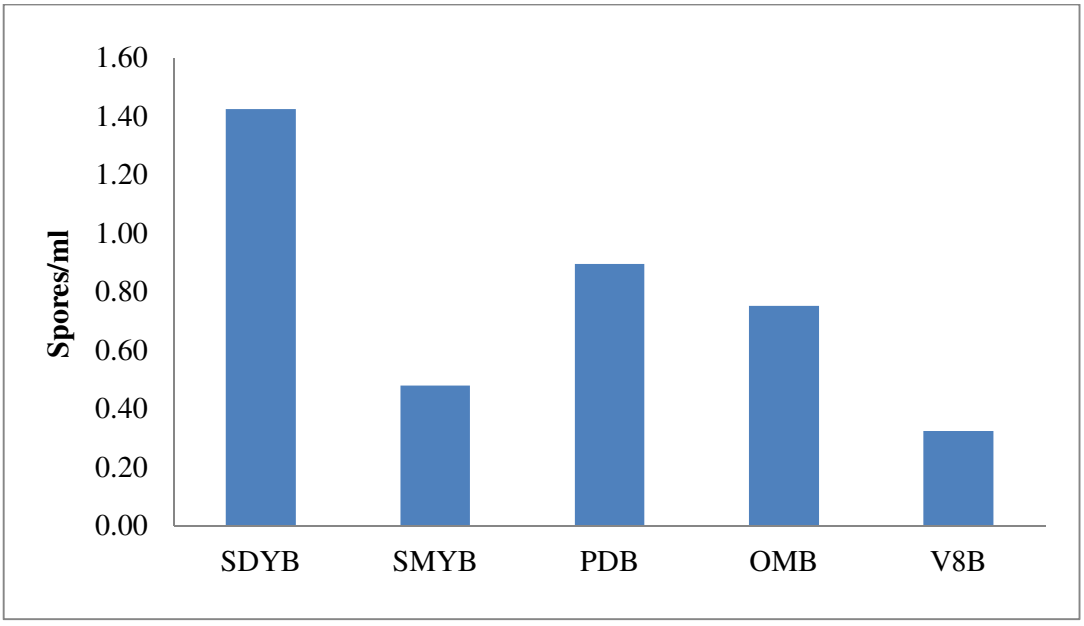

Fig. 4.27: Bar graphs illustrating various media and their respective effect of 31°C temperature on mean conidia count spore/ml (10^7) of *B. bassiana*

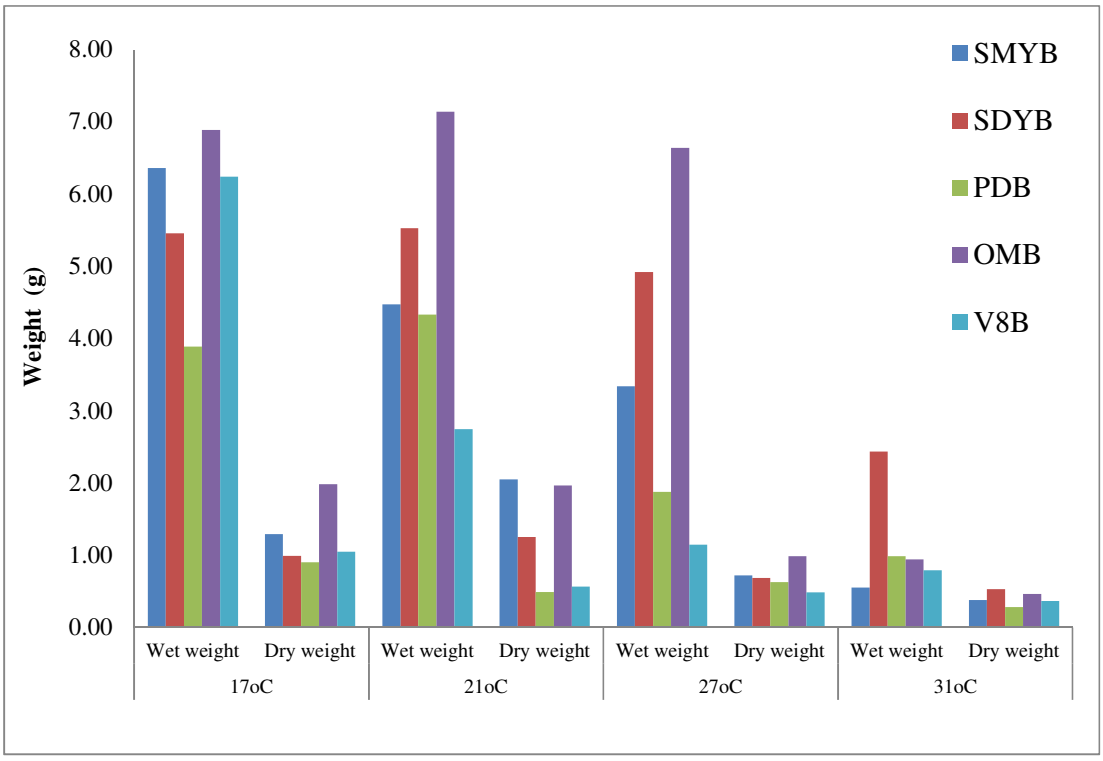

Fig. 4.28: Mean biomass wet and dry weight (g) at different temperature with response to different media

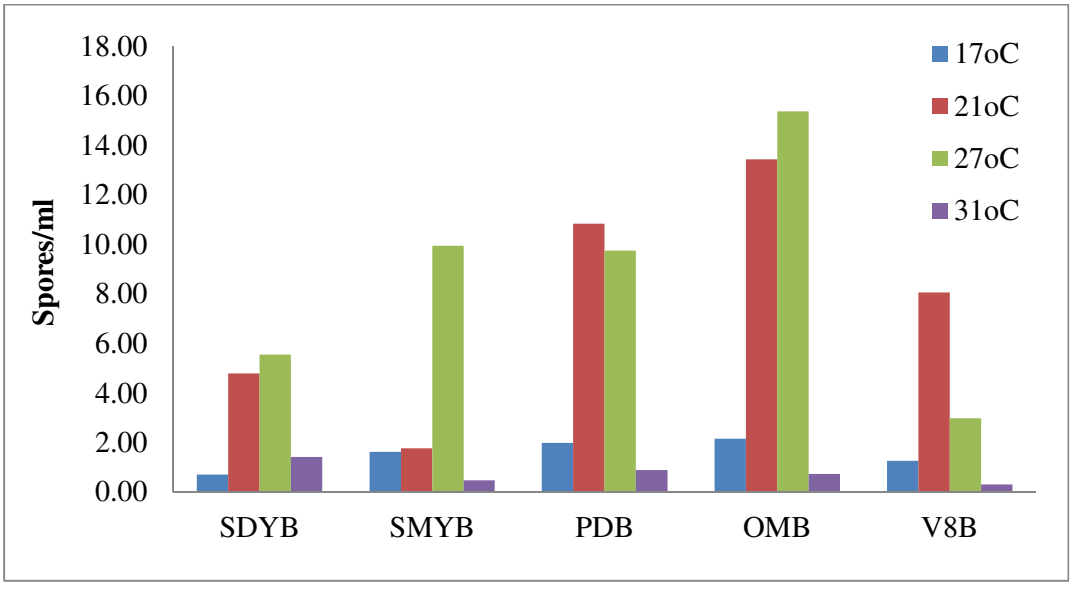

Fig. 4.29: Mean conidia/ml (10^7) at different temperature with response to different media

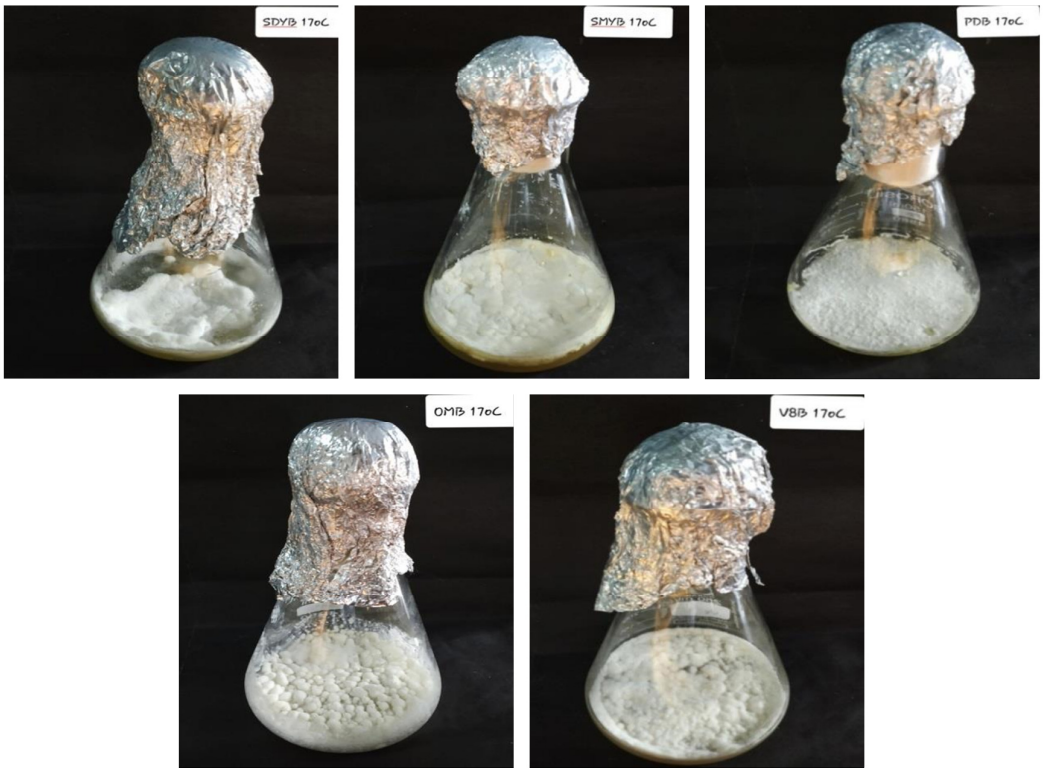

Plate 8: Liquid media SDYB, SMYB, PDB, OMB and V-8B at 17°C on 15th day

Plate 9: Liquid media SDYB, SMYB, PDB, OMB and V-8B at 21°C on 15th day

Plate 10: Liquid media SDYB, SMYB, PDB, OMB and V-8B at 27^{o}C on 15th day

Plate 11: Liquid media SDYB, SMYB, PDB, OMB and V-8B at 31^{o}C on 15th day

Therefore, the above experiments revealed **(Fig. 4.28 and 4.29),** that mass multiplication in liquid media, it would be wise to utilize OMB for getting highest biomass, followed by SDYB then SMYB, which was preceded by PDB and lastly V-8B. The best biomass weight could be achieved in a range of 17°C-21°C. But in case of conidia count getting highest conidia count spore/ml is OMB, followed by PDB then SMYB, which was preceded by V-8B and lastly SDYB. The best biomass weight and conidia count spore/ml could be achieved in a range of 21°C-27°C. Similar studies conducted by **Dhar *et al.* (2016)** conducted the study on the growth and development of *B. bassiana* and found that, the optimum temperature for growth and development for most isolates is between the 25˚C and 30˚C. At 40˚C the growth and development of *B. bassiana* came to a full stop, thus making it impossible for the fungus to develop any further. **Fargues *et al.* (1997)** confirmed the effect of temperature on 65 different isolates of *B. bassiana* that the growth was registered in over temperature range between the 8˚C to 35˚C.

4.2 Bioefficacy of *B. bassiana* conidial suspension and sunflower oil formulation against *S. litura* by 'larval atomization' method

4.2.1. Preliminary screening

Three concentrations of *B. bassiana* conidial suspension and sunflower oil formulation *viz.*1000×10^5, 500×10^5 and 75×10^5 were prepared in tap water. In order to test contact toxicity, third instar larvae were thoroughly atomized with the help of a hand atomizer. The observations on mortality were recorded at 12, 24, 36 and 48 hours after treatment (HAT) and these observations led to the selection of dosage range for the final testing.

4.2.2. Final testing

Toxicity of *B. bassiana* conidial suspension

The toxicity of *B. bassiana* conidial suspension was determined by larval atomization method against third instars larvae of *S. litura* under laboratory conditions. Six dilutions 1000×10^5, 700×10^5, 500×10^5, 250×10^5, 125×10^5 and 75×10^5 of *B. bassiana* conidial suspension were prepared in tap water. The mortality data was recorded in the toxicity trial from 12 HAT to upto 120 HAT at 12 hours interval and Duncan's multiple range test (DMRT) **(Duncan, 1955)** was performed to test the significance of the results **(Plate 12)**, The data has been presented in **Table 13 and Fig. 4.30.**

Mortality response

It is evident from the data presented in the **Table 13** that upto 24 HAT no mortality was observed at any concentration. At 36 HAT, highest two concentration i.e., 1000×10^5 and 700×10^5 showed 6.67 per cent mortality. At, 500×10^5 concentration 3.33 per cent mortality was observed at same hour after treatment. The mortality response showed an increasing trend in the later hours after treatments. At 48 HAT, mortality response was seen in every concentration except the lowest concentration 75×10^5, highest mortality 16.67 per cent was recorded at 1000×10^5concentration followed by 10 per cent at 700×10^5 and lowest at 125×10^5 i.e., 3.33 per cent. At 60 HAT again no mortality was observed in the lowest concentration. However at concentration of 1000×10^5 showed 46.67 per cent mortality was recorded. The mortality response started at 72 HAT in the lowest concentration of 75×10^5 i.e., 13.33per cent. Highest mortality response was 66 per cent at the highest concentration of 1000×10^5at 72 HAT.

100 per cent mortality was observed at 108 HAT in the highest concentration of 1000×10^5 and at 120 HAT in 700×10^5. No other concentration could cause complete 100 per cent mortality even upto 120 HAT. The lowest concentration could cause 23.33 per cent mortality upto 120 HAT.

Toxicity of *B. bassiana* sunflower oil formulation

The toxicity of *B. bassiana* sunflower oil formulation, was determined by larval atomization method against third instars' larvae of *S. litura* under laboratory conditions. Six dilutions 1000×10^5, 700×10^5, 500×10^5, 250×10^5, 125×10^5 and 75×10^5 of *B. bassiana* sunflower oil formulation were prepared in tap water. The mortality data was recorded in the toxicity trial from 12 HAT to upto 120 HAT at 12 hours interval and Duncan's multiple range test (DMRT) **(Duncan, 1955)** was performed to test the significance of the results, The data has been presented in **Table 14 and Fig. 4.31.**

Mortality response

The data presented in the **Table 14** showed that again none of the concentration could cause any mortality in the treated larve upto 24 HAT. The mortality response begins at 36 HAT, the highest two concentration i.e., 1000×10^5 and 700×10^5 showed

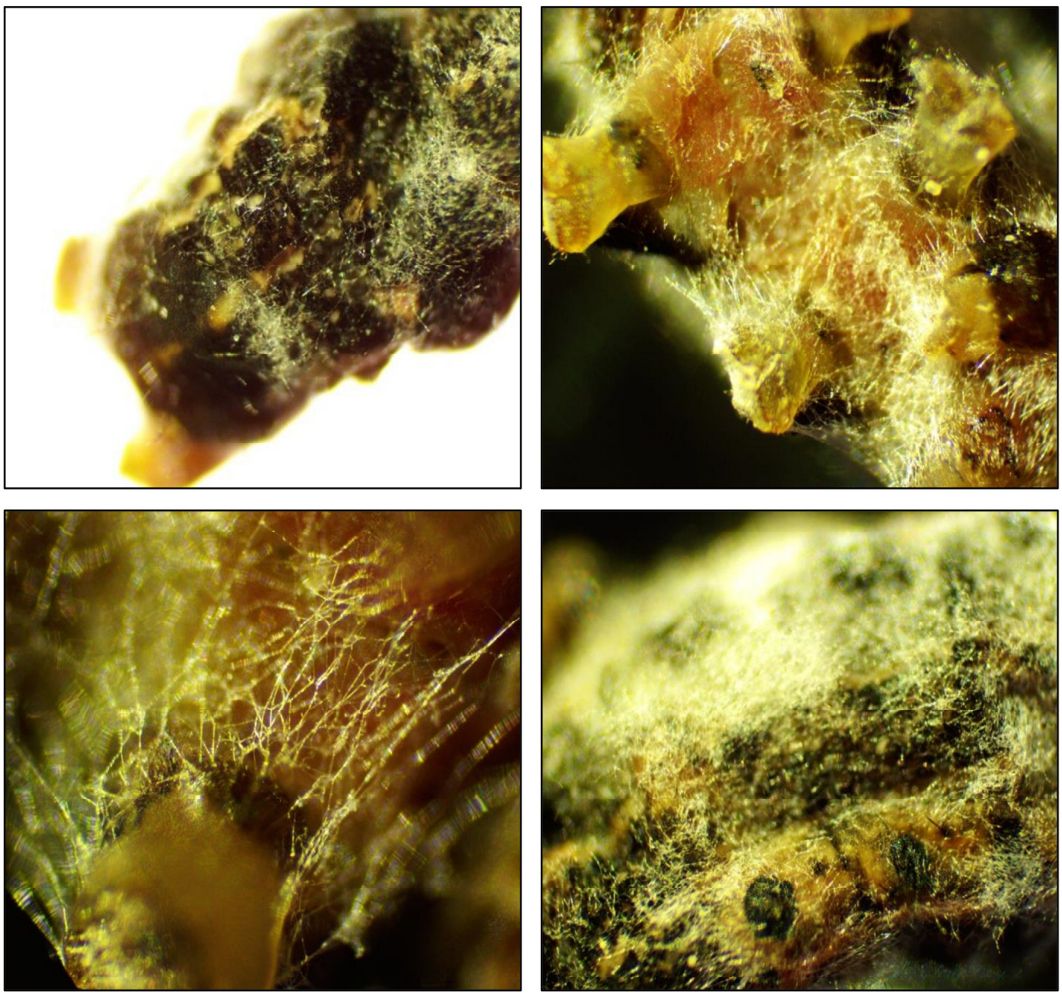

Plate 12: *B. bassiana* mycelia growth on the body of *S. litura* cadaver under microscope

some mortality response, which was 6.67 per cent for both the concentration. At 500×10^5 concentration 3.33 per cent mortality was observed at same hour after treatment. The mortality response showed an increasing trend in the later hours after treatments. At 48 HAT, mortality response was seen in all the concentrations except the lowest 75×10^5, highest being 23.33 per cent at 1000×10^5 followed by 13.33 per cent at 700×10^5 and 500×10^5 and lowest at 125×10^5 i.e., 3.33 per cent.

At 60 HAT again no mortality was observed in the lowest concentration. The mortality response started at 72 HAT in the lowest concentration of 75×10^5 i.e., 13.33per cent. Highest mortality response was 70 per cent at the highest concentration of 1000×10^5. Complete mortality was observed at 108 HAT in the highest concentration of 1000×10^5 and at 120 HAT in 700×10^5. No other concentration could cause complete 100 per cent mortality even upto 120 HAT. The lowest concentration could cause 23.33 per cent mortality upto 120 HAT.

A comparison between the two formulations indicated that there was not much difference between the contact toxicities of the *B. bassiana.* However, oil formulation showed a little higher toxicity. At 48 HAT the mortality in case of conidial suspension was 16.67 however it was 23.33 per cent at highest concentration of 1000×10^5 in case of oil formulation.

In other concentrations at various hours after treatment oil formulation is showing higher toxicity as compared to conidial suspension **(Table 13 and 14)**.

The efficacy of local isolates of *B. bassiana* was evaluated against third instar larvae of *S. litura* using the leaf spray method by **Moorthi *et al.* (2011).** At four days after treatment 66.67, 73.33 and 80.0 per cent mortality was obtained in the isolates Bb02, Bb09 and Bb10, respectively. The LC_{50} values of the various isolates were $2.1x10^6$, $3.6x10^7$ and $1.2x10^7$conidia/ml for Bb02, Bb09 and Bb10, respectively. The LT_{50} value for Bb02 and Bb09 was 4.8 days, whereas it was 4.0 days for Bb10 @10^8spore/ml. In another experiment by **Asi *et al.* (2013)** by larval dip method, the results revealed that the LC_{50} value for 3rd instar larvae was 1.11×10^7 conidia/ml for a local strain i.e., *B. bassiana* 25 at 10 days after treatment. The LT_{50} for 3^{rd} instar larvae was 187 hours in *B. bassiana* 25 at concentration of $1x10^8$conidia/ml.

Table 13: Mean mortality per cent on hours basis for different concentrations of conidial suspension for *S. litura*

Concentration	Treatment	12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	84HAT	96HAT	108HAT	120HAT
1000×10^5	**T1**	0±0^a	0±0^a	6.67±5.77^a	16.67±5.77^c	46.67±5.77^e	66±15.27^d	80±0^e	93.33±5.77^e	100±0^e	100±0^f
700×10^5	**T2**	0±0^a	0±0^a	6.67±5.77^a	10±0^{bc}	33.33±5.77^d	60±10^{cd}	70±10de	86.67±5.77^{de}	93.33±5.77^e	100±0^f
500×10^5	**T3**	0±0^a	0±0^a	3.33±5.77^a	6.67±5.77^{ab}	26.67±5.77^{cd}	46.67±11.54^c	60±10^d	73.33±11.55^d	80±10^d	86.67±5.77^e
250×10^5	**T4**	0±0^a	0±0^a	0.0±0.0^a	6.67±5.77^{ab}	20±0^{bc}	26.67±5.77^b	40±10^c	50±10^c	56.67±11.55^c	60±10^d
125×10^5	**T5**	0±0^a	0±0^a	0.0±0.0^a	3.33±5.77^{ab}	13.33±5.77^b	26.67±5.77^b	30±10^{bc}	30±10^b	33.33±5.77^b	33.33±5.77^c
75×10^5	**T6**	0±0^a	0±0^a	0.0±0.0^a	0.0±0.0^a	0±0^a	13.33±5.77^{ab}	20±10^b	20±10^b	23.33±5.77^b	23.33±5.77^b
Control (Untreated)	**T7**	0±0^a	0±0^a	0.0±0.0^a	0.0±0.0^a	0±0^a	0±0^a	0±0^a	0±0^a	0±0^a	0±0^a

Means (±SE) followed by common letters are not significantly different at p=0.05 per cent according to DMRT.

HAT = hour after treatment

Table 14: Mean mortality per cent on hours basis for different concentrations of sunflower oil formulation for *S. litura*

Concentration	Treatment	12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	84HAT	96HAT	108HAT	120HAT
1000×10^5	**T1**	0±0^a	0±0^a	6.67±5.77^a	23.33±5.77^c	50±10^e	70±17.32^d	83.33±5.77^e	93.33±5.77^e	100±0^e	100±0^f
700×10^5	**T2**	0±0^a	0±0^a	6.67±5.77^a	13.33±5.77^b	36.67±5.77^d	63.33±5.77^{cd}	73.33±5.77^{de}	86.67±5.77^{de}	93.33±5.77^e	100±0^f
500×10^5	**T3**	0±0^a	0±0^a	3.33±5.77^a	13.33±5.77^b	30±10^{cd}	50±10^d	63.33±11.55^d	73.33±11.55^d	80±10^d	86.67±5.77^e
250×10^5	**T4**	0±0^a	0±0^a	0.0±0.0^a	6.67±5.77^{ab}	23.33±5.77^{bc}	30±10^b	43.33±5.77^c	50±10^c	56.67±11.55^c	60±10^d
125×10^5	**T5**	0±0^a	0±0^a	0.0±0.0^a	3.33±5.77^a	13.33±5.77^b	26.67±5.77^b	30±10^{bc}	30±10^b	33.33±5.77^b	33.33±5.77^c
75×10^5	**T6**	0±0^a	0±0^a	0.0±0.0^a	0.0±0.0^a	0±0^a	13.33±5.77^{ab}	20±10^b	20±10^b	23.33±5.77^b	23.33±5.77^b
Control (Untreated)	**T7**	0±0^a	0±0^a	0.0±0.0^a	0.0±0.0^a	0±0^a	0±0a	0±0^a	0±0^a	0±0^a	0±0^a

Means (±SE) followed by common letters are not significantly different at p=0.05 per cent according to DMRT.

HAT = hour after treatment

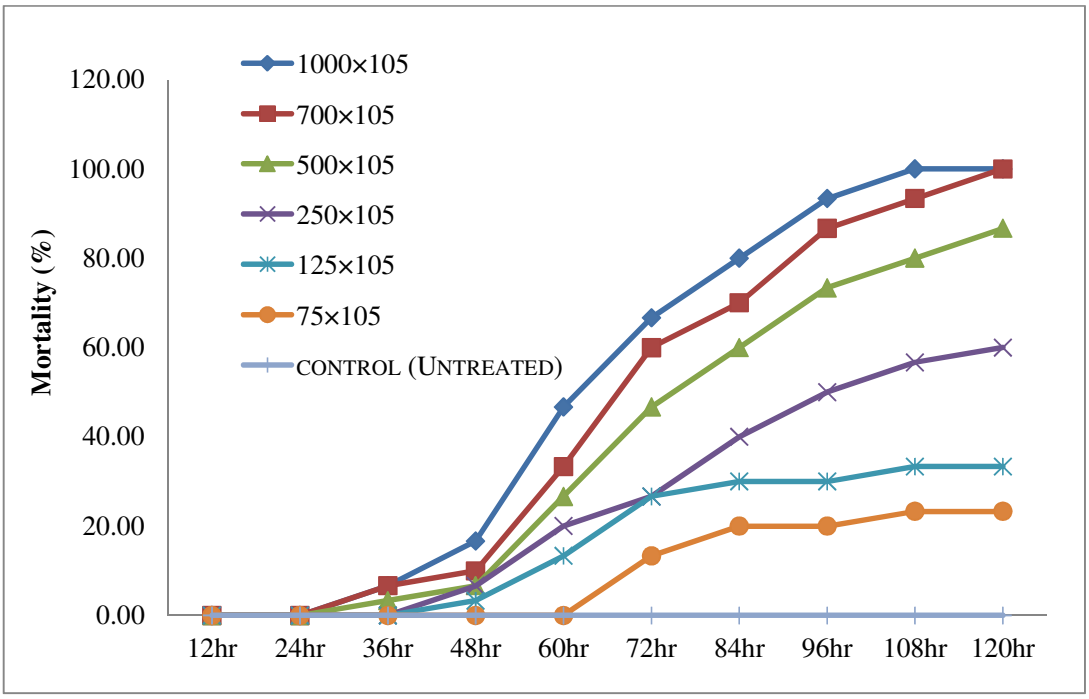

Fig. 4.30: Line graph representation of mean mortality per cent of *S. litura* in response to conidial suspension of *B.bassiana*

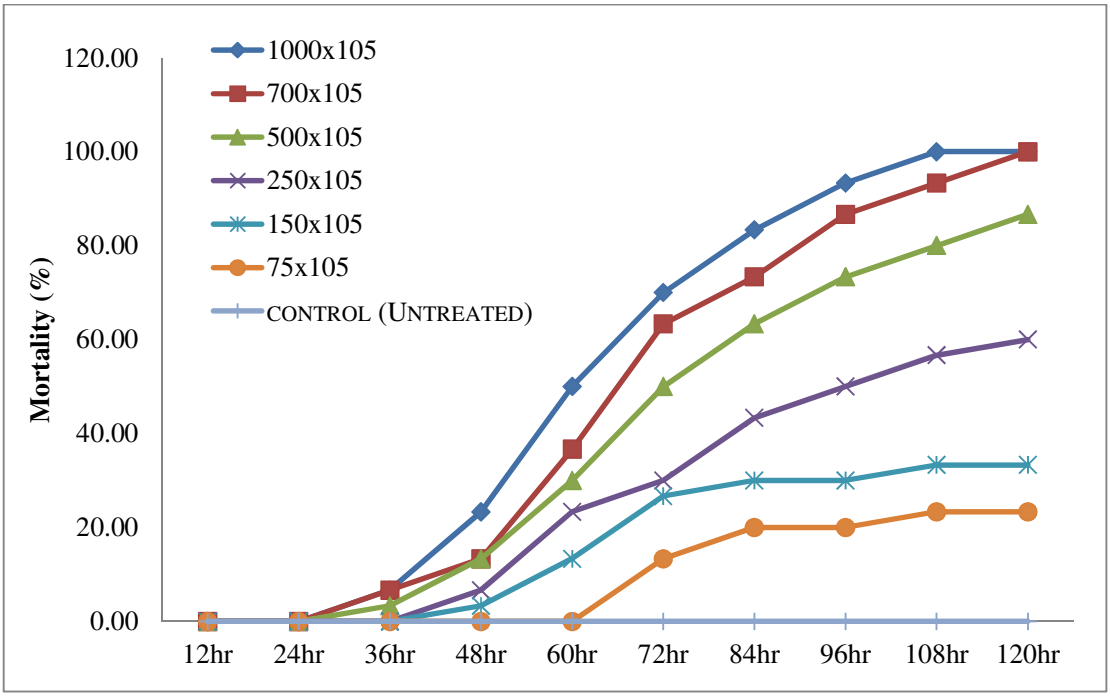

Fig. 4.31: Line graph representation of mean mortality per cent of *S. litura* in response to oil formulation of *B.bassiana*

4.3 Bioefficacy of *B. bassiana* conidial suspension and sunflower oil formulation against *G. mellonella* by 'larval atomization' method.

4.3.1 Preliminary screening

Three concentrations of *B. bassiana* conidial suspension and sunflower oil formulation *viz.*1000×10^5, 500×10^5 and 75×10^5 were prepared in tap water. In order to test contact toxicity, third instar larvae were thoroughly atomized with the help of a hand atomizer. The observations on mortality were recorded at 12, 24, 36 and 48 hours after treatment (HAT) and these observations led to the selection of dosage range for the final testing.

4.3.2 Final testing

Toxicity of *B. bassiana* conidial suspension

The toxicity of *B. bassiana* conidial suspension was determined by larval atomization method against third instar larvae of *G. mellonella* under laboratory conditions. Six dilutions 1000×10^5, 700×10^5, 500×10^5, 250×10^5, 125×10^5 and 75×10^5 of *B. bassiana* conidial suspension were prepared in tap water. The mortality data was recorded in the toxicity trial from 12 HAT upto 120 HAT at 12 hours interval and Duncan's multiple range test (DMRT) **(Duncan, 1955)** was performed to test the significance of the results **(Plate 13, Plate 14 and Plate 15)**, The data has been presented in **Table 15 and Fig. 4.32.**

Mortality response

It is evident from the **Table 15** that none of the concentration could cause any mortality response upto 12 HAT. At 24 HAT, three higher concentrations i.e., 1000×10^5, 700×10^5 and 500×10^5 start showing the mortality response highest being 6.67 per cent at 1000×10^5. At 36 HAT, a lower concentration of 250×10^5 also caused 3.33 per cent mortality, and the highest response was 13.33 at 1000×10^5. At 48 HAT every concentration showed some mortality response except the lower most 75×10^5 concentration. The highest mortality response was 26.67 per cent at 1000×10^5 concentration. In similar way an increase in the mortality response was observed with the increase in the duration after the treatment. The complete mortality (100 per cent mortality) was observed at 96 HAT at 1000×10^5 concentration. At 120 HAT 700×10^5 concentration also showed complete mortality, however, none of the other

concentration could cause complete mortality even up to 120 HAT. The lowest concentration of 75×10^5 could cause only 33.33 per cent mortality at 120 HAT.

Toxicity of *B. bassiana* sunflower oil formulation

The toxicity of *B. bassiana* sunflower oil formulation was determined by larval atomization method against third instar larvae of *B. bassiana* under laboratory conditions. Six dilutions 1000×10^5, 700×10^5, 500×10^5, 250×10^5, 125×10^5 and 75×10^5 of *B. bassiana* sunflower oil formulation were prepared in tap water. The mortality data was recorded in the toxicity trial from 12 HAT to upto 120 HAT at 12 hours interval and Duncan's multiple range test (DMRT) **(Duncan, 1955)** was performed to test the significance of the results, the data has been presented in **Table 16 and Fig. 4.33**.

Mortality response

The data presented in the **Table 16** showed that none of the concentration caused mortality upto 12 HAT. The higher three concentrations i.e. 1000×10^5, 700×10^5 and 500×10^5 showed some mortality response at 24 HAT, the highest mortality 13.33 per cent was recorded at 1000×10^5 concentration and the lowest 3.33 per cent at 500×10^5 concentration. At 36 HAT, the mortality response was also observed in the concentration of 250×10^5 i.e. 3.33per cent. At 48 HAT all the concentration showed some mortality response except the 75×10^5. An increase in the mortality response was observed with the increase in exposure period. The 100 per cent mortality was observed at 96 HAT in case of highest concentration (1000×10^5) followed by 93.33 per cent and 80 per cent mortality in700×10^5 and 500×10^5 respectively and minimum mortality 20 per cent was observed at lowest concentration 75×10^5. At 120 HAT, 100 per cent mortality was also observed at concentration 700×10^5 followed by 90 per cent and 76.66 per cent mortality in 500×10^5 and 250×10^5 concentration. The lowest concentration caused only 33.33 per cent mortality upto 120 HAT.

A comparison in the toxicity of two formulations indicated that there was not much difference between the efficacy of the formulations, however, some higher response was observed in the sunflower oil formulation. At 72 HAT the mortality response in case of conidial suspension was 73.33 per cent however at same HAT in case of sunflower oil formulation the mortality response was 83.33 per cent at the concentration of 1000×10^5. In other concentrations at various hours after treatment oil

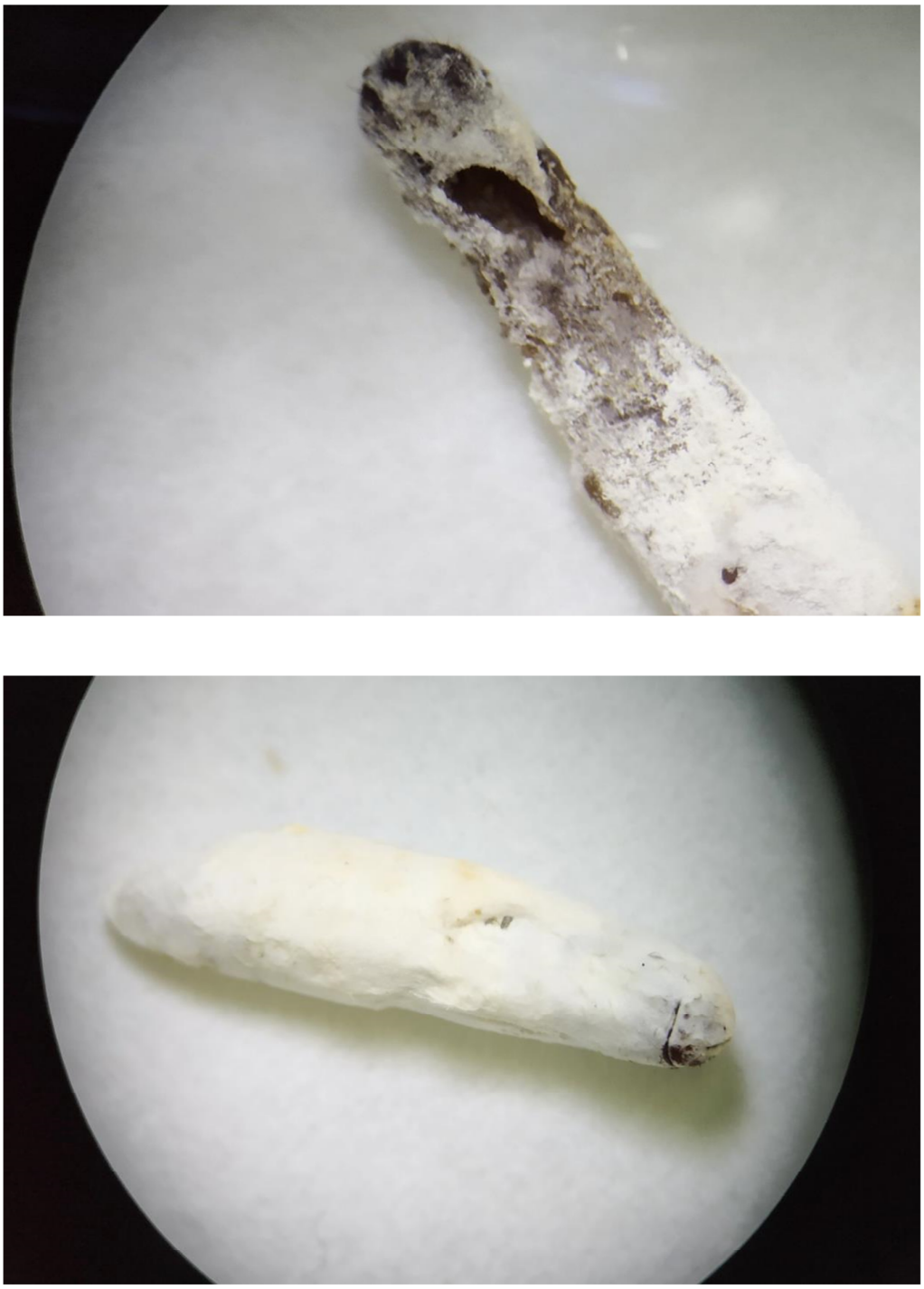

Plate 13: ***B. bassiana*** **mycelia growth on the body of** ***G. mellonella*** **cadaver under microscope**

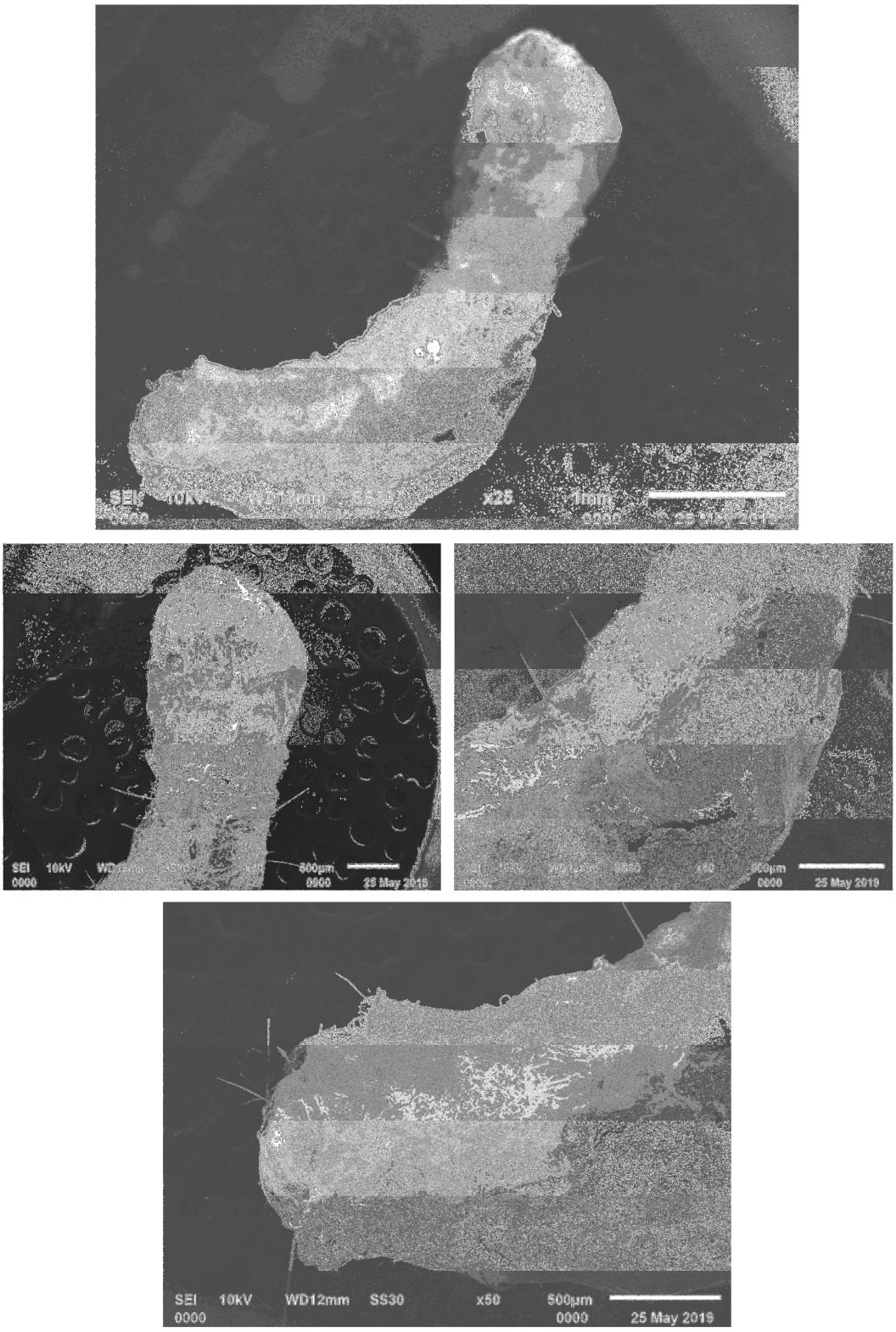

Plate 14: SEM photograph of Greater Wax Moth (*Galleria mellonella*) cadaver body covered with mycelium of *B. bassiana*

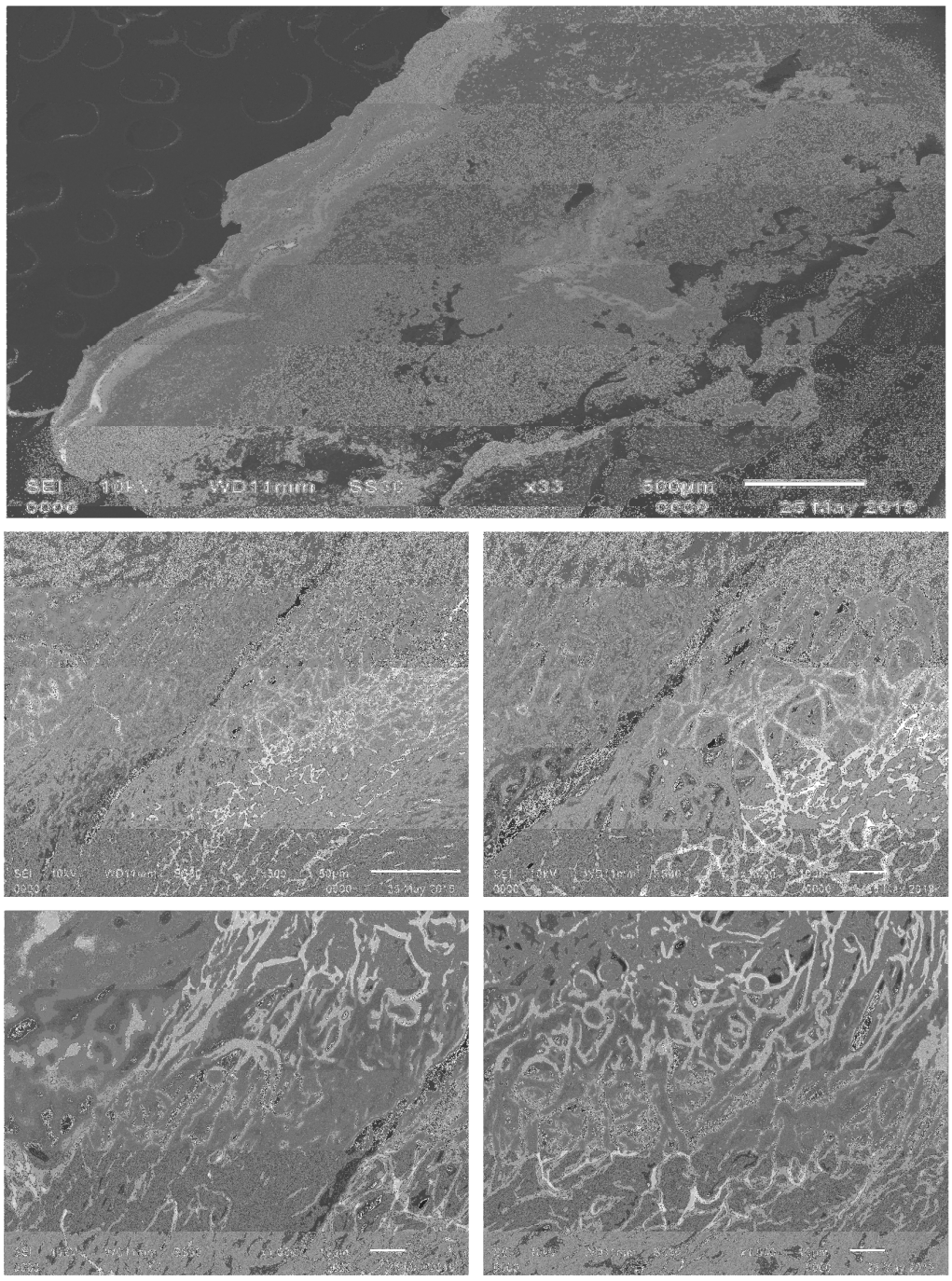

Plate 15: Greater Wax Moth (*G. mellonella*) cadaver body dissected mid dorsally to check the presence of fungal thread of *B. bassiana*. Threads are seen protruding of the body

formulation is showing higher toxicity as compared to conidial suspension **(Table 15 and 16).** Similar related studies were carried out by **Zayed (2003)** in the laboratory to determine the virulence of two *Beauveria bassiana* indigenous isolates on the last instar larvae of *Galleria mellonella* by larval dip method. The LC_{50} values of Bb1 were 8.2 x 10^{10}, 2.3 x 10^{8} and 2.2 x 10^{7} spores / ml at 4, 6 and 8 days after exposure, respectively. However, the LC_{50} values of Bb2 were 19 x10^{7}, 3.3 x10^{6} and 10^{6} spores / ml, at the same exposure periods. At conidial concentration of 2.5 x10^{6}, Bb1 isolate showed LT_{50} of 10 days, whereas for Bb2, the LT_{50} was 6.4 days. In another experiment by **Hussein *et al.* (2012),** the efficacy of different isolates of *B. bassiana* was tested against the 4th instar larvae of *G. mellonella* by topically application method. The results revealed that the isolates BbaAUMC3076 and BbaAUMC3263 caused 100 per cent mortality at concentrations of 5.5×10^{6} conidia ml^{-1} and 5.86×10^{5} conidia ml^{-1}, respectively. The LC_{50} values were 1.43×10^{3} and 1.04×10^{5} for Bba3263AUMC and Bba3076AUMC, respectively. However, in the present study the 100 per cent mortality was observed at 96 HAT when third instar larvae of *G. mellonella* were atomized with 1000×10^{5} concentration and at 120 HAT at a concentration of 700×10^{5} in case of sunflower oil as well as in conidial suspension formulation.

Table 15: Mean mortality per cent on hours basis for different concentrations of conidial suspension for *G. mellonella*

Concentration	Treatment	12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	84HAT	96HAT	108HAT	120HAT
1000×10^5	**T1**	0±0^{a}	6.67±5.77^{b}	13.33±5.77^{b}	26.67±11.54^{d}	56±11.54^{e}	73.33±15.27^{d}	93.33±5.77^{d}	100±0.0^{e}	100±0.0^{e}	100±0^{e}
700×10^5	**T2**	0±0^{a}	3.33±5.77^{ab}	6.67±5.77^{ab}	20±10^{cd}	30±0^{d}	60±10^{d}	80±10^{d}	93.33±11.55^{de}	96.67±5.77^{e}	100±0^{e}
500×10^5	**T3**	0±0^{a}	0±0^{a}	3.33±5.77^{a}	13.33±5.77^{bc}	23.33±5.77^{cd}	40±10^{c}	60±17.32^{c}	80±10^{d}	83.33±15.28^{de}	90±10de
250×10^5	**T4**	0±0^{a}	0±0^{a}	3.33±5.77^{a}	6.67±5.77^{ab}	16.6±5.77^{c}	26.6±5.77^{bc}	40±0^{b}	60±10^{c}	73.33±20.82^{cd}	76.67±25.17^{d}
125×10^5	**T5**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	3.33±5.77^{ab}	13.33±5.77^{bc}	23.33±5.77^{b}	36.67±15.28^{b}	50±17.32^{c}	53.33±15.28^{c}	56.67±11.55^{c}
75×10^5	**T6**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	3.33±5.77^{ab}	13.33±5.777^{ab}	16.67±5.77^{a}	20±10^{b}	26.67±5.77^{b}	33.33±5.77^{b}
Control (Untreated)	**T7**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Means (±SE) followed by common letters are not significantly different at p=0.05 per cent according to DMRT.

HAT = hour after treatment

Table 16: Mean mortality per cent on hours basis for different concentrations of sunflower oil formulation for *G. mellonella*

Concentration	Treatment	12HAT	24HAT	36HAT	48HAT	60HAT	72HAT	84HAT	96HAT	108HAT	120HAT
1000×10^5	**T1**	0±0^{a}	13.33±5.77^{b}	13.33±5.77^{c}	30±10^{c}	60±10^{f}	83.33±5.77^{f}	96.67±5.77^{d}	100±0.0^{e}	100±0.0^{e}	100±0^{e}
700×10^5	**T2**	0±0^{a}	6.67±5.77^{a}	10.00±0.00^{bc}	20±10^{bc}	33.33±5.77^{e}	60±10^{e}	80±10^{d}	93.33±11.55^{de}	96.67±5.77^{e}	100±0^{e}
500×10^5	**T3**	0±0^{a}	3.33±5.77^{a}	3.33±5.77^{ab}	10±0^{ab}	26.67±5.77^{de}	43.33±5.77^{d}	60±20^{c}	80±10^{d}	83.33±15.28^{de}	90±10de
250×10^5	**T4**	0±0^{a}	0±0^{a}	3.33±5.77^{ab}	6.67±5.77^{a}	20±10^{cd}	30±10^{c}	40±10^{b}	60±10^{c}	73.33±20.82^{cd}	76.67±25.17^{d}
125×10^5	**T5**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	3.33±5.77^{a}	13.33±5.77^{bc}	23.33±5.77^{bc}	36.67±15.28^{b}	50±17.32^{c}	53.33±15.28^{c}	56.67±11.55^{c}
75×10^5	**T6**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	3.33±5.77^{ab}	13.33±5.777^{b}	16.67±5.77^{a}	20±10^{b}	26.67±5.77^{b}	33.33±5.77^{b}
Control (Untreated)	**T7**	0±0^{a}	0±0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}

Means (±SE) followed by common letters are not significantly different at p=0.05 per cent according to DMRT.

HAT = hour after treatment

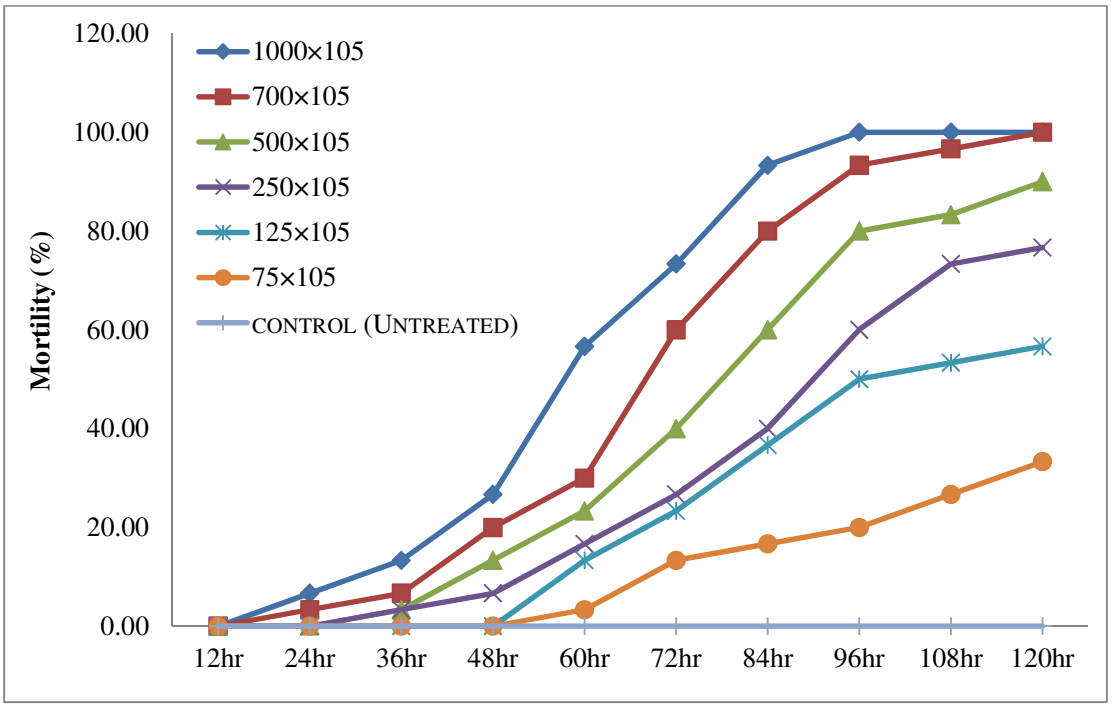

Fig. 4.32: Bar graph representation of mean mortality per cent of *G. mellonella* in response to conidial suspension of *B.bassiana*

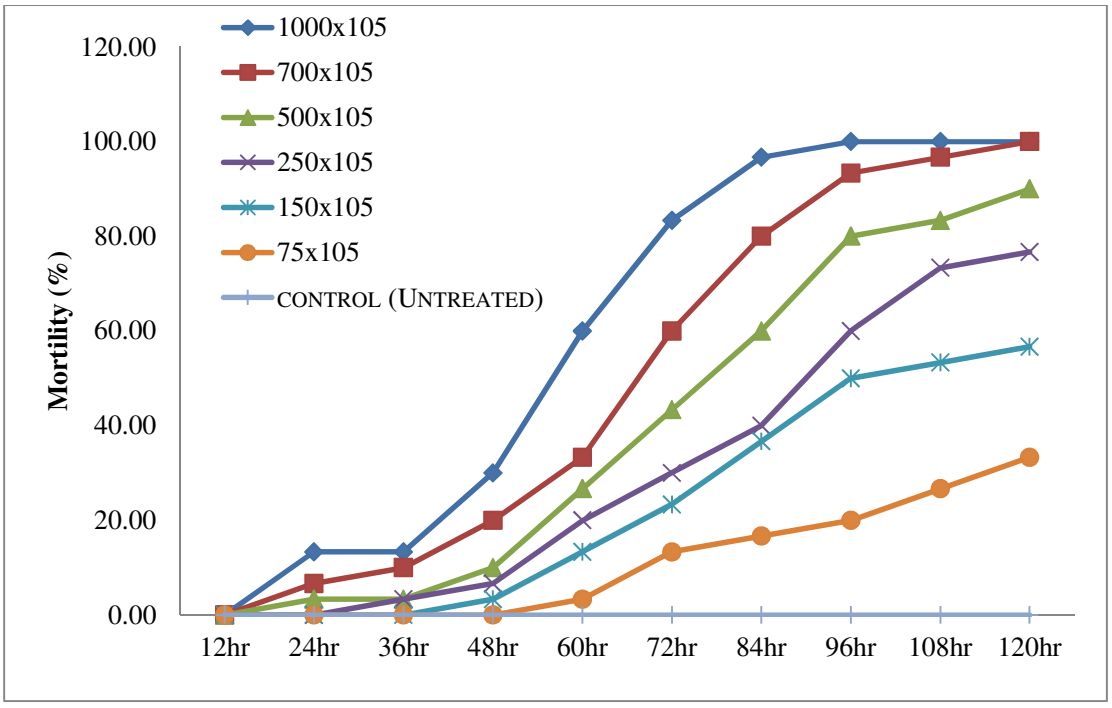

Fig. 4.33: Bar graph representation of mean mortality per cent of *G. mellonella* in response to oil formulation of *B.bassiana*

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

Present study on mass multiplication and formulation of entomopathogenic fungi (EPF), *Beauveria bassiana* (Balsamo) Vuillemin along with their pathogenicity against lepidopteran insect pest, i.e. Tobacco caterpillar (*S. litura*) and Greater Wax Moth larve (*G. mellonella*) was conducted in Insect Pathology Laboratory, Department of Entomology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar. All the silent findings are presented below in this chapter.

The grains which were used for the study for mass multiplication of *B. bassiana* were rice, sorghum, and maize. Among these broken grains, rice showed the best results followed by sorghum and maize. The highest biomass (0.62 g), conidia count ($10.92x10^7$conidia/ ml) and, per cent germination (86.94 per cent) was recorded in rice followed by sorghum biomass (0.54 g), conidia count ($7.35x10^7$conidia/ ml) and, per cent germination (77.43 per cent). The lowest results were obtained from maize *viz.* biomass (0.37 g), conidia count ($6.05x10^7$ conidia/ml) and, per cent germination (72.44 per cent). Hence, rice was best grain source for the mass multiplication of *B. bassiana* followed by sorghum and lowest was maize recorded for growth.

Among different synthetic media tested for mass multiplication of *B. bassiana* was studied on five different media *viz.* Potato Dextrose Agar (PDA), Sabouraud's Dextrose Agar+1% Yeast (SDAY), Sabouraud's Maltose Agar+1% Yeast (SMAY), Oatmeal Agar (OMA), and V-8 Juice Agar (V-8) at temperature 17°C, 21°C, 27°C and 31°C, the best media at 17°C on the basis of mean radial growth, mean conidia/ml, and mean germination percentage was OMA (6.88 cm, $1.92x10^7$ conidia/ml, and 79.62 per cent), and the second best was V-8 (6.83 cm, $1.37x10^7$conidia/ml, and 76.27 per cent), followed by SMAY (6.30 cm, $1.08x10^7$conidia/ml, and 75.17per cent) and then PDA (5.03 cm, $0.79x10^7$conidia/ml, and 72.65 per cent). The least preferable was SDAY (3.94 cm, $0.59x10^7$conidia/ml, and 72.60 per cent). At 21°C on the basis of all parameter studies, the best media achieved was OMA (7.29 cm, $11.18x10^7$conidia/ml, and 82.42per cent), the second best media was V-8 (6.82 cm, $6.77x10^7$conidia/ml, and 79.38 per cent), and then it was PDA (6.80 cm, $5.38x10^7$ conidia/ml, and 79.13per cent), which was followed by SDAY (5.57 cm, $3.97x10^7$ conidia/ml, and 75.56per

cent). The least suitable media was SMAY (3.57 cm, $1.55x10^7$conidia/ml, and 74.27per cent). Even at temperature 27°C, OMA proved to be the best media (6.44 cm, $12.85x10^7$ conidia/ml, and 86.74per cent) and followed by V-8 (4.70 cm, $8.51x10^7$ conidia/ml, and 75.00per cent), PDA (4.98 cm, $6.12x10^7$ conidia/ml, and 74.42 per cent) and it was led by SDAY (4.65 cm, $4.91x10^7$ conidia/ml, and 77.74 per cent). The least preference should be given to SMAY (4.43 cm, $2.52x10^7$conidia/ml, and 72.53 per cent). Lastly, the temperature 31°C, it was concluded that the best media was OMA (4.34 cm, $3.85x10^7$ conidia/ml, and 83.72 per cent), the second best alternative media was V-8 (3.52 cm, $2.43x10^7$ conidia/ml, and 78.6 3per cent), and the third best media was SMAY (3.23 cm, $1.62x10^7$ conidia/ml, and 78.46 per cent) which was followed by PDA (3.20 cm, $1.24x10^7$ conidia/ml, and 80.29 per cent). And the least good media was SDAY (2.89 cm, $0.68x10^7$ conidia/ml, and 78.69 per cent).

The counter part of the above mentioned media were taken i.e. liquid based or broth media (SDYB, SMYB, PDB OMB and V-8B) to find out the best growth and development of the EPF. The observations for mean wet and mean dry weight of the biomass, and mean conidia/ml were taken. All these media were studied at 17°C, 21°C, 27°C and 31°C. Other extreme temperatures were also studies in preliminary basis like 15°C and 35°C which did not show any growth and no sporulation, respectively. Here, all the great importance was given to the sporulation.

At 17°C, the data for mean wet and mean dry and mean conidia/ml was recorded. The best media obtained was OMB (6.89 g, 1.98 g and $2.16x10^7$ conidia/ml) followed by PDB (3.89 g, 0.90 g and $2.00x10^7$ conidia/ml), SMYB (5.46 g, 0.99 g and $1.63x10^7$ conidia/ml), and V-8B (6.24 g, 1.05 g and $1.27x10^7$ conidia/ml). The least observations were obtained from SDYB (6.36 g, 1.29 g and $0.72x10^7$ conidia/ml). At 21°C, the best media obtained was OMB (7.14 g, 1.96 g and $13.44x10^7$ conidia/ml) followed by PDB (4.33 g, 0.49 g and $10.84x10^7$ conidia/ml), V-8B (2.74 g, 0.56 g and $8.06x10^7$ conidia/ml). This was followed by SDYB (4.47 g, 2.05 g and $4.80x10^7$ conidia/ml). The least good media was SMYB (5.53 g, 1.25 g and $1.77x10^7$ conidia/ml). At 27°C, the best media figured was OMB (6.64 g, 0.99 g and $15.24x10^7$ conidia/ml) followed by PDB (1.88 g, 0.63 g and $9.96x10^7$ conidia/ml), SMYB (4.92 g, 0.69 g and $9.76x10^7$ conidia/ml), and SDYB (3.34 g, 0.73 g and $5.56x10^7$ conidia/ml). The least

results were obtained from V-8B (1.15 g, 0.49 g and $3.00x10^7$ conidia/ml). At 31°C, the best media obtained was SDYB (0.55 g, 0.38 g and $1.42x10^7$ conidia/ml) it was preceded by PDB (0.99 g, 0.29 g and $0.89x10^7$ conidia/ml), then it was OMB (0.95 g, 0.47 g and $0.75x10^7$ conidia/ml). This was lead by SMYB (2.44 g, 0.53 g and $0.48x10^7$ conidia/ml). The least results were from V-8B (0.80 g, 0.37 g and $0.32x10^7$ conidia/ml). The best media and temperature which was identified and ultimately specified from the above mentioned data and experiments was OMA at 21°C. OMA media showed better results in comparison with the other four media. It was followed by V-8, PDA, and SDAY. The least figures were obtained from SMAY, keeping all the experiments in consideration these specification were made. The experiments for mass multiplication in liquid media advise that it would be wise to utilize OMB for getting highest biomass and conidial count, followed by SDYB, SMYB, PDB and lastly by V-8B. The best wet and dry weight biomass and conidia count could be achieved, respectively, in a range of 17°C-21°C and 21°C-27°C.

After that, when media was ready with a hold of good conidia/ml, various concentrations of sunflower oil formulation and conidial suspension were prepared with the help of Nuebauer Haemocytometer for the bioefficacy test of *B. bassiana* against Greater Wax moth (*G. mellonella*) and Tobacco Caterpillar (*S. litura*). Variegated concentrations were set as treatments *viz.*$1000x10^5$, $700x10^5$, $500x10^5$, $250x10^5$, $125x10^4$ and $75x10^4$ conidia/ml. For *G. mellonella* in sunflower oil formulation, the initial mortality was observed at 24 HAT, in the highest three concentration of $1000x10^5$, $700x10^5$, $500x10^5$ where observed mortality were found 13.33per cent, 6.67 per cent and 3.33 per cent respectively. The 100 per cent mortality was observed at 96 HAT and 120 HAT in the concentration of $1000x10^5$ and 700×10^5 concentration, respectively. The lowest concentration caused only 33.33 per cent mortality up to 120 HAT and the least mortality was observed at the concentration of $75x10^5$ (33.33per cent) within 120 HAT. In case of conidial suspension first mortality was observed at 24 HAT, in the highest two concentrations of $1000x10^5$, $700x10^5$ where observed mortality were found 6.67 per cent and 3.33 per cent, respectively. The 100 per cent mortality was observed at 96 HAT and 120 HAT in the concentration of $1000x10^5$ and 700×10^5 concentration respectively and the least mean mortality was observed at the concentration of $75x10^5$ (33.33 per cent) within 120 HAT. The similar concentrations were made for sunflower oil formulation against the *S. litura.* It was found that, the first

mortality was observed at 36 HAT, in the highest three concentration of $1000x10^5$, $700x10^5$, $500x10^5$ where observed mortality were found 6.67 per cent, 6.67 per cent and 3.33 per cent respectively. The 100 per cent mortality was observed at 108 HAT and 120 HAT in the concentration of $1000x10^5$ and 700×10^5 concentration respectively. And the least mortality was observed at the concentration of $75x10^5$ (23.33 per cent) within 120 HAT. In case of conidial suspension first mortality was observed at 36 HAT, in the highest three concentrations of $1000x10^5$, $700x10^5$ where the observed mortality were found 6.67 per cent 6.67 per cent and 3.33 per cent respectively. The 100 per cent mortality was observed at 108 HAT and 120 HAT in the concentration of $1000x10^5$ and 700×10^5 concentration respectively and the least mean mortality was observed at the concentration of $75x10^5$ (23.33 per cent) within 120 HAT.

Since the establishment of the fact that fungi pathogenic to insects can be key components in the fight against insect pests in agriculture, several large scale researches have been undertaken by governments, institutions, organizations and individuals to explore their potentials. To date, a number of mycoinsecticide have been developed and are being used against many insect pests of economic importance in a number of countries. However, use of mycoinsecticide in pest management is generally moving at a slow pace even in the developed countries where production of mycoinsecticide began more than five decades ago. In spite of this, mycoinsecticide are gradually becoming popular. While acknowledging limitations, one can still argue that, use of mycoinsecticide is likely to rise if research is focus on; improving its performance under challenging environmental conditions, formulations that will increase persistence, longer shelf life, ease of application, pathogen virulence and wider spectrum of action.

Formulations of *B. bassiana* can thus serve as an effective broad spectrum biocontrol agent for various crops. Since the spores are key component for developing any formulation, in the present study, it was concluded that rice based and Oatmeal agar and Oatmeal broth media at temperature range 21-27^{0}C could be the best substrate for mass multiplication of *B. bassiana.* The mortality data against insect pests indicated that oil based formulations are best to manage test insects in terms of feeding efficiency and mortality. Therefore it is the need of hour to develop advanced oil based formulation that will increase persistence, longer shelf life, ease of application, pathogen virulence and wider spectrum of action.

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VITA

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ABSTRACT

Present study on mass multiplication and formulation of entomopathogenic fungi (EPF), *Beauveria bassiana* (Balsamo)Vuillemin along with their pathogenicity against lepidopteran insect pest, i.e. Tobacco caterpillar (*Spodoptera litura*) and Greater Wax Moth larve (*Galleria mellonella*) was conducted in Insect Pathology Laboratory, Department of Entomology, College of Agriculture, , G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2017-19.

Experiment conducted for the mass multiplication of *B. bassiana* on grain based media showed that Rice was best substrate on the basis of mean biomass, mean conidial count, and mean germination per cent (0.62 g, 10.92×10^7 conidia/ml and 86.94 %) followed by Sorghum and Maize. For synthetic media the same parameters were studied for mass multiplication of *B. bassiana* and it was found that best media were Oatmeal Agar Media (OMA) and Oatmeal Broth (OMB) within a range of temperature 21°C- 27 °C. For bioassay the developed conidial suspension and Sunflower oil formulation were evaluated on Greater Wax Moth (*Galleria mellonella*) and Tobacco Caterpillar (*Spodoptera litura*). In Greater Wax Moth comparison in the toxicity of two formulations indicated that there was not much difference between the efficacy of the formulations, however some higher response was observed in the sunflower oil formulation. At 72 hours after treatment (HAT) the mortality response in case of conidial suspension was 73.33% however at same 72 HAT in case of sunflower oil formulation the mortality response was 83.33 % at the concentration of 1000×10^5. In other concentrations at various hours after treatment oil formulation is showing higher toxicity as compared to conidial suspension and in tobacco caterpillar comparison between the two formulations indicated that there was not much difference between the contact toxicities of the *B. Bassiana,* however oil formulation showed a little higher toxicity. At 48 HAT the mortality in case of conidial suspension was 16.67%, however it was 23.33% at highest concentration of 1000×10^5 in case of sunflower oil formulation. In other concentrations at various hours after treatment sunflower oil formulation is showing higher toxicity as compared to conidial suspension.

Mycoinsecticide formulations are the leading effective products for controlling various insect pests. Being highly potent agent, Chemical firms are diversifying their portfolio to such microbial agents as the degradation of soil and resistance in insects has made synthetic chemicals more questionable. Need of the hour is to discover biological entities to control insect pests which will help to develop new horizons for Integrated Pest Management (IPM) modules.

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शोध का विषय	:	**कीटरोगकारी कवक ब्यूवेरिया बैसियाना(बालसामो) वुइलमिन से निर्मित फार्मूलेशन एवं निर्मित फार्मूलेशन का लेपिडोप्टेरान गण के कीटो में प्रभाव**			
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सारांश

वर्तमान अध्ययन कीटरोगकारी कवक, ब्यूवेरिया बैसियाना(बालसामो) वुइलमिन के वृहत गुणन और फार्मूलेशन के निर्माण एवं इन फार्मूलेशन के उपयोग द्वारा हानिकारक कीटो जैसे तम्बाकू की सुंडी (स्पोडोप्टेरा लिटुरा) और बृहत्तर मोम पतंग सुंडी(गैलेरिया मेलोनेला) के रोकथाम पर केंद्रित है। प्रस्तुत शोध कीट विकृति विज्ञान प्रयोगशाला, कीट विज्ञान विभाग, कॉलेज ऑफ एग्रीकल्चर, जीबी पंत यूनिवर्सिटी ऑफ एग्रीकल्चर एवं टेक्नोलॉजी, पंतनगर, उत्तराखंड में 2017-19 के दौरान किया गया।

बी. बैसियाना के वृहत गुणन के लिए किए गए प्रयोग से पता चला है कि चावल माध्यम जैवभार, औसत बीजाणु गणना, और औसत अंकुरण प्रतिशत (0.62 ग्राम, 10.92×10^7 बीजाणु / एमएल और 86.94%) के आधार पर सबसे अच्छा सब्सट्रेट था और उसके बाद ज्वार और मक्का थे। बी. बैसियाना के वृहत गुणन हेतु कृत्रिम मीडिया के लिए समान मापदंडों पर किये गए अध्ययन से यह पाया गया कि तापमान 21^oC - 27^oC की एक सीमा के भीतर, सर्वश्रेष्ठ मीडिया ओटमील अगर माध्यम(ओ.म.ए.) और ओटमील ब्रोथ(ओ.म.बी.) थे।

बी. बैसियाना के विभिन्न निर्मित फार्मूलेशन के परिक्षण हेतु विकसित बीजाणु निलंबन और सूरजमुखी तेल फार्मूलेशन का मूल्यांकन वृहत्तर मोम पतंग (गैलेरिया मेलोनेला) और तम्बाकू की सुंडी (स्पोडोप्टेरा लिटुरा) पर की गई। वृहत्तर मोम पतंग में दो फार्मूलेशन की विषाक्तता में तुलना ने संकेत दिया कि फार्मूलेशन की प्रभावकारिता के बीच बहुत अंतर नहीं था, हालांकि सूरजमुखी तेल फार्मूलेशन में कुछ उच्च प्रतिक्रिया देखी गई थी।

उपचार के 72 घंटे पश्चात बीजाणु निलंबन के मामले में मृत्यु दर 73.33% थी, लेकिन सूरजमुखी तेल फार्मूलेशन के 1000×10^5 की सांद्रता पर उसी 72 घंटे पश्चात मृत्यु दर प्रतिक्रिया 83.33% थी। उपचार के बाद विभिन्न घंटों में अन्य सांद्रताओं में तेल फार्मूलेशन, बीजाणु निलंबन की तुलना में अधिक विषाक्तता दिखा रहा है और तम्बाकू की सुंडी में दोनों फार्मूलेशन के बीच तुलना ने दर्शाया कि बी. बैसियाना के संपर्क विषाक्तता के बीच बहुत अंतर नहीं था, हालांकि तेल फार्मूलेशन में थोड़ा अधिक विषाक्तता दिखाई दी। उपचार के 48 घंटे पस्चात बीजाणु निलंबन के मामले में मृत्यु दर 16.67% थी, हालांकि सुरजमुखी तेल फार्मूलेशन के मामले में 1000×10^5 के उच्चतम सांद्रता पर यह 23.33% थी। उपचार के बाद विभिन्न घंटों में अन्य सांद्रता में सूरजमुखी तेल फार्मूलेशन बीजाणु निलंबन की तुलना में उच्च विषाक्तता दिखा रहा है।

विभिन्न कीटों को नियंत्रित करने के लिए कवक कीटनाशी प्रमुख प्रभावी उत्पाद हैं। अत्यधिक शक्तिशाली प्रतिनिधि होने के नाते, रासायनिक कंपनियां अपने संग्रहण में ऐसे जीवाणु प्रतिनिधि को विविधता प्रदान कर रही हैं क्योंकि मिट्टी की गिरावट और कीड़ों में प्रतिरोध ने कृत्रिम रसायनों को अधिक संदिग्ध बना दिया है। कीट नाशिजीवों को नियंत्रित करने के लिए जैविक अस्तित्व की खोज करना समय की आवश्यकता है जो एकीकृत कीट प्रबंधन (आई.पी.एम) मापांक के लिए नए क्षितिज विकसित करने में मदद करेगा।

(रेनू पाण्डेय)
सलाहकार

(रजनीश राय)
लेखक