Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato

THESIS

Submitted to the

Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur

In partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

AGRICULTURE
(ENTOMOLOGY)

By

P.SWATHI

Department of Entomology
College of Agriculture, Jabalpur - 482004
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (MP)

2018

CERTIFICATE – I

This is to certify that the thesis entitled ***"Developing effective formulation of Beauveria bassiana (Balsamo) Vuillemin for management of insect pest complex on tomato"*** *submitted in partial fulfilment of the requirement for the degree of* ***DOCTOR OF PHILOSOPHY IN AGRICULTURE (Entomology)*** *of the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur is a record of the bonafide research work carried out by* ***Ms. P.Swathi*** *under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instructions.*

All the assistance and help received during the course of the investigation have been acknowledged by her.

Place: Jabalpur
Date: / / 2018

(Dr.S.B.Das)
Chairman of the Advisory Committee

THESIS APPROVED BY THE STUDENT'S ADVISORY COMMITTEE

Committee	Name	Signature
Chairman	**Dr. S.B.Das**	
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Member	**Dr. A. K. Bhowmick**	
Member	**Dr. (Smt.) Vibha**	
Member	**Dr. H. L. Sharma**	

Thesis is approved by

(Dr. A. K. Bhowmick)
Professor and Head

CERTIFICATE – II

This is to certify that the thesis entitled ***"Developing effective formulation of Beauveria bassiana (Balsamo) Vuillemin for management of insect pest complex on tomato"*** *submitted by* ***Ms. P.Swathi*** *to the Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur in partial fulfilment of the requirements for the degree of* ***Ph.D. in the subject Entomology*** *of the Department of* ***Entomology*** *has been, after evaluation, approved by the External Examiners and by the Student's Advisory Committee after an oral examination on the same.*

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External Examiner

MEMBER OF THE STUDENT'S ADVISORY COMMITTEE

Committee	Name	Signature
Chairman	**Dr. S.B.Das**	.....
Member	**Dr.(Smt.) P.N.Ganga Visalakshy**	.....
Member	**Dr. A. K. Bhowmick**	.....
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Declaration and Undertaking by the Candidate

I, **P. Swathi** D/o **Shri Padavala Ramu** certify the work embodied in thesis entitled **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"** is my own first time bonafide work carried out by me under the guidance of **Dr. S. B. Das**, Professor, Department of Entomology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) and under the guidance of **Dr. P. N. Ganga Visalakshy**, Principal Scientist, Division of Entomology and Nematology, ICAR-Indian Institute of Horticultural Research, Bengaluru (Karnataka) during *kharif* 2016-17 and 2017-18.

The matter embodied in the thesis has not been submitted for the award of any other degree/diploma. Due credit has been made to all the assistance and help.

I, undertake the complete responsibility that any act of misinterpretation, mistakes and errors of fact are entirely of my own.

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Date : / / 2018
Place: Jabalpur

Dr. S.B.Das
(Major Advisor)

P.Swathi
(Student)

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Place : Jabalpur

Date : **(P.Swathi)**

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Symbol	Abbreviation	Stand for
@		At the rate of
#		Numbers
()		Parenthesis
%		Percentage
±		Plus or minus
/		Per
√		Square root
	µg	Microgram
	^{0}C	Degree centigrade
	2nd	Second
	3rd	Third
	ANOVA	Analysis of Variation
	BOD	Biological Oxygen Demand
	Bt	*Bacillus thuringiensis*
	CABS-65	Calcium alkyl benzene sulphonate-65
	cal	Calculated
	CB	Cost Benefit
	CD	Critical difference
	CDA	Czapek Dox Agar
	CDB	Czapek Dox Broth
	cfu /CFU	Colony forming units
	cm	Centimetre
	CMC	Carboxy Methyl Cellulose
	CRD	Complete Randomized Design
	D	Days
	DAT	Days after treatment
	df	degree of freedom
	DOR	Directorate of Oilseed Research
	E	East
	et al	Co-workers
	etc.	And for the rest
	ETL	Economic Threshold Level
	g	Gram
	g^{-1}	Per gram
	ha	Hectare
	Ha Bb	*Helicoverpa armigera Beauveria bassiana*
	ha^{-1}	Per hectare
	hrs	Hours
	i.e	That is

	ICAR	Indian Council of Agricultural Research
	IIHR	Indian Institute of Horticultural Research
	In-vitro	Under controlled conditions
	ITCC	Indian Type Culture Collection
	Kg or kg	Kilogram
	Km/hr	Kilometre per hour
	L or l	Litre
	LC	Lethal concentration
	LC_{50}	Median lethal concentration that kills 50% population
	LC_{95}	Median lethal concentration that kills 95% population
	LT	Lethal time
	LT_{50}	Median lethal time that kills 50% population
	M	Million
	m^2	Metre square
	max.	Maximum
	mg	Milligram
	min	Minimum
	ml	Millilitre
	ml^{-1}	Per millilitre
	mm	Millimetre
	MMT	Million metric tonnes
	MSS	Mean sum of squares
	MT	Metric tonnes
	N	North
	NBAIIM	National Bureau of Agriculturally Important Microorganisms
	no.	Number
	NS	Non-Significant
	PDA	Potato Dextrose Agar
	PDB	Potato Dextrose Broth
	psi	Pounds per square inch
	RBA	Rose Bengal Agar
	RBD	Randomized Block Design
	RH	Relative humidity
	Rs.	Rupees
	S.N.	Serial number
	SC	Suspension concentrates
	SDA	Sabouraud Dextrose Agar
	SDB	Sabouraud Dextrose Broth
	SDS	Sodium dodylsulphate
	SEm±	Standard error of mean
	SS	Sum of squares

	SW	Standard Week
	T	tonnes
	Temp.	Temperature
	Tr.	Treatment
	Tween-20	Polyoxyethylene (20) sorbitan monolaurate
	Tween-40	Polyoxyethylene (20) sorbitan monopalmitate
	Tween-60	Polyoxyethylene (20) sorbitan monostearate
	Tween-80	Polyoxyethylene (20) sorbitan monooleate
	viz.	Namely
	x^2	Chi square

LIST OF FIGURES

INTRODUCTION

Vegetables play a major role in Indian agriculture by providing food, nutritional and economic security and more importantly, producing higher returns per unit area and time. During recent years, the interest in vegetable production has increased rapidly as a result of greater appreciation of the food value of vegetables. India is the world's largest producer of vegetable next to China sharing 10.70 per cent in the world's vegetable production (Horticultural Statistics at a Glance 2015).

Among various vegetables, tomato (*Solanum lycopersicum* L.) is the most common and extensively grown vegetable all over the country and occupies an important place in the food basket of Indian consumers. In India during 2015-16, tomato was grown in an area of 0.791 million(M) ha with 17.39 M tonnes (T) production and productivity of 22 T/ha. Madhya Pradesh ranks first in tomato production in India with an area of about 0.070 M ha, production of 2.17 MT and 31.00 T/ha productivity followed by Karnataka with an area of about 0.064 M ha with production and productivity of 2.03 MT and 31.58 T/ha, respectively (Horticultural Statistics at a Glance 2015).

A wide range of insects and mites attack tomato and they form the major limiting factors in its successful cultivation. The major insect pest complex which causes economic losses of tomato are whitefly (*Bemisia tabaci* Gennadius), fruit borer (*Helicoverpa armigera* Hubner) and spider mites, (*Tetranychus urticae* Koch) to a minor extent and a recent invasive pest, South American tomato leaf miner(*Tuta absoluta* Meyrick) (Gulya, 2014 and Taram et al. 2016).

Tuta absoluta is an important emerging pest of tomato in India posing a serious threat to tomato production and is considered as a key pest of both greenhouse and open-field tomato causing up to 100 per cent devastating losses to tomato production (Arturo et al. 2012 and Sabbour, 2014).

Fruit borer, *Helicoverpa armigera* (Hubner) causes damage to the developing fruits and results in yield loss ranging from 20 to 60 per cent (Kumar, 2014).

Bemisia tabaci (Gennadius) is a serious, cosmopolitan sucking pest as it imparts direct damage to the crop by desapping and also acts as a vector for transmission of leaf curl virus disease in tomato (De Barro, 1995; Riley and Palumbo, 1995 ; Jones, 2003). Continuous feeding affects the physiology of plant leading to detrimental effect on all stages of the crop. More important is the role of *B.tabaci* as a vector of several Gemini viruses such as leaf curl virus of tomato and okra, tobacco leaf curl virus and yellow mosaic virus of beans (Muniyappa and Veeresh 1984; Saikia and Muniyappa, 1989; Harrison et al.1991, Markham et al.1994). Yield losses due to direct and indirect damage caused by whiteflies were reported to the extent of 20 to 100 per cent in tomato (Rapisarda and Garzia, 2002).

Development of resistance, elimination of natural enemies and environmental disharmony due to most of the chemical insecticides has led to large upsurges of the pest during the last decade and also the invasion of new pest has necessitated the search for alternatives to chemical control. During export there is also a risk of rejection of whole consignment due to presence of pesticide residues (Naik, 2008). Hence, organic management of the pests is the only alternative left as demand is ever increasing for organically produced agricultural commodities all-round the globe. Other than botanicals, utilization of biological control agents has been considered as a safe alternative for pest management. The use of insect pathogens gained general acceptance as a realistic goal in insect pest management.

The use of entomopathogenic fungi is an environmental-friendly alternative to plant protection chemicals (Balazy, 2004). The success of fungal entomopathogens as a biological control agent depends not only on its high efficacy against insect pests, but also on its low virulence against non-target insects (Thungrabeab and Tongma, 2007).

Fungi subsume a heterogeneous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Beauveria* spp., *Metarhizium* spp., *Nomuraea rileyi, Lecanicillium lecanii* and *Hirsutella* spp. (Yadav and Neeraj, 2012). Insect-

pathogenic fungi that act by contact and with no records of resistance

developed so far could be a viable alternative (Revathi et al. 2011).

Beauveria bassiana is exploited in greenhouse and outdoor crops as a tool for control of many agricultural arthropod pests, including whiteflies, aphids, thrips, psyllids, weevils and mealybugs (Shah and Goettel, 1999). *B.bassiana* can have many effects on susceptible insects, including moulting disruption, growth reduction, interference with development and oviposition and high mortality, particularly in immature insect (Mitchell et al. 2004). Being facultative and amenable for easy multiplication on large scale, they offer great scope to develop as potent biopesticide (Lingappa and Patil, 2002).

Faster and luxuriant growth of the fungus can only be obtained when grown on suitable substrate. Locally available and agricultural wastes were found to be excellent substrates for on-farm production of antagonists (Kumar and Palakshappa, 2009). Fungal spores are living organisms and their viability diminishes with time depending on environmental conditions and for the commercial production of fungal spores, there is need to obtain an ideal cheap and highly productive culture medium (Moore et al. 2000). Hence, an attempt was also made to determine the most suitable and locally available substrate and media for use in the mass multiplication of the fungus.

The use of microbial agents in the management of insect pests is becoming a common practice in many countries. The development of suitable formulation is essential for successful utilization of myco-insecticides. However, lack of adequate formulation technologies for most microbial control agents is still a major problem. Formulation of mycoinsecticides must be compatible with the agent, that enhance its performance and ideally it must maintain an adequate shelf-life of the agent in order to be successful (Derakhshan et al. 2008).

To enhance the myco-pesticidal efficacy, appropriate formulation is needed that stabilizes the productivity, distribution and storage of the fungus. Formulation greatly enhance the efficacy and persistence of entomopathogens (Navon and Ascher 2000). Biological and physical properties of the formulation must remain stable for at least one year, but preferably for more than 18 months for commercialization to take place (Couch and Ignoffo, 1981). As the shelf life of microbial pesticide is an

important factor for effective insect control (Consolo et al. 2003) therefore, an attempt was made to develop a formulation with at least one year shelf life.

Keeping in view the important role of eco-friendly way to manage tomato insect-pest complex, the present research program was carried out with the following objectives:

1. Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay.
2. Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*.
3. Compatibility of virulent isolate of *B. bassiana* with various additives.
4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulations against insect-pest complex on tomato under protected conditions.

REVIEW OF LITERATURE

The literature according to the objectives of the present study are reviewed and presented in this chapter.

2.1. Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay

Beauveria spp. is characterized by having conidiophores consisting of whorls and dense clusters of sympodial, short and globose or flask shaped conidiogenous cells with apical denticulate rachi giving a distinct zig-zag appearance and one celled conidia (Samson et al. 1988).

Uma Devi et al. (2008) reported that *B. bassiana* based biopesticide can be used as a broad spectrum insecticide against a myriad of insect pests.

a. Fruit borer, *Helicoverpa armigera*

Rathod (2002) used *B. bassiana* (1.18×10^4, 10^6, 10^8 and 10^{10} spores ml^{-1}) to control *Helicoverpa armigera* eggs and larvae (first, second, third, fourth and fifth instars) on groundnut. Larval instars, second and third were most susceptible to the pathogen than the other instars and the susceptibility decreased with age. However, the fungus was pathogenic to all stages of the pest (2 to 72% morality) at 1.18×10^{10} spores ml^{-1}.

Dhembare and Siddique (2004) tested the pathogenicity of *B. bassiana* against *H. armigera* and found that the larval mortality increased with difference in the spore concentration.

Sridevi et al. (2004) tested the effect of *B. bassiana* (1.6×10^5 to 2.5×10^5 spores ml^{-1}) against third instar larvae of *H. armigera* and recorded 60.4% to 75.3 % larval mortality.

Vinod and Chowdhry (2004) recorded that susceptibility of *H.armigera* larval stages were positively associated with the spore concentration of both, *B.bassiana* and *Metarhizium anisopliae*. *B. bassiana and M. anisopliae* revealed highest larval mortality (96.60% and 87.00%) at highest concentration ($1x10^9$ spores ml^{-1}), respectively.

Gundannavar et al. (2006) reported that the mortality of *H. armigera* larvae generally increased with difference in concentration of *B. bassiana*.

Quesada et al. (2006) explained that the efficiency of the entomopathogenic fungi began clearly after 48 hrs after inoculation and the hyphae penetrated the integument, trachea and the epithelial cells. After 72 hrs, the fat tissues were damaged and lethality reached to 100% after 96 hrs.

Rijal et al. (2008) reported that the number of *H. armigera* larvae observed in plots treated with *M. anisopliae* and *B. bassiana* were significantly lesser than the control plots during vegetative, flowering and pod setting stage of chickpea and the yield was also higher in entomopathogenic fungi treated plots.

Prasad et al. (2010) evaluated *B. bassiana* against the most damaging fourth instar larvae of *H. armigera*. They reported that a dose dependent mortality was observed which went up to 76.70% at highest dose of 0.25 ml ($1x10^8$ spores ml^{-1}).

Prasad and Nilofer (2010) evaluated different concentrations (0.1, 0.125, 0.2 and 0.25 x 10^8 conidia ml^{-1}) of fungus *B. bassiana* against third instar larvae of *H.armigera* and recorded 86.70% mortality at highest dose. Besides high mortality, treated larvae expressed various morphological abnormalities such as extensive hairy growth, swollen body covered with fungal mycelium.

Tyagi et al. (2010) evaluated the mortality of *H. armigera* on different age group of larvae (2, 4, 6, 8 and 12 days old) with four concentrations ($1x10^9$, $1x10^8$, $1x10^7$ and $1x10^6$ spores ml^{-1}) of *B.bassiana*. They reported that the mean mortality achieved in different larval age group was 87.2, 72.6, 54.4, 46.5 and 40.7%, respectively. They concluded that the susceptibility of the larvae was negatively associated with the larval age.

Mishra and Simon (2012) evaluated the efficacy of *B. bassiana*, against *H.armigera* with five different concentrations (0.15%, 0.20%, 0.25, 0.30% and 0.35%). A significant dose dependent mortality was observed up to 74.75% with highest dose of 0.35%.

Prabhukarthikeyan et al. (2013) evaluated four different spore concentrations of *B.bassiana* ($1x10^2$, $1x10^4$, $1x10^6$ and $1x10^8$ conidia ml^{-1}) against third instar larvae of *H. armigera*. Spore concentration of $1x10^8$

conidia ml^{-1} registered maximum mortality of 73.33% with least LT_{50} value of 4.59 days.

Karkar et al. (2014) reported the highest dose of *B. bassiana* 10^8 conidia g^{-1} @ 3.5 g/ litre was most effective dose among five doses (1.5, 2.0, 2.5, 3.0 and 3.5 g/litre) tested against third instar larvae of *H. armigera*. They concluded that mortality increased with difference in the dose of *B. bassiana*.

Phukon et al. (2014) evaluated the efficacy of three commercial bio-pesticides, two based on insect pathogenic fungi *viz.*, *B. bassiana* and *M. anisopliae* and one botanical-neem oil in comparison with chemical-cypermethrin against the tomato fruit borer, *H. armigera*. They concluded that *B. bassiana* and *M. anisopliae* could be effectively used as pest management option in production of organic tomato to reduce the pest population below economic threshold level and increased yield.

Qayyum et al. (2015) evaluated the effect of endophytic colonized *B. bassiana* against second and fourth instar larvae of *H. armigera* at 1, 3 and 5 weeks post plant inoculation. They reported that second instar larvae were more susceptible than fourth instar larvae at all time intervals evaluated and concluded that endophytic colonization of *B. bassiana* has potential as an effective strategy to control *H. armigera* in tomato.

Pandey and Das (2016) evaluated seven bio-pesticides against *H.armigera*. Among the bio-pesticides, *B.bassiana* @ 1litre ha^{-1} ($1x10^{12}$ spores ml^{-1}) was found to be most effective with least larval population (6.68 larvae / 5 plants) on pigeonpea.

b. South American tomato leaf miner, *Tuta absoluta*

Shalaby et al. (2013) evaluated four concentrations (10^7, 10^8, 10^9 and 10^{10} spores ml^{-1}) of *Bacillus thuringiensis* var. *kurstaki* (Btk), *B. bassiana* and *M. anisopliae* on *T. absoluta* eggs and larvae (neonate "newly hatched", second and third instar larvae). They concluded that *B. bassiana* and *M. anisopliae* has a potential effect on both egg and neonate larvae followed by Btk which had least effect on eggs and moderate effect on neonate and third instar larvae of *T. absoluta*.

Sabbour (2014) evaluated three microbial control agents, *Bt var kurstaki*, *B. bassiana* and *M. anisopliae* against *T.absoluta* under laboratory and green house conditions. He reported that under laboratory conditions, the

LC_{50} values for *Bt* was 243.9 µg ml^{-1} and 129.4 x 10^4 and 98.7 x 10^4 spores ml^{-1} for *B.bassiana* and *M.anisopliae* against *T.absoluta* respectively, while under green house conditions, the LC_{50} values for corresponding microbial control agents were 211µg ml^{-1} and 102 x 10^4 and 100 x 10^4 spores ml^{-1}, respectively.

Klieber and Reineke (2016) reported epiphytic and endophytic activity of *B.bassiana* against *T.absoluta*. High mortality rates and significantly reduced longevity of larvae were observed with *B. bassiana* propagules present on the surface as an epiphyte. They concluded that *B.bassiana* expresses different modes of action again target insect pests, that will help to design efficient management strategies for invasive pests like *T. absoluta*.

Tadele and Emana (2017) evaluated three different concentrations (2.5 x 10^7, 2.5 x 10^8 and 2.5 x 10^9 conidia ml^{-1}) each of *B. bassiana* and *M.anisopliae* against *T. absoluta* larvae under laboratory and glasshouse conditions. They reported that highest mortality of 95.83% and 84.04% was recorded at highest concentration of 2.5 x 10^9 conidia ml^{-1} of *B. bassiana* and *M. anisopliae,* respectively. They concluded that isolates of *B. bassiana* and *M. anisopliae*, at 2.5 x 10^9 conidia ml^{-1} are promising for integrated control of *T. absoluta* larvae.

c. Whitefly, *Bemisia tabaci*

Ramos et al. (2000) evaluated the susceptibility of eggs and first instar nymphs of *B. tabaci* to *B. bassiana* at concentration of 1x10^8 conidia ml^{-1} and reported 62 to 71% nymphal mortality. However, the eggs were not susceptible to *B. bassiana*.

Nymphal stages of *B. tabaci* are highly susceptible to infection by a number of fungi including *B. bassiana* (Vincentini et al. 2001).

James et al. (2003) found that second and third instar of the silver leaf whitefly, were the most susceptible stages, as *B. bassiana* conidia readily germinated on the cuticle of these instars.

Kuang et al. (2005) studied the efficacy of *B.bassiana* against nymphal instars of *B. tabaci* under *in-vitro* conditions. They reported that immersion of *B. tabaci* in the fungal suspension of 1 x 10^8 conidia ml^{-1} caused 84.88, 86.81, 55.94 and 38.78% mortality of the first to fourth instar nymphs, respectively.

The probit analysis showed that the LT_{50} values of *B. bassiana* were 4.14, 3.78, 6.24 and 7.59 days for the respective stages of nymphs.

Candido et al. (2006) reported that the efficacy of *B. bassiana* against *B. tabaci* significantly increased with time and the mean mortality of nymphs at 8 days after inoculation was 52.3 ± 7.3% and 91.8 ± 5.8% on cotton and cucumber, respectively.

Quesada et al. (2006) evaluated 25 native *B. bassiana* isolates and a commercially available *B. bassiana* based myco-insecticide for virulence to fourth instar nymphs of *B. tabaci* at concentration of $1x10^7$ conidia ml^{-1}. All the isolates were pathogenic, whereas mortality rates varied from 3 to 85%. A second series of bioassay was conducted on 10 selected isolates using four 10-fold concentrations ranging from $1x10^5$ to $1x10^8$ conidia ml^{-1}. Median lethal concentrations (LC_{50}) of the four most virulent isolates varied from 1.1 x 10^5 to 6.2 $x10^6$ conidia ml^{-1} and average survival time of the treated nymphs ranged from 5.9 to 7.4 days.

Torrado-León et al. (2006) evaluated the effect of entomopathogenic fungi *B. bassiana* on *B. tabaci*. They reported sub lethal effects on four nymphal stages of *B.tabaci* and due to fungal infection, observed impaired fertility, production of malformations or external variations and reduced survival of the later generations. Further, almost 30% of the imagos resulting from treated nymphs were unable to detach completely from the exuvia. A gradual reduction in the mortality rates between subsequent generations was also observed.

Al-Deghairi (2008) reported that differential mortality of *B. tabaci* to *B.bassiana* is based on the stage of insect and concentration of conidia. Infection level to eggs was only 4.49%, even with higher conidial concentration (6 x 10^6 conidia ml^{-1}). Whereas, it was highest with first and second instars (42.04%) followed by third and fourth instars (35.93%) and concluded that nymphs were highly susceptible to fungal treatment compared to eggs.

Aref et al. (2009) evaluated fungal suspensions of 1 x 10^4, 10^5, 10^6, 10^7 and 10^8 conidia ml^{-1} on second-instar nymphs of *B. tabaci* on four different host plants. They reported that ten days after inoculation with $1x10^8$ conidia ml^{-1}, survival of nymphs were significantly influenced by host plant species,

with 4.2±0.7, 9.6±0.4, 13.4±0.8 and 24.3±0.9% on cucumber, eggplant, tomato and cabbage, respectively. They concluded that virulence differed depending on host plant species with LC_{50} values of 4.6×10^4, 1.6×10^5, 4.2×10^5 and 2.1×10^6 conidia ml^{-1} on cucumber, eggplant, tomato and cabbage, respectively.

Ramazeame (2012) evaluated entomopathogens against *B. tabaci* adults and recorded a mortality of 98.33, 85.00, 72.33 and 95.00% after 72 hrs after treatment with *B. bassiana, Lecanicillium lecanii, M. anisopliae* and *Paecilomyces farinosus*, respectively and concluded that among the entomopathogens, *B.bassiana* was found to be highly effective against whitefly.

Al-alawi et al. (2014) evaluated 32 isolates of *B. bassiana* against the fourth nymphal instar and adult whitefly. They reported that the four isolates along with a commercial isolate were highly virulent to the whitefly causing more than 80% mortality with LC_{50} values of 3.16×10^6, 1.17×10^6, 9.33×10^5, 5.62×10^5 and 7.76×10^5, respectively. Further, greenhouse trials showed that the isolates BAU018 and BAU019 were as virulent as the commercial isolate GHA and suggested that the two isolates may be developed as microbial insecticides for effective and safe management of *B. tabaci*.

Zafar et al. (2016) evaluated different isolates of *B. bassiana* against different life stages of *B. tabaci* on different hosts. They reported that *B.bassiana* isolate, Bb-01 at $2x10^8$ conidia ml^{-1} was most effective and recorded highest mortality of eggs and nymphs (65.30% and 88.82%, respectively) with LC_{50} values, 2.4×10^7 and 2.7×10^6 spores ml^{-1}, respectively on *Gossypium hirsutum* in comparison to other hosts.

2.2. Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*

Several attempts have been made to multiply the entomopathogenic fungi using semi-synthetic media and solid substrates in order to cut down the cost of production. Simple and cost effective mass production technology is required to make it a highly acceptable bioagent. Some of the reviews are presented below.

Ibrahim and Low (1993) reported rice and coconut water as the most suitable loose-solid and liquid media, respectively for the growth and sporulation of *B. bassiana*.

Sharma et al. (2002) observed rice as the best solid substrate for spore production and viability of *B. bassiana*. They further reported that the fungus also grows equally well on maize or other grains.

Mondal and Bhattacharya (2004) investigated growth and sporulation of Pantnagar (PI) and Delhi (DI) isolates of *B. bassiana* on twenty-one culture media. The media were prepared from broken grains of pulses (bengal gram, black gram, cowpea, french bean, green gram, lentil, pea and pigeonpea), cereals (maize, pearl millet, rice, sorghum and wheat), oil seeds (groundnut and soybean), agricultural by-products (chopped sesbania and soybean stems, maize cob and sugarcane bagasse) and on potato dextrose broth (PDB) and Sabouraud's dextrose broth (SDB). They reported that sporulation of PI isolate was highest on pea, very good on SDB followed by PDB and french bean, fairly good on 13 other media, poor on the two stem media and extremely poor on bagasse and maize cob media. However, dry matter production was highest on groundnut and cowpea media, though significantly ($p=0.01$) different from lentil, SDB, pea and PDB.

Siwach and Jaipal (2004) studied various industrial wastes like rice bran, wheat bran, maize bran, press mud, rice husk, bagasse and three liquid media *viz.,* carrot broth, potato broth, sugar mill effluent for the mass production of *B. bassiana*. They recorded maximum fungal sporulation in wheat bran and maize bran with conidial count of 5.53 and 5.52 x 10^7 conidia ml^{-1}, respectively.

The effect of different grain media (barley, finger millets, maize, sorghum, soybean and wheat) on the sporulation of *B. bassiana* was studied by Pandey and Kanaujia (2005). They reported that highest spore production (5.39 x 10^7 conidia ml^{-1}) and spore viability (86.60%) was obtained from finger millet media.

Sahayaraj and Namasivayam (2008) evaluated various agricultural products and by-products such as grains, vegetable wastes, seeds, rice husk, sawdust and liquid media such as coconut water, rice and wheat-washed

water and rice-cooked water for mass production of three entomopathogenic fungi, namely *B. bassiana, P. fumosoroseus* and *V. lecanii*. Among the grains, wheat supported maximum spore production for *B. bassiana*. Similarly carrot, jack seeds and ladies finger also supported good growth and sporulation of all the three tested fungi. Among the liquid media, coconut water supported maximum growth and sporulation.

Padmanabhan et al. (2009) evaluated five different substrates for mass production of *B. bassiana viz.*, ragi flour, maize flour, sorghum flour, rice chaffy grains and wheat bran. They reported that maximum spore production (6×10^{10} cfu g^{-1}) was obtained with rice chaffy grains.

Kalidas (2010) reported rice powder broth as the most suitable media for carrying out mass multiplication of *B. bassiana* than PDB.

Karanja et al. (2010) evaluated four different solid substrates machicha (a waste product obtained from traditional brew consisting maize, millet or sorghum flour) broken maize, rice and maize husk for mass production of *B. bassiana* and *M. anisopliae* strains. They reported that the higher spore production was observed on broken maize and rice as compared to machicha and maize husk.

Rajanikanth et al. (2010) evaluated five substrates, *viz.*, sorghum, rice bran, rice husk, pressmud and bagasse for large scale multiplication of *B. bassiana*. They reported that sorghum as the most suitable substrate as it yielded highest conidial count and the conidial viability. And stated that it may be due to the presence of rich source of carbon and adequate source of nitrogen, which are essential for higher growth and sporulation.

Karthikeyan and Selvanarayanan (2011) evaluated three synthetic media *viz.,* Potato Dextrose Agar (PDA), Czapek's Dox Agar (CDA), Rose Bengal Agar (RBA) and three natural substrates *viz.,* water hyacinth, rice bran and spent mushroom paddy straw as culturing medium for *B.bassiana*. They concluded that *B. bassiana* attained maximum colony growth, highest spore density and biomass production of 42.00 mm, 4.52×10^7 spores ml^{-1} and 466.33 mg, respectively in the synthetic media PDA ; while among the natural substrates, rice bran amended media achieved highest colony growth (46.33mm), spore density (4.86×10^7spores ml^{-1}) and biomass (485.00 mg).

Kumar 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 tested, sugarcane bagasse was most economical.

Rajanikanth et al. (2011) reported sorghum as the most suitable substrate for large scale mass multiplication of *B. bassiana* and also virulent as the conidia harvested from sorghum recorded highest mortality of *Spodoptera litura*.

Suasa-ard et al. (2011) reported that broken unpolished rice pieces were the most suitable food media for the mass multiplication of *B. bassiana*. Conidia harvested after 7,14, 21 and 28 days incubation were 2.14 x 10^9, 3.06 x 10^9, 3.86 x 10^9 and 4.13 x 10^9 spores ml^{-1}, respectively.

Six naturally entomopathogenic fungal isolates (2 isolates of *B. bassiana* and one each of *M. anisopliae, M. flavoviride, Icariotis tenuipes* and *P. lilacinus* collected from natural habitats of Northern Thailand) were grown on five cereal grains *viz.* rice, wheat, rye, corn and sorghum in order to measure the linear growth and spore production by Mar and Lumyong (2012). They found that among all the grains, rice yielded the highest amount of *B. bassiana* spores.

Gangwar (2013) evaluated different substrates for mass multiplication of *B. bassiana*. They reported that sorghum grains were found to be the best, as it recorded maximum colony forming units (6.32 x 10^9 cfu), followed by broken maize grains (5.77 x 10^9 cfu) and barnyard millet (5.08 x 10^9 cfu), respectively.

Rishi et al. (2013) reported that *B.bassiana* grew better on sorghum grains at normal room temperature of 25-28^{0}C with relative humidity of 60-65%. The spore load varied from 500-550 spores ml^{-1} as compared to other media which ranged from 400 - 450 spores ml^{-1}.

Yadav et al. (2013) reported that rice was the best solid substrate for spore production and viability of entomopathogenic fungi, *B. bassiana* and *M. anisopliae*.

Kaur and Joshi (2014) evaluated rice, wheat, maize, sorghum, mini potato, rice bran and rice straw for mass production of three *B.bassiana*

strains. The results revealed rice as the most suitable substrate as it yielded highest conidial counts and colony forming units (31.0 x 10^5 conidia g^{-1} and 30.5 x 10^5 cfu g^{-1}, respectively) whereas it was minimum in rice straw (12.2 x 10^5 conidia g^{-1} and 9.5 x 10^5 cfu g^{-1}, respectively).

Prasad and Pal (2014) reported that the maximum spore yield of *B.bassiana* (2.78 x 10^8 spores ml^{-1}) was obtained in farm yard manure followed by SDB (2.46 x 10^8 spores ml^{-1}), while lowest number of spores of *B. bassiana, M. anisopliae* and *V. lecanii* was obtained in sugarcane bagasse (0.65 x 10^8 spores ml^{-1}).

Babul et al. (2016) reported that rice had registered maximum spore production (8.13 x 10^9 conidia g^{-1} of substrate) of *B.bassiana* when kept under natural day light at 28±2°C for 10 days.

2.3.Compatibility of *B. bassiana* with various additives

Vidhyasekaran and Muthamilan (1995) used CMC as adhesive in the preparation of different *P. fluorescens* based formulations and recovered a population of more than 10^7 cfu g^{-1} even after 240 days of storage.

Formulation of mycoinsecticides must be compatible with the additives/ adjuvants, that enhances its performance and ideally it must maintain an adequate shelf-life of the formulation in order to be successful (Derakhshan et al. 2008).

Tanuja et al. (2010) stated that the negative impact of surfactants to microorganism is probably due to increased cell permeability and amino acids leakage through inner membrane.

Chakravarty and Kalita (2011) reported that CMC as adhesive consistently contributed to significantly higher viable population (1x10^6 cfu g^{-1}) of *P. fluorescens* even after 120 days after storage.

Mishra et al. (2013) evaluated compatibility of *B. bassiana* with four surfactants; SDS (sodium dodyl sulphate), CABS-65 (calcium alkyl benzene sulphonate), Tween 20 (polyethylene sorbitan monolaureate) and Tween 80 (polyoxyethylene sorbitan monoleate) at six different concentrations (0.1%, 0.5%, 1%, 2.5%, 5% and 10%). Incubated spores showed decrease in concentrations due to conversion of spores to hyphae. The surfactant effect on spores was more pronounced with SDS and CABS-65, where significant

deterioration and loss in viability of the incubated spores were observed. They further reported that the effect of Tween-20 and Tween-80 were comparatively less inhibiting .

Usha et al. (2014) studied the compatibility of *B.bassiana* with insecticides (Chlorpyrifos, Imidacloprid, Monocrotophos and Quinalphos), fungicides (Mancozeb, Sulphur, Copper oxychloride and Carbendazim) and botanicals (Herbastim, Exodos, Biospark and Neem gold) at three different concentrations (0.1%, 0.5% and 1%). The study revealed that chlorpyrifos was highly detrimental to all the isolates even at low concentrations, while all the botanicals were compatible with *B. bassiana* isolates.

Sangamithra et al. (2015) evaluated the compatibility of six oils *viz.,* neem (*Azadirachta indica*), pongamia (*Millettia pinnata*), castor (*Ricinus communis*), mahua (*Madhuca longifolia*), corn (*Zea mays*) and paraffin with *B. bassiana*. Paraffin oil 10% recorded highest colony diameter (6.50cm) in comparison to control (8.70cm). While fungus sporulation was good in corn oil 10 % (1.83 x 10^8 spores ml^{-1}) followed by paraffin oil (1.80 x 10^8 spores ml^{-1}). They concluded that oils were compatible (except pongamia oil and paraffin oil) and may be encouraged because of its rheology that may suit well for formulation.

Petlamul et al. (2017) reported that *B.bassiana* has the ability to produce cellulolytic enzymes on CMC and thus the ability of cellulose degradation to carbon source led to their growth.

2.4. Shelf life studies of different *B. bassiana* formulations and to evaluate the effective formulations against insect-pest complex on tomato under protected conditions

a. Preparation of *B.bassiana* formulations

To enhance the mycopesticide efficacy, appropriate formulation is required which stabilizes the productivity, distribution and storage of the fungus. Fungal formulations are mainly available in the form of dry products, suspensions and emulsions. The formulation has been reported to greatly enhance the efficacy and persistence of entomopathogenic fungus (Navon and Ascher 2000).

(i) Talc based formulation

Talc-based fungal formulations were found to be more effective and cheaper in pest control (Radjacommare et al. 2002, Rajendran et al. 2007, Saravanakumar et al. 2007 and Kavino et al. 2007).

(ii) Oil based formulation

Several vegetable oils such as sunflower, groundnut, safflower and cotton seed oils, petroleum based oils such as kerosene and diesel have been used for the preparation of formulation of entomopathogenic fungus, *M.anisopliae* and *N.rileyi* (Nahar et al. 2003). They reported that combination of sunflower oil + diesel (3:7) formulations with *M.anisopliae* and *N.rileyi* resulted in higher chickpea yields *i.e.,*16.64 q/ha and 12.65 q/ha, respectively.

Vimala Devi and Hari (2009) formulated two isolates of *B. bassiana* - ITCC 4513 and HaBb DOR as suspension concentrates (SC) in groundnut, sunflower and mineral oils and observed that performance of both the isolates were superior to unformulated. They concluded that the lipophilic nature of the *B.bassiana* conidia helps them to easily suspend in the oils.

The effects of different vegetable oil formulations on the temperature tolerance and storage duration of *B. bassiana* conidia were studied by Mola and Afkari (2012). They reported that there was a significant difference between tolerance of conidia to different vegetable oil formulation and further observed that the lowest effects on storage and germination of conidia were established at 25^0C with olive oil formulation.

b. Shelf life studies of *B. bassiana* formulations

Powder formulations were prepared with conidia of the entomopathogenic fungus *B. bassiana* strain 447 by Alves et al.(1996). The carrier materials used in the formulations included talc hydrous magnesium silicate, silica gel, powdered rice and corn starch and were stored in plastic containers under three conditions: ambient temperature (15°C to 38°C), refrigerator (6°C to -2°C) and freezer (-10°C to -7°C). Formulations stored under ambient temperature conditions completely lost viability after 8 months. Unformulated conidia stored under ambient conditions were totally unviable after 2 months. All formulations stored under refrigerator and freezer conditions maintained 100% viability for 7 years.

Shelf life is the major constraint in commercial production of myco-pesticides. It varies in response to many factors such as temperature, fungal species and life stage, water availability, production and harvest methods (Jones and Burges 1998).

Conidia can be applied in several formulations, the maintenance of conidial viability in formulations during storage is crucial for obtaining successful insect control in the field. Some studies reported that successive cultures of entomopathogenic fungi in artificial media cause phenotypic alteration (changes in colour, growth and morphology) and degeneration that leads to decay in sporulation, metabolites and virulence of the fungi (Ibrahim et al. 2002, Ryan et al. 2002 and Butt et al. 2006).

Puzari et al. (2003) studied virulence and conidial density of *B.bassiana* at room temperature and observed that the virulence (90.97%) and conidial density (39.4 x 10^7 conidia ml^{-1}) did not differ up to 90 days, after which it declined significantly to 82.20% and 30.27 x 10^7 conidia ml^{-1}, respectively, on 120 days of storage. With an increase in age, the viability and infectivity decreased significantly and percentage of dried conidia was 100 beyond 180 days.

Simková (2009) studied the impact of different carriers and storage temperatures on conidia of the fungus *B. bassiana*. The spores were formulated in three types of carriers (one nutritive and two inert carriers) and stored at three different temperatures *viz.*, 22^0C, 4^0C and -20°C. The initial germination rate was 97.67% and after storage at 4°C for 90 days the germination rate was 97.33%. On the contrary, worse results were mostly achieved in unformulated conidia stored at 22°C which had germination rate of 12.33% after 90 days of storage.

Kalidas (2010) reported that growth of *B.bassiana* on PDB was fast and was used for talc formulation. The number of spores in the talc formulated cultures dropped from 10^6 to 10^3 (minimal number required to keep it viable) in 18 months, and so it's use is recommended within this period from packing.

Kaur and Joshi (2014) studied the viability of *B. bassiana* spores at different storage temperature and reported that talc based formulation at refrigeration temperature as the best and the spores were viable upto three months of storage.

c. To evaluate the effective formulations against insect-pest complex on tomato under protected conditions

The studies made with respect to tomato insect-pest complex is scanty, hence an attempt has been made to collect the information in other crop ecosystems and are presented below.

Forschler and Nordin (1989) stated that commercial wettable powder formulation of entomopathogenic fungus *B. bassiana* (AGB 6178) when applied at $2x10^9$, $2x10^{10}$ and $1x10^{11}$ conidia/m^2 against cotton wood borer, *Plectodera salcator* (F.) (Coleoptera: Cerambicidae) caused more than 60% infection in the adult beetles.

Zhang et al. (1992) reported that the wettable powder formulation of *B. bassiana* @ 5 $x10^9$ spores g^{-1} when applied against Asian corn borer, *Ostrinia furnacalis* under laboratory condition showed 95% mortality with LC_{50} and LT_{50} values of 7.3 x 10^5 spores ml^{-1} and 113.6 hrs, respectively.

Kaaya and Hassan (2000) reported that *B. bassiana* and *M. anisopliae* oil formulations ($1x10^9$ conidia ml^{-1}) caused 100% larval mortality of Brown ear tick, *Rhipicephalous appendiculatus* Neum and tropical bont tick, *Amblyomma variegates* (F.) whereas, mortality in nymphs and adults varied between 80 to 100% and 80 to 90%, respectively.

Nankinga and Moore (2000) reported that single application of *B. bassiana* wettable powder formulation prepared from maize @ 3 $x10^9$ conidia g^{-1} proved to be most effective in reducing the banana weevil population which ranged from 65 to 72% within 8 weeks of application.

Orozcosatho et al. (2000) reported that commercial wettable powder formulation of *B.bassiana*, Mycotrol-R registered higher mortality of whitefly nymphs and adults as compared to other commercial formulation of *B.bassiana i.e.,* Naturalis-L and insecticide endosulfan.

Silva and Silva (2001) studied the comparative performance of three *B. bassiana* formulations *viz.,* mineral oil suspension, water suspension, combination of mineral oil and water against cotton boll weevil. They reported that aqueous formulation of *B. bassiana* was as efficient as the mineral oil suspension.

The commercial entomopathogenic fungal based formulation Bio-power (*B. bassiana*) recorded significantly higher mortality of *Plutella xylostella* ranging between 6.7 to 86.7 % (Sood et al. 2001) and 47.6 to 83.2 % (Ramarethinam et al. 2002) on cauliflower in greenhouse conditions.

Vimala Devi et al. (2002) revealed that oil formulation of *N. rileyi* with triton-x-100 significantly reduced *Spodoptera litura* population on groundnut and castor under field conditions.

Eshwaramoorthy et al. (2003) reported that in laboratory bioassay, pressmud formulation of *B. bassiana* caused higher mortality of third instar grubs of *Holotrichia serrata* (Fab.) than lignite formulations.

Manjula et al. (2003) evaluated different oil formulations of *B. bassiana* against *B. tabaci* and concluded that among the different formulations, ground nut oil formulation recorded 100% mortality of adults followed by coconut oil formulation (64.40%) at 72 hrs after inoculation.

Prithiva et al. (2017) evaluated different formulations of *B. bassiana viz.,* crude, talc and oil based against *B. tabaci* on tomato under microplot conditions. They concluded that oil formulation was most effective and recorded 45.86% reduction in whitefly population, followed by talc (29.62%) and crude formulations (21.63 %).

MATERIAL AND METHODS

This chapter includes details of the material used and methodology followed during the course of present investigation. In accordance with the objectives, the studies were divided into four sections as detailed below.

1. Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci)* through laboratory bioassay.
2. Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*.
3. Compatibility of virulent isolate of *B. bassiana* with various additives.
4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulation/s against insect-pest complex on tomato under protected conditions.

3.1 Location

The *in-vitro* studies of the present investigation entitled, **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"** were carried at Bio-control laboratory (Insect Pathology laboratory) and field experiments were conducted in the Polyhouse during *Kharif* seasons of 2016-17 and 2017-18 at Division of Entomology and Nematology, ICAR-Indian Institute of Horticulture Research (IIHR), Bengaluru.

3.2 Climatic condition

Bengaluru also known as "Silicon Valley of India" the capital of Karnataka is the center of India's high-tech industry, situated between 12.97^0 N; 77.56^0 E altitude. A landlocked city, located in the heart of the Mysore plateau at an average elevation of 920 metres above the mean sea level (www.bbmp.gov.in).

Bengaluru is basically dry tropical savanna climate, with distinct wet and dry seasons of relatively equal duration. Temperature extremes varies between minimum temperature of 14^0C during winter and maximum

temperature of 33^{0}C during summer months. The average annual rainfall of about 859 mm, mostly received between June to September and November to December. The average annual relative humidity is 65.2%, while average monthly relative humidity ranges from 45% in March to 79% in August (www.karnataka.com).

3.3 Climatic condition during the crop season

The weather condition during the course of study under protected conditions from June, 2016 to October, 2016 and June, 2017 to October, 2017 are presented in Appendix I and Appendix II.

3.4 *Beauveria bassiana* isolates

Table 1: Source of *Beauveria bassiana* isolates

***B.bassiana* isolates (I)**	**Location**
I - 1	Obtained from NBAIIM , Mau, Uttar Pradesh
I - 2	IIHR isolate-1 ,Bengaluru, Karnataka
I - 3	IIHR isolate-2 ,Bengaluru, Karnataka
I - 4	Isolated from *Bombyx mori* larva from Nellore, Andhra Pradesh
I - 5	Obtained from NBAIIM, Mau, Uttar Pradesh
I - 6	IIHR isolate-3, Bengaluru, Karnataka
I - 7	IIHR isolate-4, Bengaluru, Karnataka

*__NBAIIM :__ National Bureau of Agriculturally Important Microorganisms

3.4.1. Maintenance of cultures

All the *Beauveria bassiana* isolates were maintained on Potato Dextrose Agar (PDA) slants. The stock cultures were maintained at 4°C, till further use (Kaur, 2013).

3.4.2. Preparation of conidial suspension

The fungal isolates were grown on PDA media for 10-14 days in petri dishes (Plate 1) and flasks, while aqueous spore suspensions of various spore concentration was prepared using sterile distilled water. Media was inoculated with 0.1ml of spore suspension in 250ml Erlenmeyer flasks. The seeded flasks were incubated for 15 days at 27±1ºC. The conidia were harvested by scrapping and were suspended in sterile aqueous 0.1% Tween 80 solution (Luz et al. 1998). A sample

of suspension was quantified through plate count method (Roberts and Humber, 2012). A suspension containing $1x10^9$ spores ml^{-1} was prepared and concentrations of $1x10^8$, $1x10^7$, $1x10^6$ and $1x10^5$ spores ml^{-1} were obtained through serial dilutions (Geroh et al. 2015) .The spore suspension was vortexed for 5 minutes to produce uniform conidial suspension. The viability of conidia was evaluated according to studies reported by Alves (1998).

Plate 1: Pure culture of *Beauveria bassiana* on PDA

3.5. Insect cultures

For bioassay studies the insect cultures were maintained under laboratory and protected conditions.

3.5.1. *Helicoverpa armigera* rearing

Helicoverpa armigera (Hubner) larvae were collected from tomato fields and brought into the laboratory and reared in artificial diet (Plate 2) for second generation population as per standard procedures given by Krishnareddy and Hanur (2015) at a temperature of 27±1°C and 60±10% relative humidity. Freshly emerged second instar larvae from the culture were used for experiments (Plate 3).

3.5.2. *Tuta absoluta* rearing

Colony of *Tuta absoluta* was established in the laboratory from the pupae collected from field and maintained on tomato plants under caged condition in Polyhouse (Plate 4 and Plate 5). For the bioassay purpose, newly emerged adults were collected using an aspirator and were provided 10%

honey as food source and terminal twigs of tomato for egg laying (Hussein et al. 2014 and Abdel et al. 2015).

3.5.3. *Bemisia tabaci* rearing

Bemisia tabaci adults were collected from field by using aspirator. They were released and maintained on tomato plants under cages in polyhouse (Plate 4). The whiteflies were allowed to develop and multiply on tomato plants. The third instar nymphal stage of *B. tabaci* were utilised for bioassay studies.

3.6.1. Screening isolates of *B. bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci)* through laboratory bioassay studies

Acquiring promising isolate of *B. bassiana* for biological insect management is the prime factor. For this, various isolates of *B. bassiana* were obtained from different sources (Table 1) and screening was carried out under *in-vitro* conditions to identify promising isolate/s against tomato insect- pest complex.

Experimental details:

Designs	: CRD and Factorial CRD
Replications	: 4
Treatments(Main)	: 8 (7 *B.bassiana* isolates + 1 control)
Doses (Sub-treatments)	: 5 ($1x10^5$ to $1x10^9$ spores ml^{-1})
Methodology	: Bioassay method

Seven isolates of *B.bassiana* with five doses *i.e.,* $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ and $1x10^9$ spores ml^{-1} were selected along with one control set. Ten second instar larvae of *H. armigera*, *T. absoluta* and third instar nymphs of *B. tabaci* were used in the study. Each concentration was replicated four times and water spray treatment served as control.

Bioassay method for *H. armigera*

The immersion method was used for *H. armigera* larvae as described by Goettel and Inglis (1997). Ten second instar larvae were immersed individually for 10 seconds in 10 ml of different fungal suspension and larvae

dipped in water served as control. Treated larvae were allowed to crawl freely on filter paper to remove excess moisture before placing them individually into the petri dishes with filter papers to maintain sufficient moisture which was moistened on the first day and then on every 2nd day with distilled water. Soaked chickpea seeds (pesticide free) were added as food source for the larvae which were regularly replaced by fresh ones. The petri dishes were kept in BOD at 27±1°C and 65±5% RH (Plate 6a).

Bioassay method for *T. absoluta*

Fresh tender tomato leaves were kept in petri dishes and ten second instar larvae were released per each replication and allowed to feed on them. Then the *T.absoluta* mined leaves were dipped into 10 ml of different concentrations of *B.bassiana* and were transferred onto petri dishes with filter papers to maintain sufficient moisture while a set of untreated mined leaves served as control. The treated leaves were used only once at the beginning of the bioassay. Subsequently, the larvae were fed with untreated fresh leaves when needed (Shalaby et al. 2013). The petri dishes were kept in BOD at 27±1°C and 65±5% RH. The larval mortality was observed at 24 hours interval till five days after treatment (Plate 6b).

Bioassay method for *B. tabaci*

Fresh leaves of tomato with ten third instar nymphs of *B.tabaci* per replication were kept in petri dishes. Leaves were sprayed with 10 ml of different concentrations of *B. bassiana* and were transferred onto petri dishes with filter papers to maintain sufficient moisture, while a set of untreated leaves served as control. The petri dishes were kept in BOD at 27±1°C and 65±5% RH. The mortality of nymphs was observed at 24 hours interval till five days after treatment (Plate 6b).

Observations recorded for bioassay studies

Insect mortality and sporulation on cadavers (Plate 7a, b and c) was assessed daily over five days for all the insect pests. The corrected mortality was obtained using Abbott's formula (Abbott, 1925).

$$\text{Corrected percent mortality} = \frac{P_t - P_c}{(100 - P_c)} \times 100$$

Where, P_t - Observed mortality in a treatment; P_c -Observed mortality in control (untreated check)

The median lethal concentration (LC_{50}) values were obtained using probit analysis (Finney, 1962). Further, the mortality data obtained at different concentrations and time intervals was analyzed using one way ANOVA after transforming the per cent mortality data into arcsin transformation (Gomez and Gomez,1984).

3.6.2. Identification of an economic substrate for mass production of the promising isolate/s of *B. bassiana*

Design : CRD

Replications : 3

Treatments : 9

The different solid substrates and liquid media's selected for mass production of *B. bassiana* spores are presented in Table 2 and Plate 8 and 9.

Table 2 : Details of solid substrates and liquid media's selected for mass production of *B.bassiana*

Treatment codes	Treatments
I	**Solid substrates**
T_1	Maize (*Zea mays* L.)
T_2	Chickpea (*Cicer arietinum* L.)
T_3	Sorghum (*Sorghum vulgaris* L.)
T_4	Ragi (*Eleusine coracana* (L.) Gaertn
T_5	Rice (*Oryza sativa* L.)
T_6	Neem cake (*Azadirachta indica*) A.Juss
II	**Liquid media**
T_7	Potato Dextrose Broth (PDB)
T_8	Czapek Dox Broth (CDB)
T_9	Sabouraud Dextrose Broth (SDB)

I. Solid substrates

a) Whole grains

Whole grains of maize, *Zea mays* (L.), chickpea, *Cicer arietinum* L, sorghum, *Sorghum vulgare* L., ragi , *Eleusine coracana* (L.) Gaertn and rice,

Oryza sativa (L.) were used for mass multiplication of *B. bassiana* at 28ºC in BOD. Hundred grams of each substrate was washed and soaked in water for overnight, except rice which was soaked for 3 hours before starting the experiment. The excess water was drained by decanting and further shade drying was done for half an hour to remove the excess moisture. The substrates were packed separately in individual 250 ml conical flask and were plugged with non-absorbent cotton and autoclaved at 15 psi for 30 min. After cooling, 5 mm fungal disc was inoculated into each flask aseptically under laminar air flow chamber. Flasks were incubated in BOD incubator at 28^{0}C. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the grains and to break the mycelial mat.

b) Cakes

100 g of neem cake was taken in a 250 ml conical flask and to it 50 ml of sterile distilled water was added and plugged with non-absorbent cotton. The substrate was sterilized in an autoclave at 15 psi for 30 min. After cooling, 5 mm fungal disc of *B. bassiana* was inoculated into each flask under laminar air flow chamber. Flasks were incubated in BOD incubator at 28ºC. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the cake pieces and to break the mycelial mat.

II. Liquid media

The constituents and methodology for preparation of different liquid media are as follows:

a) Potato dextrose broth (PDB)

To 100 g of peeled and sliced potato, 250 ml distilled water was added in a 1L beaker and boiled till the potatoes became soft. The contents of the beaker were filtered through muslin cloth to squeeze out all the liquid. 10g of dextrose was dissolved in water and added to the extract and the volume was made upto 500 ml. 100 ml of the extract per flask was dispensed in three conical flasks of 250 ml capacity and was plugged with non-absorbent cotton. These flasks were sterilized in an autoclave at 15 psi pressure for 30 min. After cooling, 5 mm fungal disc of *B. bassiana* was inoculated into each flask under laminar air flow chamber. Flasks were incubated in BOD incubator at 28ºC.

b) Sabouraud's dextrose broth (SDB)

250 ml of distilled water was taken into 1L beaker, in which 20 g of dextrose and 10 g of peptone was added and the volume was made to 750 ml. In three conical flasks each of 250 ml capacity, 100 ml of the extract per flask was dispensed and was plugged with non-absorbent cotton and autoclaved at 15 psi pressure for 30 minutes. After cooling, 5 mm fungal disc of *B. bassiana* was inoculated into each flask under laminar air flow chamber. Flasks were incubated in BOD incubator at 28ºC.

c) Czapek Dox Broth (CDB)

Czapek Dox Broth is a synthetic readymade media and the components includes Sucrose 30.00g, Sodium nitrate 3.00g, Dipotassium phosphate 1.00g, Magnesium sulphate 0.50g, Potassium chloride 0.50g and Ferrous sulphate 0.01g. CDB @ 35g /L was dissolved in distilled water, and 100 ml / flask of this media was dispensed into 3 conical flasks of 250 ml capacity. The flasks were sterilized in an autoclave at 15 psi pressure for 30 min. After cooling, 5 mm fungal disc of *B. bassiana* was inoculated into each flask under laminar air flow chamber. Flasks were incubated in BOD incubator at 28^0C.

Determination of Colony Forming Units on substrate

The spore count of the fungus grown on various substrates was estimated through plate count method. For this purpose 10g or 10ml homogenous grain or solution samples were drawn from each replicate of uniformly sporulated flask and transferred to 100 ml sterilized distilled water containing Tween 80 solution (0.05%) in 250 ml conical flask. The flasks were then shaken on mechanical shaker for 10 minutes and the contents were filtered through double layered muslin cloth. Counting of spore's were made after the serial dilution of the suspension using plate count method (Hokkanen and Lynch 1998).

The diluted samples were plated @ 1ml per plate on PDA media. The plates were gently rotated for uniform spreading of the spore suspension and incubated at 28^0C in the BOD. The counts of the colony forming units (CFU) were recorded on the 7^{th} day after plating. These observations were recorded at an interval of 10, 20 and 30 days after inoculation of the fungus.

Determination of conidial count

To record the conidial count, method proposed by Sahayaraj and Namasivayam (2008) and Rajanikanth et al. (2010) were followed with some little modifications.

3.6.3 To study the compatibility of promising isolates/s of *B. bassiana* with various additives

Design : CRD

Replications : 3

Treatments : 13

Concentrations : 3 (0.1%, 0.5% and 1.0%)

Table 3 : Additives selected for compatibility studies with *B. bassiana* (Plate 10)

Tr. Code	Category	Treatments	Importance	References
T_1	Wetting agents and Emulsifiers	Tween-20	Helps to rehydrate spores that are stored dry and helps to disperse clumps	Burges (2012)
T_2		Tween-40		
T_3		Tween-60		
T_4		Tween-80		
T_5		Triton-X		
T_6	Humectants	Glycerol	Delays the evaporation of the liquid and favours spore germination	Kubicek and Druzhinina (2007)
T_7	Desiccants	Kaolite	Regulates water availability to microorganisms and also helps in absorption of harmful metabolic by-products	Agnes(1971)
T_8		Silica gel		
T_9	Crude/ Refined oils	Sunflower oil	Improves spore survival and reduces sensitivity to UV radiations	Mishra et al. (2013)
T_{10}		Neem oil		
T_{11}		Pongamia oil		
T_{12}	Detergent carrier	Carboxyl Methyl Cellulose (CMC)	Enhances the ability of *B. bassiana* to produce cellulolytic enzymes	Petlamul et al. (2017)
T_{13}	Control			

Methodology : Poisoned food technique

Studies on compatibility of different additives with *B. bassiana* was evaluated by poisoned food technique in Potato Dextrose Agar (PDA) medium. For this purpose, 25 ml of PDA media having additives of different concentrations (0.1%,0.5% and 1.0%) was poured into petri dishes aseptically and allowed to solidify under laminar flow cabinet. A 5 mm disc of fungus was taken from 7 day old culture of *B. bassiana* and placed at the center of the petri dishes containing PDA incorporated with different additives. Growth media (PDA) without additives served as control. The plates were incubated in BOD at 28^0C and the diameter of the growing culture was recorded on the 10^{th} day after inoculation. The data was expressed as growth inhibition of *B. bassiana* in additives as proposed by Hokkanen and Kotiluoto (1992):

$$X = \frac{Y - Z}{Z} \times 100$$

Where X, Y, Z stands for percentage of growth inhibition, radial growth of fungus in control and radial growth of fungus in poisoned medium, respectively.

3.6.4. Shelf life studies of different formulations of *B. bassiana* and to evaluate the effective formulations against insect-pest complex on tomato under protected conditions

Formulation will help to retain the biological activity of the active ingredient, to prevent in-store replication of any contaminant microorganisms and to ease the handling of the product.Formulation can be defined as "The mixture of the candidate bio-pesticide and material that affect its chemical, physical and biological properties". So, by keeping in mind the importance of formulation, this objective is taken up.

A. Shelf life studies of different formulations of *B. bassiana*

The development of a suitable formulation is essential for successful utilization of mycoinsecticides. Experimental details are as follows:

Design : CRD

Replications : 3

Treatments : 3 (2 Solid formulations -Talc, Wettable powder based and 1 Liquid formulation - Aqueous based)

Materials used

Wettable formulations and liquid formulation of *B.bassiana* were prepared under *in-vitro* conditions with the most virulent isolate *i.e.*, I-4 using different carriers *viz.,* talc, CMC and vegetable oil (sunflower oil).

Powder based formulation of *B. bassiana*

Methodology proposed by Kaur (2013) was followed with slight modifications which is as follows:

Two types of wettable formulations were prepared by using rice as the basic substrate with talc powder and Carboxy methyl cellulose (CMC) as carriers. *B.bassiana* was mass multiplied on rice and after 30 days of inoculation the conical flasks showing full sporulation were decanted and transferred in the trays for drying, followed by crushing of the grains to fine powder.

a) Carrier : Talc powder : 100g of dried powdered rice grains containing *B. bassiana* conidia was mixed with talc powder in the proportion of 1:2 and dried under aseptic conditions for 24 hrs.

b) Carrier : Carboxy methyl cellulose (CMC) : CMC was added at the rate of 1g/kg powdered rice grains containing *B. bassiana* conidia.

After thorough mixing these formulations were packed into sterilized polyethylene bags and were stored under ambient conditions (Prasad and Rangeshwaran ,1999).

Liquid based formulation of *B. bassiana*

For preparing the liquid based formulation, Potato Dextrose Broth (PDB) was used as base material. To 1L of PDB, 10g of CMC was added followed by autoclaving at 15 psi at 121^0C for 30 min. Four discs of 5 mm diameter of *B. bassiana* (I-4) were added to the broth under aseptic conditions in the laminar airflow chamber. After 30 days of inoculation, 25 ml of the carrier (sunflower oil) and Tween- 80 solution (0.05%) was added to the fully grown culture in the broth. This was mixed thoroughly and stored in sterile screw cap bottle under room conditions (Devi and Hari , 2009).

Determination of colony forming units

Colony forming units of the formulations were assessed by plate count method. The shelf life studies were carried out initially prior to storage and later at monthly interval and continued upto 13 months as the formulated bio-product should at least have 12 months shelf life to get registered under Central Insecticide Board (CIB).

For determining the number of colony forming units of the formulations, 1g or 1ml of wettable or aqueous formulations were homogenized in 9 ml sterile water followed by serial dilutions and later the aliquots of the dilutions were placed on PDA to determine the CFU's (Geroh et al. 2015).

While virulence and viability of the formulations were tested at every three months interval against 2nd instar larvae of *H. armigera* under *in-vitro* conditions.

B. To evaluate the effective formulations against insect-pest complex on tomato under protected conditions

The formulation/s of *B.bassiana* that retained spore viability (shelf life) even after six months of storage were evaluated for their virulence against insect-pest complex on tomato under protected conditions during *kharif* 2016-17 and 2017-18.

For conducting the studies on efficacy of *B. bassiana* formulations, the experiment was laid with the following details:

Design	:	RBD
Replications	:	4
Plot size	:	2.40 x 2.40m
Crop	:	Tomato
Variety	:	NS-501
Spacing: Row x Plant	:	60 x 45 cm
Treatments	:	2 spore concentration ($2x10^8$ and $2x10^9$ cfu/ml or g) of the effective formulation/s along with a control

Table 4: Treatment details of *B. bassiana* formulation for the management of tomato insect pest complex under protected conditions

Treatment code	Treatments	
	Formulations	**Spore concentration (cfu/g or ml)**
T_1	Wettable powder	2 x 10^8 cfu / g
T_2	Wettable powder	2 x 10^9 cfu / g
T_3	Aqueous based	2 x 10^8cfu / ml
T_4	Aqueous based	2 x 10^9 cfu / ml
T_5	Control	

Date of transplanting : 25/05/2016 and 27/05/2017

Number of spraying : 5

Initiation of spray : When ETL is observed for *H.armigera*, *T.absoluta* and *B.tabaci* (as proposed by Gautam et al. 2018; Sridhar et al.2015 and Afzal et al.2014, respectively)

Frequency of spraying: At 10 days interval after first spray

Methodology for field observations

Pre- count observations was taken before spraying and at 3, 7 and 10 days after spraying.

Observations on fruit borer, *H.armigera* larvae were recorded as proposed by Jeyarani et al. (2010) on five tagged plants per treatment per replication.

Observations on live mines of *T.absoluta* (larvae) were recorded as suggested by Sridhar et al. (2016). Larval counts were recorded on three leaves per plant (*viz.,* upper , middle and bottom of the plant canopy) and five such plants were observed per treatment per replication.

Observations on whitefly, *B.tabaci* were recorded as proposed by Prithiva et al. (2017). Nymphal counts were recorded on nine leaves per plant (*viz.,* three leaves each from upper, middle and bottom of the plant canopy) and from each leaf 3 cm^2 area was observed and 27 cm^2 leaf area per plant and five such tagged plants were observed per treatment per replication.

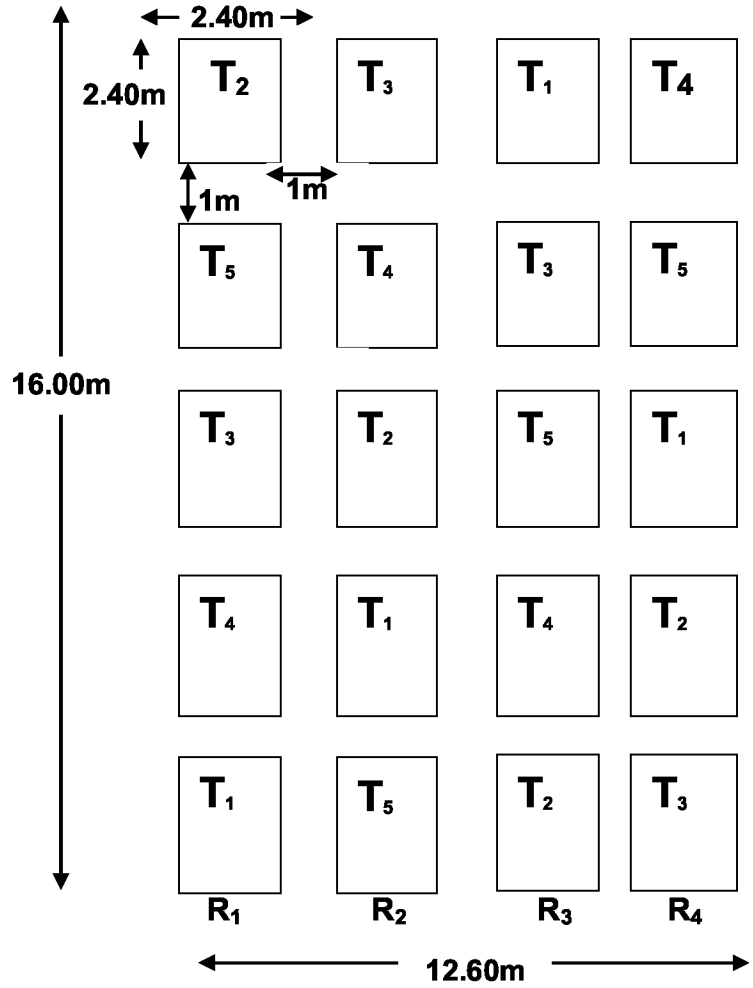

Figure 1: Layout plan of experimental trial under protected conditions

3.7 Statistical Analysis

The data obtained from the laboratory and field experiments were statistically analysed following standard procedures (Gomez and Gomez, 1984).

I. Analysis of variance – Complete Randomized Design (CRD)

Analysis of different variables was carried out to know the inherent variability` amongst all the treatments. The analysis of variance of different observations has been given in appendix and the skeleton of ANOVA for CRD is presented in the table given below :

Skeleton of Analysis of Variance (ANOVA)

Sources of variation	df	SS	MSS	F calculated	F table	
					5%	1%
Treatments	t-1	SSt	SSt/t-1=MSSt	MSSt / MSSe		
Error	N-t	SSe	SSe/n-t=MSSe	-	-	-
Total	N-1	TSS	-	-	-	-

t = Number of treatments

N = Total number of observations

df = Degrees of freedom

SS = Sum of squares

SSt =Sum of squares of treatments

SSe= Sum of squares of error

TSS= Total sum of squares

MSS = Mean sum of squares

MSSt=Mean sum of squares of treatments

MSSe= Mean sum of squares of error

The 'F' test was applied to check the overall significance of various treatments in general and comparison of individual treatment was made with the help of critical difference at 5 % level of significance, which was calculated as given below :-

$$\text{SEm}\pm \text{ for treatment 't'} = \sqrt{\frac{\text{MSSe}}{\text{No. of replications}}}$$

SEd for treatment = SEm x √2

CD for treatment = SEd x 't' value at 5% error degree of freedom

Where,

SEm± = Standard Error of treatment means

SEd = Standard Error of difference between two treatments

CD = Critical difference

t = ' t' value at 5 % level at error df

II. Analysis of variance- Factorial CRD

Analysis of different variables was carried out to know the inherent variability amongst all the treatments. The data were statistically analyzed and the analysis of variance has been given in appendix and the skeleton of ANOVA for Factorial CRD is presented in the table given below:

Sources of variation	df	SS	MSS	F calculated	F table	
					5%	1%
Isolates (I)	I-1	SSI	SSI / I-1 = MSSI	MSSI / MSSe		
Spore concentration (S)	S-1	SSS	SSS / S-1= MSS	MSSS / MSSe		
I x S	(I-1)(S-1)=i	SSi	SSi / i = MSSi	MSSi / MSSe		
Error	I x S (r-1)	SSe	SSe /IS (n-1) = MSSe	-	-	-
Total	ISr-1	TSS	-	-	-	-

Where,

I = Number of isolates

S = Spore concentration

i = Isolates x spore concentration interaction

r = Number of replications

df = Degrees of freedom

SS = Sum of squares

SSI = Sum of squares of isolates

SSS = Sum of squares of spore concentration

SSi = Sum of squares of interaction of isolates x spore concentration

SSe = Sum of squares of error

TSS = Total sum of squares

MSS = Mean sum of squares

MSSI = Mean sum of squares of isolates

MSSS = Mean sum of squares of spore concentration

MSSi = Mean sum of squares of interaction between isolates and spore concentration

MSSe = Mean sum of squares of error

The 'F' test was applied to check the overall significance of various treatments in general and comparison of individual treatment was made with the help of critical difference at 5 % level of significance, which was calculated as given below:-

Mean standard error

SEm± for isolate

$$= \sqrt{\frac{\text{MSSe}}{\text{r x No.of spore concentration}}}$$

SEm± for spore concentration

$$= \sqrt{\frac{\text{MSSe}}{\text{r x No. of isolates}}}$$

SEm± for interaction

$$= \sqrt{\frac{\text{MSSe}}{r}}$$

Critical difference (CD) :

CD = SEd x 't' value at 5% error degrees of freedom

where,

MSSe = Mean sum of square of error

r = Number of replications

t = ' t' value at 5 % level at error df

SEm± = Standard error of mean

CD = Critical Difference

III. Analysis of variance- Randomised Complete Block Design (RCBD)

The data were subjected to statistical analysis after tabulation. The percentage data were transformed to their angular values. The data so

obtained were analysed using the analysis of variance techniques as given below:

Skeleton of "Analysis of Variance" (ANOVA):

Sources of variance	df	SS	MSS	F calculated	F table	
					5%	1%
Replications	(r-1)	SSR	SSR/ r-1= MSSr	MSSr / MSSe		
Treatments	(t-1)	TSS	SST/ t-1= MSSt	MSSt / MSSe		
Error	(r-1) (t-1)	SSE	SSE/(r-1)(t-1)= MSSe	-	-	-
Total	(r.t-1)	-	-	-	-	-

Where,

r = Number of replications

t = Number of treatments

df = Degrees of freedom

SS = Sum of squares

SSR = Sum of squares of replication

TSS = Sum of squares of treatment

SSE = Sum of squares of error

MSS = Mean sum of squares

MSSr = Mean sum of squares of replication

MSSt = Mean sum of squares of treatment

The 'F' test was applied to check the overall significance of various treatments in general and comparison of individual treatment was made with the \4help of critical difference at 5 % level of significance, which was calculated as given below:-

$$SEm\pm = \sqrt{\frac{MSSe}{\text{No. of replications}}}$$

$$CD = SEm \pm \times \sqrt{2} \times t \text{ at } 0.05$$

where,

MSSe = Mean sum of squares error

t = ' t' value at 5 % level at error df

SEm± = Standard error of mean

CD = Critical Difference

IV. Fruit damage by *H. armigera* and *T. absoluta*

Percentage of fruits damaged by *H.armigera* and *T.absoluta* was calculated for different treatments as per the following formula:

$$\text{Fruit damage by } H.armigera \text{ / } T.absoluta\ (\%) = \frac{\text{No. of fruits damaged by } H.armigera \text{ or } T.absoluta}{\text{Total number of fruits observed}} \times 100$$

V. Fruit yield:

Fruit yield was calculated for different treatments as per the following formula:

Yield (kg/ha) = Conversion factor x fruit yield / plot (kg)

Where,

$$\text{Conversion factor} = \frac{10000}{\text{Net plot size}} \text{ in sq. m.}$$

RESULTS

The findings of the experiment on **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"** are described in this chapter under respective objectives.

4.1 Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay

The experiment on screening of seven isolates of *B.bassiana* against tomato insect pest complex was carried under *in-vitro* conditions at five different concentrations with four replications and the data presented in Tables 5 to 28.

4.1.1. Mortality of *Helicoverpa armigera* larvae (2^{nd} instar)

4.1.1. a. Spore concentration : 1 x 10^5 spores ml^{-1}

4.1.1. a. i. At 24 hours after spraying

Differences in the *H.armigera* larval mortality among different *B. bassiana* isolates tested were not significant. Among them, isolate I-1 recorded highest larval mortality (2.50%), whereas no mortality was recorded in other isolates including control and similar trend was recorded in corrected *H.armigera* larval mortality (Table 5).

4.1.1. a. ii. At 48 hours after spraying

Perusal of the data in Table 5, revealed that at 48 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (7.50%), followed by isolate I-1 (5.00%), but both were at par with each other. While no larval mortality was recorded in the remaining isolates and control.

Perusal of the data in Table 5 revealed that at 48 hours after spray there was significant difference in the corrected larval mortality and the trend was similar as observed in larval mortality (Table 5).

4.1.1. a. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *H.armigera* larval

mortality among different isolates were significant. Among them, isolate I-4 recorded highest larval mortality (17.50%), followed by isolate I-3 (12.50%), but both were at par with each other. The next effective isolates were I-1 and I-5 (both recorded 5.00% mortality), followed by I-2, I-6, I-7 and control (all of them recorded 2.50% mortality), but non-significant difference were observed among the isolates (Table 5).

Perusal of the data in Table 5 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Isolate I-4 was found to be most effective as it recorded highest corrected larval mortality (15.28%), followed by I-3 (10.00%), but both were at par with each other. The next effective isolates were I-1 and I-5 and both of them recorded 2.50% corrected larval mortality, while there was no mortality in isolates I-2, I-6 and I-7, but all were statistically at par with each other.

4.1.1. a. iv. At 96 hours after spraying

At 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (27.50%), followed by I-2 (22.50%), I-1, I-3 and I-5 registered 20.00% larval mortality, but all were statistically at par with each other. The least effective isolates were I-6 and I-7 with 7.50% larval mortality, while in control it was 5.00%, but there was no significant difference among them (Table 5).

Perusal of the data in Table 5 revealed that at 96 hours after spray there was significant differences in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (23.61%), followed by isolate I-2 (18.61%), I-3 and I-5 (15.83%) and I-1(15.28%), but they did not differ significantly from each other. The lowest effective isolates were I-6 and I-7 (both registered 2.50% larval mortality), but both the isolates were at par with each other.

4.1.1. a. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *H.armigera* larval mortality among the different isolates were significant. Among them, isolate I-4 recorded highest larval mortality (50.00%), followed by isolate I-1 (47.50%) and I-5 (32.50%), but all were statistically at par with each other.The next effective isolate was I-3 (27.50%), followed by I-2 (22.50%), I-7 (20.00%) and

I-6 (12.50%), but there was no significant difference among these isolates. However, larval mortality recorded in control was 7.50% (Table 5).

Perusal of the data in Table 5 revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate I-4 was found to be highly effective as it recorded highest corrected larval mortality (46.11%), I-1 (43.89%), I-5 (26.94%), but these isolates didn't differ significantly from each other. The next effective isolate was I-3 (21.94%), followed by I-2 (16.39%) and I-7(13.61%), but all were at par with each other. Lowest corrected larval mortality was recorded in I-6 (5.28%).

4.1.1. b. Spore concentration : 1 x 10^6 spores ml^{-1}

4.1.1. b. i. At 24 hours after spraying

Differences in the *H.armigera* larval mortality among different *B. bassiana* isolates tested were not significant. Among the isolates I-1 , I-2 and I-4 recorded highest larval mortality (2.50%), respectively, whereas no mortality was recorded in other isolates including control and similar trend was exhibited in corrected larval mortality (Table 6).

4.1.1. b. ii. At 48 hours after spraying

At 48 hours after treatment, the differences in the *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-1 and I-4 were found to be most effective as they recorded highest larval mortality (12.50%), followed by isolates I-2, I-3 and I-5 (all recorded 7.50% larval mortality), but all were statistically at par with each other. The next effective isolates were I-6 and I-7 (with 2.50% larval mortality), whereas no larval mortality was recorded in control. Computation of corrected larval mortality exhibited similar trend as in larval mortality (Table 6).

4.1.1. b. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (30.00%), followed by isolates I-2, I-3 and I-5 (22.50%), but all were statistically at par with each other. The next effective isolates were I-6 and I-7 (both of them registered 10.00% mortality), followed by isolate I-1 (7.50%), however, there was no significant difference among them, but were significantly superior to control (2.50%)(Table 6).

Perusal of the data in Table 6 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (27.78%), followed by isolate I-2 and I-5 (both recorded 20.56% mortality), followed by I-3 (20.28%), but there was no significant difference among these isolates. The next effective isolates were I-6 and I-7 (with 7.50% mortality), followed by I-1 (5.00%), but all were at par with each other.

4.1.1. b. iv. At 96 hours after spraying

Perusal of the data in Table 6 revealed that at 96 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-1 and I-4 were found to be most effective as they recorded highest larval mortality (47.50%), followed by I-5 (45.00%), but all were statistically at par with each other. The next effective isolate was I-3 (32.50%), followed by I-2 and I-6 (both registered 30.00% mortality), but there was no significant difference among these isolates. The lowest effective isolate was I-7 (20.00%), but was significantly superior to control (5.00%).

Perusal of the data in Table 6 revealed that at 96 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest *H.armigera* corrected larval mortality (44.72%), followed by I-1 (44.44%), I-5 (42.22%) and I-3 (28.89%), but all were statistically at par with each other. The next effective isolate was I-6 (25.83%), followed by I-2 (25.83%) and I-7 (15.56%), but there was no significant difference among these isolates.

4.1.1. b. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *H.armigera* larval mortality among the different isolates were significant. Among them, isolate I-1 and I-4 (both recorded 65.00% mortality), but were statistically at par with each other.The next effective isolate was I-5 (47.50%), followed by I-3 and I-6 (with 42.50% mortality), I-2 (37.50%) and I-7 (35.00%), but there was no significant difference among these isolates. However, the larval mortality recorded in control was 7.50% (Table 6).

Perusal of the data in Table 6, revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-1 and I-4 were highly effective as both of them recorded highest corrected larval mortality (62.22%), but they did not differ significantly from each other. The next effective isolate was I-5 (42.22%), followed by I-3 and I-6 (37.50%), I-2 (32.50%) and I-7 (29.17%), but no significant differences were observed between these isolates.

4.1.1. c. Spore concentration : 1 x 10^7 spores ml^{-1}

4.1.1. c. i. At 24 hours after spraying

Differences in the *H.armigera* larval mortality among different *B. bassiana* isolates tested were not significant. Among the isolates, I-4 recorded highest larval mortality (7.50%), followed by I-1 and I-2 (5.00%), I-3, I-5 and I-7 (all recorded 2.50% mortality), while no mortality was recorded in I-6 and control. Corrected larval mortality exhibited similar trend as observed in larval mortality (Table 7).

4.1.1. c. ii. At 48 hours after spraying

Perusal of the data in Table 7 revealed that at 48 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (20.00%), followed by I-2 and I-5 (15.00%) and I-1 (12.50%), but all were at par with each other. The next effective isolate was I-3 (10.00%), followed by I-6 and I-7 (both recorded 7.50% mortality), but all were statistically at par with each other. While no larval mortality was recorded in control.

Computation of corrected larval mortality at 48 hours after spray revealed that there was no significant difference among the isolates and the trend was similar as observed in larval mortality (Table 7).

4.1.1. c. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (40.00%), followed by isolates I-1, I-2 and I-3 (27.50%), but all were statistically at par with each other. The next effective isolate was I-5 (25.00%), followed by I-7 (15.00%), but both were at par with

each other. Whereas, lowest larval mortality was recorded in I-6 (10.00%), but was significantly superior to control (2.50%) (Table 7).

Perusal of the data in Table 7 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Highest corrected larval mortality was recorded in isolate I-4 (38.06%), followed by isolate I-1 (25.56%), I-2 and I-3 (both registered 25.28% corrected larval mortality) and I-5 (22.78%), but they didn't differ significantly from with each other. The next effective isolate was I-7 (12.78%), followed by I-6 (7.50%), but non-significant differences were observed between them (Table 7).

4.1.1.c .iv. At 96 hours after spraying

Perusal of the data in Table 7 revealed that at 96 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-1 and I-4 were found to be most effective as they recorded highest larval mortality (57.50%), followed by isolates, I-2 (45.00%) and I-5 (42.50%), but all were statistically at par with each other. The next effective isolate was I-3 (40.00%), followed by I-6 (32.50%) and I-7 (30.00%), but there was no significant difference among these isolates. Whereas, larval mortality recorded in control was 5.00%.

Perusal of the data in Table 7 revealed that at 96 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-1 was found to be most effective as it recorded highest corrected larval mortality (55.28%), followed by I-4 (54.72%) and I-2 (41.94%), but all were statistically at par with each other. The next effective isolate was I-5 (39.44%), followed by I-3 (36.94%), I-6 (29.17%) and I-7 (26.39%), but there was no significant difference among these isolates.

4.1.1. c. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *H.armigera* larval mortality among the different isolates were significant. Among the isolates I-4 and I-1 recorded highest larval mortality (72.50%), followed by I-3 (60.00%) and I-5 (57.50%), but all were statistically at par with each other.The next effective isolate was I-2 (47.50%) followed by I-6 (42.50%) and I-7 (40.00%), but there was no significant difference among these isolates. However, larval mortality recorded in control was 7.50% (Table 7).

Perusal of the data in Table 7 revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolates, I-1 and I-4 were found to be highly effective as both of them recorded highest larval mortality (70.28%), but they did not differ significantly from each other. The next effective isolate was I-3 (56.94%), followed by I-5 (54.17%) and I-2 (42.78%), but all were statistically at par with each other.The next effective isolate was I-6 (37.78%), followed by I-7 (35.00%), however non-significant difference was observed between these isolates.

4.1.1. d. Spore concentration : 1 x 10^8 spores ml^{-1}

4.1.1. d. i. At 24 hours after spraying

Perusal of the data in Table 8 revealed that at 24 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (15.00%), followed by I-1 (10.00%), I-3 (7.50%) and I-5 (7.50%), but all these were statistically at par with each other. The next effective isolates were I-2 and I-6 and both of them recorded 5.00% larval mortality followed by I-7 (2.50%), but they did not differ significantly from each other. While no larval mortality was recorded in control.

Perusal of the data in Table 8 revealed that at 24 hours after spray there was significant difference in the corrected larval mortality and exhibited similar trend as observed in larval mortality.

4.1.1. d. ii. At 48 hours after spraying

Perusal of the data in Table 8 revealed that at 48 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (35.00%), followed by I-1 and I-2 (both registered 20.00% mortality), but all were at par with each other. The next effective isolates were I-3 and I-5 (both recorded 17.50% larval mortality), followed by I-6 and I-7 (10.00%), but there was no significant difference between these isolates. While no larval mortality was recorded in control and similar trend was observed in corrected larval mortality.

4.1.1. d. iii. At 72 hours after spraying

Perusal of the data in Table 8 revealed that at 72 hours after spray, the differences in the mean larval mortality among different isolates were

significant. Among the isolates, I-4 recorded highest larval mortality (55.00%) and was significantly superior than all the other isolates. This was followed by I-1 (40.00%), I-2 (32.50%) and I-3 and I-5 (both recorded 30.00% mortality), but they were statistically at par with each other. The next effective isolate was I-6 (20.00%), followed by I-7 (17.50%), but there was no significant difference among them. The mortality recorded in control was 2.50%.

Perusal of the data in Table 8 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (53.89%).The next effective isolate was I-1 (38.06%), followed by I-2 (30.83%) and I-3 and I-5(recorded 28.33% mortality), but there was no significant difference among these isolates. The next effective isolate was I-6 (17.78%), followed by I-7 (15.28%), but all were statistically at par with each other .

4.1.1. d. iv. At 96 hours after spraying

Perusal of the data in Table 8 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (72.50%), followed by I-1(65.00%), but both were statistically at par with each other. The next effective isolates were I-3 and I-5 (both recorded 50.00% mortality), followed by I-2 (47.50%) and I-6 and I-7 (both of them recorded 37.50% larval mortality), but there was no significant difference among these isolates. Larval mortality recorded in control was 5.00%.

Perusal of the data in Table 8 revealed that at 96 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (71.11%), followed by isolate I-1 (63.06%), but both were statistically at par with each other. The next effective isolates were I-3 and I-5 with corrected larval mortality of 47.22% followed I-2 (44.72%), I-7 (33.89%) and I-6 (33.61%), however no significant difference was observed among these isolates.

4.1.1. d. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *H.armigera* larval mortality among the different isolates were significant. Among the isolates, I-4

recorded highest larval mortality of 90.00%, followed by I-1 (82.50%), but both were statistically at par with each other. The next effective isolate was I-3 (72.50%), followed by I-5 (65.00%), I-2 and I-7 (both registered 57.50% mortality) and I-6 (50.00%), but there was no significant difference among these isolates. Larval mortality recorded in control was 7.50% (Table 8).

Perusal of the data in Table 8 revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate I-4 was found to be highly effective as it recorded highest larval mortality (89.72%), followed by I-1 (81.39%), but they did not differ significantly from each other. The next effective isolate was I-3 (70.56%), followed by I-5 (62.22%), I-2 (53.89%), I-7 (53.61%) and I-6 (45.56%), but all were at par with each other.

4.1.1. e. Spore concentration: 1 x 10^9 spores ml^{-1}

4.1.1. e. i. At 24 hours after spraying

Perusal of the data in Table 9 revealed that at 24 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (17.50%), followed by isolate I-1 (12.50%), I-7 (10.00%) and I-2, I-3, I-5 and I-6 (all registered 7.50% larval mortality), but all of them were at par with each other. While no larval mortality was recorded in control. Similar trend was observed in corrected larval mortality.

4.1.1. e. ii. At 48 hours after spraying

Perusal of the data in Table 9 revealed that at 48 hours after spray, the differences in the mean *H.armigera* larval mortality among different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (42.50%) and was significantly superior than all other isolates. The next effective isolates were I-1, I-2, I-3 and I-5 (with 22.50% mortality), followed by I-7 (15.00%) and I-6 (12.50%), but all these isolates were at par with each other. While no larval mortality was recorded in control. Similar trend was observed in corrected larval mortality.

4.1.1. e. iii. At 72 hours after spraying

Perusal of the data in Table 9 revealed that at 72 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (70.00%)

and was significantly superior than all other isolates. The next effective isolate was I-1 (52.50%), followed by I-2 (45.00%), I-3 (42.50%) and I-5 (37.50%), but all were statistically at par with each other. The next effective isolates were I-6 and I-7 (both of them recorded 25.00% mortality). Larval mortality recorded in control was 2.50%.

Perusal of the data in Table 9 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (69.17%) and was significantly superior than all the other isolates .The next effective isolate was I-1 (51.67%), followed by I-2 (43.61%), I-3 (41.11%) and I-5 (36.11%), but there was no significant difference among these isolates. The next effective isolate was I-6 (23.06%), followed by I-7 (22.78%), but both of these were statistically at par with each other.

4.1.1. e. iv. At 96 hours after spraying

Perusal of the data in Table 9 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest larval mortality (90.00%), followed by I-1 (85.00%), but both were statistically at par with each other. The next effective isolate was I-5 (70.00%), followed by I-2 and I-3 (both of them registered 57.50% mortality) and I-7 (47.50%), but there was no significant difference among these isolates. The next effective isolate was I-6 (42.50%) and was significantly superior to control (5.00%).

Perusal of the data in Table 9 revealed that at 96 hours after spray there was significant differences in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (89.17%), followed by isolate I-1 (84.44%), but both were statistically at par with each other. The next effective isolate was I-5 (68.61%), followed by isolates I-2 and I-3, both recorded 55.00% mortality, followed by I-7 (44.74%), but no significant difference were observed between these isolates. Corrected larval mortality recorded in I-6 was 39.44%.

4.1.1. e. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *H.armigera* larval mortality among the different isolates were significant. Among the isolates, I-4

recorded highest larval mortality (100.00%) followed by isolate I-1 (95.00%), but there was no significant difference among both the isolates. The next effective isolate was I-5 (80.00%), followed by I-3 (77.50%) and I-6 (65.00%), but all were at par with each other. The next effective isolates were I-2 and I-7 (both recorded 62.50% mortality), but non-significant differences were observed between them. Larval mortality recorded in control was 7.50% (Table 9).

Perusal of the data in Table 9, revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be superior than all the other isolates as it recorded 100.00% corrected larval mortality followed by I-1 (94.44%), and they differed significantly from each other. The next effective isolate was I-5 (78.33%), followed by I-3 (75.83%) and I-6 (61.94%), but all were at par with each other. The next effective isolate was I-2 (59.44%), followed by I-7 (50.28%), but they did not differ significantly from each other.

4.1.2. Mortality of *Tuta absoluta* (2^{nd} instar)

4.1.2. a. Spore concentration: 1 x 10^5 spores ml^{-1}

4.1.2. a. i. At 24 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B. bassiana* isolates tested were not significant. No larval mortality was recorded in all the isolates, including control (Table 10).

Computation of the corrected larval mortality at 24 hours after spray was not possible as there was no larval mortality observed (Table 10).

4.1.2. a. ii. At 48 hours after spraying

Perusal of the data in Table 10 revealed that at 48 hours after spray, the differences in the mean *T.absoluta* larval mortality among different isolates were not significant. No larval mortality was recorded in all isolates including control, hence corrected larval mortality was not computed.

4.1.2. a. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *T.absoluta* larval mortality among different isolates were not significant. Among the isolates I-4 recorded highest larval mortality (7.50%), followed by isolates I-2, I-3, I-5 and I-6 (all these four isolates recorded 5.00% larval mortality), I-1, I-7 and control (2.50% larval mortality), respectively (Table 10).

Corrected larval mortality at 72 hours after spray revealed that there was no significant difference among the isolates. However, highest corrected larval mortality was recorded in isolate I-4 (5.00%), followed by I-2, I-3, I-5 and I-6 (with 2.50% corrected mortality), while no corrected larval mortality was observed in I-1 and I-7 (Table 10).

4.1.2. a. iv. At 96 hours after spraying

Perusal of the data in Table 10 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were not significant. Among the isolates I-4 was found to be most effective as it recorded highest larval mortality (15.00%), followed by I-3 (12.50%), I-2 and I-5 (both registered 10.00% larval mortality), I-6 and I-7 (both exhibited 7.50% mortality) respectively, while 5.00% larval mortality was recorded both in I-1 and control .

Computation of the corrected larval mortality at 96 hours after spray revealed that there was no significant difference among the isolates. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (10.56%), followed by I-3 (7.78%), I-5 (5.28%), I-2 (5.00%), I-6 and I-7 (both recorded 2.50% larval mortality), respectively. However, no corrected mortality was observed in I-1 (Table 10).

4.1.2. a. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, isolate I-4 recorded highest larval mortality (35.00%), followed by isolate I-3 (22.50%) and I-2 (20.00%), but all were at par with each other.The next effective isolate was I-7 (17.50%), followed by I-5 (15.00%), I-1 and I-6 (both registered 12.50% mortality), but there was no significant difference among these isolates. While larval mortality recorded in control was 7.50% (Table 10).

Perusal of the data in Table 10 revealed that at 120 hours after spray there was significant increase in the corrected larval mortality. Isolate I-4 was found to be highly effective as it recorded highest corrected larval mortality (30.00%), followed by isolate I-3 (16.39%) and I-2 (13.61%), but these isolates did not differ significantly from each other. The next effective isolate

was I-7 (11.11%) followed by I-5 (7.78%), I-6 and I-1(both recorded 5.28% corrected mortality), but all were at par with each other.

4.1.2. b. Spore concentration : 1 x 10^6 spores ml^{-1}

4.1.2. b. i. At 24 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B. bassiana* isolates tested were not significant. No mortality was recorded in all the isolates along with the control, hence corrected larval mortality was not computed (Table 11).

4.1.2. b. ii. At 48 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B. bassiana* isolates tested were not significant. Highest larval mortality was recorded in isolate I-4 (7.50%), followed by I-2 and I-3 (with 5.00% mortality), I-6 and I-5 (both recorded 2.50% mortality), respectively. Whereas no larval mortality was recorded in I-1, I-7 and control. Computation of corrected larval mortality revealed that it was not significant and similar trend was observed as in larval mortality (Table 11).

4.1.2. b. iii. At 72 hours after spraying

Perusal of the data in Table 11 revealed that at 72 hours after spray, the differences in the *T.absoluta* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most effective as it recorded highest larval mortality (17.50%), followed by I-2 (15.00%), I-5 and I-6 (with 10.00% mortality) , but all these isolates were statistically at par with each other. The next effective isolates were I-1 and I-3 (both exhibited 7.50% larval mortality), followed by I-7 (5.00%) and control (2.50%), but these did not differed significantly from each other.

Computation of corrected larval mortality at 72 hours after spray revealed that there was significant difference among the isolates. Among the isolates, I-4 was found to be the most effective as it recorded highest corrected larval mortality (15.28%), followed by I-2 (12.78%), I-6 and I-5 (both registered 7.50% mortality), but there was no significant difference among these isolates. The next effective isolates were I-1 and I-3 (with 5.00% mortality), followed by isolate I-7 (2.50%), but all were at par with each other (Table 11).

4.1.2. b. iv. At 96 hours after spraying

At 96 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (32.50%), followed by I-2 and I-6 (both registered 20.00% mortality), but all the isolates were at par with each other. The next effective isolate was I-7 (17.50%), followed by isolates I-5 and I-3 (both recorded 15.00% mortality), but there was no significant difference among these isolates. While 7.50% mortality was recorded in I-1 and control, and were statistically at par with each other (Table 11).

Perusal of the data in Table 11 revealed that at 96 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (27.22%) and was significantly superior than all other isolates. The next effective isolates were I-6 and I-2 (both recorded 13.61% mortality), followed by I-5 (12.50%), I-7 (10.83%) and I-3 (8.06), but all were at par with each other. While no corrected mortality was recorded in isolate I-1.

4.1.2. b. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (45.00%). The next effective isolates were I-1 and I-6 (both recorded 32.50% mortality), followed by I-2 (30.00%), followed by I-3 and I-7 (both registered 27.50% mortality), but all were at par with each other. The least effective isolate was I-5 (22.50%). However, 10.00% larval mortality was recorded in control (Table 11).

Perusal of the data in Table 11 revealed that at 120 hours after spray there was no significant difference in the corrected larval mortality. Isolate, I-4 was found to be highly effective as it recorded highest corrected larval mortality (38.47%), followed by I-1 and I-6 (both registered 24.51% larval mortality), followed by I-2 (21.74%), I-5 (20.00%), I-3 and I-7 (both recorded 18.96 % mortality), respectively.

4.1.2. c. Spore concentration : 1 x 10^7 spores ml^{-1}

4.1.2. c. i. At 24 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B.*

bassiana isolates tested were not significant. Among the isolates, I-4 recorded highest larval mortality (5.00%), followed by isolates I-3, I-6 and I-7 (all recorded 2.50% mortality), whereas no mortality was recorded in I-1, I-2 and I-5 including control. Computation of corrected larval mortality showed that the extend and trend was similar to that as observed in larval mortality (Table 12).

4.1.2. c. ii. At 48 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B. bassiana* isolates tested were not significant. Among the isolates, I-4 recorded highest larval mortality (12.50%), followed by I-2, I-3, I-5, I-6 and I-7 (all registered 5.00% mortality), I-1 (2.50%) and no mortality in control, respectively. Computation of corrected larval mortality showed that the extend and trend was similar to that as observed in larval mortality (Table 12).

4.1.2. c. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *T.absoluta* larval mortality among different isolates were significant. Among the isolates, I-2 and I-4 recorded highest larval mortality (20.00%), followed by isolates I-1, I-3, I-5 and I-6 (registered 12.50% mortality), but all were statistically at par with each other. The next effective isolate was I-7 (7.50%), followed by control (2.50%), but they did not differed significantly from each other (Table 12).

Computation of the corrected larval mortality at 72 hours after spray revealed that there was no significant difference among the isolates. However, highest corrected larval mortality was recorded in isolate I-2 and I-4 (17.78%), followed by I-1 and I-5 (10.28%), I-3 and I-6 (10.00%),respectively. While 5.00% mortality was recorded in I-7 ((Table 12).

4.1.2. c .iv. At 96 hours after spraying

Perusal of the data in Table 12 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most effective as it recorded highest larval mortality (42.50%), followed by I-2 (37.50%), I-1 and I-7 (both recorded 27.50% mortality) and I-5 (25.00%), but all were statistically at par with each other. The next effective isolates were I-3 and I-6 (both registered 22.50% mortality), but there was no significant difference among them. However, lowest larval mortality was recorded in control (7.50%).

Perusal of the data in Table 12 revealed that at 96 hours after spray there was no significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (38.06%), followed by I-2 (32.50%), I-5 (22.78%), I-1 (21.94%), I-7 (21.39%) and lowest in I-3 and I-6 (both recorded 15.55% mortality), respectively .

4.1.1. c. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *T. absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (47.50%), followed by I-2 (45.00%), I-1 (37.50%), I-3 (37.50%), I-6 (35.00%), I-5 and I-7 (both registered 32.50%), but all were statistically at par with each other. However, control recorded 10.00% mortality (Table 12).

Perusal of the data in Table 12, revealed that at 120 hours after spray there was no significant increase in the corrected larval mortality. Isolate, I-4 was found to be highly effective as it recorded highest larval mortality (41.67%), followed by I-2 (38.89%), I-1, I-3 and I-5 (all recorded 30.56%), I-6 (27.78%) and I-7 (25.00%), respectively.

4.1.2. d. Spore concentration : 1 x 10^8 spores ml^{-1}

4.1.2. d. i. At 24 hours after spraying

Differences in the *T.absoluta* larval mortality among different *B. bassiana* isolates tested were not significant. Among the isolates, I-4 recorded highest larval mortality (10.00%), followed by isolates, I-2, I-3, I-5, I-6 and I-7 (all registered 5.00% larval mortality), I-1 (2.50%),respectively. While no mortality was recorded in control. Computation of corrected larval mortality revealed that it was non-significant and the extend and trend was similar to the larval mortality (Table 13).

4.1.2. d. ii. At 48 hours after spraying

Perusal of the data in Table 13 revealed that at 48 hours after spray, the differences in the mean *T.absoluta* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most effective as it recorded highest larval mortality (17.50%), followed by isolates I-3 and I-6 (both recorded 10.00% mortality), but all these isolates were statistically at par with each other. The next effective isolates were I-1, I-

2, I-5 and I-7 (7.50% larval mortality), but there was no significant among them. While no larval mortality was recorded in control.

Computation of the corrected larval mortality at 48 hours after spray revealed that there was no significant difference among the isolates and followed the similar trend as observed in larval mortality (Table 13).

4.1.2. d. iii. At 72 hours after spraying

Perusal of the data in Table 13 revealed that at 72 hours after spray, the differences in the mean *T.absoluta* larval mortality among different isolates were significant. Among the isolates, I-2 recorded highest larval mortality (27.50%), followed by isolates I-1 and I-4 (with 25.00% larval mortality), I-3 (20.00%) and I-5 and I-7 (both registered 17.50% mortality), but all were at par with each other. The least effective isolate was I-6 (15.00%), while 2.50% mortality was recorded in control.

Perusal of the data in Table 13 revealed that at 72 hours after spray there was no significant increase in the corrected larval mortality. Isolate I-2 was found to be most effective as it recorded highest corrected larval mortality (25.28%), followed by I-1 and I-4 (both registered 23.06% mortality), I-3 (17.78%), I-5 and I-7 (recorded 15.56% mortality),while lowest larval mortality was recorded in I-6 (12.50%), respectively.

4.1.2. d. iv. At 96 hours after spraying

Perusal of the data in Table 13 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most effective as it recorded highest larval mortality (55.00%), followed by I-2 (42.50%), but both were at par with each other. The next effective isolates were I-1, I-5 and I-7 (with 32.50% larval mortality), followed by I-3 and I-6 (both exhibited 27.50% mortality), but they did not differed significantly from each other. However, 7.50% mortality was registered in control .

Perusal of the data in Table 13 revealed that at 96 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be most effective as it recorded highest corrected larval mortality (51.67%), followed by isolate I-2 (37.50%), but both were at par with each other. The next effective isolates were I-1 , I-5 and I-7 (with 26.94% larval

mortality), followed by I-3 and I-6 (both registered 21.39% mortality), but there was no significant difference among these isolates.

4.1.2. d. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (65.00%), followed by isolate I-2 (60.00%), but there was no significant difference among the isolates. The next effective isolates were I-1 and I-7 (both of them recorded 47.50% larval mortality), followed by I-3 (45.00%), I-5 and I-6 (both registered 40.00% mortality), but all were at par with each other. However, larval mortality recorded in control was 10.00% (Table 13).

Perusal of the data in Table 13, revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate, I-4 was found to be highly effective as it recorded highest corrected larval mortality (61.11%), followed by I-2 (55.56%), but both were at par with each other. The next effective isolates were I-1 and I-7 (both recorded 41.67% mortality), I-3 (38.89%), I-5 and I-6 (both registered 38.33% mortality), but there was no significant difference among these isolates.

4.1. 2. e. Spore concentration : 1 x 10^9 spores ml^{-1}

4.1.2. e. i. At 24 hours after spraying

At 24 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (12.50%), followed by I-3, I-6 and I-7 (with 10.00% mortality), I-1 and I-2 (both recorded 7.50% larval mortality), but there was no significant difference among these isolates. The least effective isolate was I-5 (5.00%), while no mortality was recorded in control (Table 14).

Computation of corrected larval mortality at 24 hours after spray revealed that there was no significant difference among the isolates and similar trend was observed as in larval mortality.

4.1.2. e. ii. At 48 hours after spraying

Perusal of the data in Table 14 revealed that at 48 hours after spray, the differences in the mean *T.absoluta* larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most

effective as it recorded highest larval mortality (30.00%), followed by isolate I-5 (20.00%), I-1, I-3 and I-6 (with 17.50% larval mortality), but all were at par with each other. The next effective isolates were I-7 and I-2 (both recorded 15.00% mortality), while no larval mortality was recorded in control .

Computation of corrected larval mortality at 48 hours after spray revealed that there was no significant difference among the isolates and exhibited similar trend of mortality as in larval mortality (Table 14).

4.1.2. e. iii. At 72 hours after spraying

Perusal of the data in Table 14 revealed that at 72 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, isolate I-4 recorded highest larval mortality (47.50%), followed by I-1 and I-2 (both recorded 40.00% mortality) , but all were statistically at par with each other. The next effective isolate was I-5 (32.50%), followed by I-3 (27.50%), I-6 and I-7 (with 22.50% larval mortality), but all the isolates were at par with each other. While, 2.50% mortality was recorded in control.

Perusal of the data in Table 14 revealed that at 72 hours after spray there was significant difference in the corrected larval mortality. Isolate I-4 was found to be most effective as it recorded highest corrected larval mortality (46.11%), followed by I-1 and I-2 (both recorded 38.33% mortality), but there was no significant difference among these isolates. The next effective isolate was I-5 (30.56%), followed by I-3 (25.56%), I-7 and I-6 (both registered 20.56% mortality), but all were statistically at par with each other.

4.1. 2. e. iv. At 96 hours after spraying

Perusal of the data in Table 14 revealed that at 96 hours after spray, the differences in the mean larval mortality among different isolates were significant. Among the isolates, I-4 was found to be the most effective as it recorded highest larval mortality (75.00%) and was significantly superior than all other isolates. The next effective isolates were I-1 and I-2 (both recorded 55.00% mortality),I-5 (45.00%), but all were statistically at par with each other. The next effective isolates were I-6 and I-7 (both registered 37.50% mortality), followed by I-3 (32.50%), but there was no significant difference among these isolates. However, in control 7.50% mortality was observed.

Perusal of the data in Table 14 revealed that at 96 hours after spray there was significant increase in the corrected larval mortality. Isolate I-4 was found to be most effective as it recorded highest corrected larval mortality (73.06%). The next effective isolates were I-1 and I-2 (both recorded 51.39% mortality), I-5 (43.33%), but all were at par with each other. The next effective isolates were I-7 and I-6 (with 32.33% mortality), followed by I-3 (27.22%), but all were statistically at par with each other.

4.1.2.e. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *T.absoluta* larval mortality among the different isolates were significant. Among the isolates, I-4 recorded highest larval mortality (80.00%). The next effective isolates were I-1 and I-2 (recorded 62.50% larval mortality), I-5 (60.00%), I-7 (57.50%), I-3 and I-6 (with 52.50% mortality), but there was no significant difference among these isolates. However, larval mortality recorded in control was 10.00% (Table 14).

Perusal of the data in Table 14 revealed that at 120 hours after spray there was significant difference in the corrected larval mortality. Isolate I-4 was found to be significantly superior that all the other isolates as it recorded highest corrected larval mortality (77.78%). The next effective isolates were I-1 and I-2 (both registered 58.33% mortality), followed by I-5 (55.56%), I-7 (52.78%), I-3 and I-6 (both recorded 43.41% mortality), but all were at par with each other.

4.1.3. Mortality of *Bemisia tabaci* (3rd instar nymph)

4.1.3. a. Spore concentration : 1 x 10^5 spores ml^{-1}

4.1.3. a. i. At 24 hours after spraying

Differences in the *B. tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. No mortality was recorded in all the isolates, including control, hence it was not possible to compute corrected nymphal mortality (Table 15).

4.1.3. a. ii. At 48 hours after spraying

Perusal of the data in Table 15 revealed that at 48 hours after spray, the differences in the mean *B. tabaci* nymphal mortality among different isolates were not significant. No mortality was recorded in all the isolates,

including control. Hence, computation of the corrected nymphal mortality was not possible as there was no nymphal mortality observed .

4.1.3. a. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *B. tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-4 and I-6 recorded highest nymphal mortality (7.50%), followed by I-1, I-2, I-3, I-5 and I-7, all these isolates including control recorded 5.00% nymphal mortality (Table 15).

Computation of the corrected nymphal mortality at 72 hours after spray revealed that there was no significant difference among the isolates. However, highest corrected nymphal mortality was recorded in isolate I-4 and I-6 (2.50%), while no corrected nymphal mortality was observed in I-1, I-2, I-3, I-5 and I-7 (Table 15).

4.1.3. a. iv. At 96 hours after spraying

Perusal of the data in Table 15 revealed that at 96 hours after spray, the differences in the nymphal mortality among different isolates were not significant. Among the isolates, I-1 was found to be most effective as it recorded highest nymphal mortality (12.50%), followed by isolates I-2, I-3, I-4 and I-7(all registered 10.00% mortality), I-5, I-6 and control (7.50%), respectively .

Computation of the corrected nymphal mortality at 96 hours after spray revealed that there was no significant difference among the isolates. Isolate I-1 was found to be most effective as it recorded highest corrected nymphal mortality (5.28%), followed by I-2, I-3, I-4 and I-7 (all of them registered 2.50% mortality), while no mortality was recorded in I-5 and I-6 (Table 15).

4.1.3. a. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *B. tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-1, I-2 and I-7 recorded highest nymphal mortality (15.00%), followed by isolates I-3, I-4, I-5 and I-6 (12.50% mortality), while lowest was recorded in control (10.00%) (Table 15).

Perusal of the data in Table 15 revealed that at 120 hours after spray

there was no significant difference in the corrected nymphal mortality. Isolates, I-1, I-2 and I-7 were found to be highly effective as they recorded highest nymphal mortality (5.56%), followed by isolates I-3, I-4, I-5 and I-6 with 2.78% mortality, respectively.

4.1.3. b. Spore concentration : 1 x 10^6 spores ml^{-1}

4.1.3. b. i. At 24 hours after spraying

Differences in the *B.tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. No mortality was recorded in all the isolates along with the control, hence corrected nymphal mortality was not computed (Table 16).

4.1.3. b. ii. At 48 hours after spraying

Differences in the *B.tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. No mortality was recorded in all the isolates along with the control, hence corrected nymphal mortality was not calculated (Table 16).

4.1.3. b. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *B.tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-1, I-2, I-4 and I-7 recorded highest nymphal mortality (10.00%), followed by I-3, I-5 and I-6 (all of them registered 7.50%), while 5.00% nymphal mortality was registered in control (Table 16).

Computation of corrected nymphal mortality at 72 hours after spray revealed that there was no significant difference among the isolates. However, highest corrected nymphal mortality was recorded in isolates I-1, I-2, I-4 and I-7 (all of them recorded 5.28% mortality), while isolates I-3, I-5 and I-6 registered 2.78% mortality (Table 16).

4.1.3. b. iv. At 96 hours after spraying

Perusal of the data in Table 16 revealed that at 96 hours after spray, the differences in the mean nymphal mortality among different isolates were not significant. Among the isolates, I-1 was found to be most effective as it recorded highest nymphal mortality (20.00%), followed by isolate I-4 (17.50%), I-2 (15.00%), I-7 (12.50%), I-3 (10.00%), I-5 and I-6 including control, recorded 7.50% mortality, respectively.

Perusal of the data in Table 16 revealed that at 96 hours after spray

there was no significant difference in the corrected nymphal mortality. Isolate, I-1 was found to be most effective as it recorded highest corrected nymphal mortality (13.33%), followed by isolate I-4 (10.83%), I-2 (7.78%), I-7 (5.28%) and I-3 (2.50%), respectively.Whereas, no corrected nymphal mortality was recorded in isolates I-5 and I-6.

4.1.3. b. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *B.tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-1 recorded highest nymphal mortality (27.50%), followed by isolate I-4 (25.00%), I-3 and I-7 (recorded 17.50% mortality), I-2, I-5 and I-6 with 15.00% and lowest in control (10.00%), respectively (Table 16).

Perusal of the data in Table 16 revealed that at 120 hours after spray there was no significant difference in the corrected nymphal mortality. Isolate I-1 was found to be highly effective as it recorded highest corrected nymphal mortality (19.44%), followed by isolate I-4 (16.67%), I-3 and I-7 (both registered 8.33% mortality), respectively. However, isolates I-2, I-5 and I-6 were least effective as they recorded 5.56% mortality.

4.1.3. c. Spore concentration : 1 x 10^7 spores ml^{-1}

4.1.3. c. i. At 24 hours after spraying

Differences in the *B.tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. No mortality was recorded in all the isolates along with the control, hence corrected nymphal mortality was not computed (Table 17).

4.1.3. c. ii. At 48 hours after spraying

Differences in the *B.tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. Among the isolates, I-1, I-2, I-4 and I-7 recorded highest nymphal mortality (2.50%), while no mortality was registered in I-3, I-5 and I-6 including control (Table 17).

Computation of the corrected nymphal mortality at 48 hours after spray revealed that there was no significant difference among the isolates and exhibited similar trend as observed in nymphal mortality (Table 17).

4.1.3. c. iii. At 72 hours after spraying

At 72 hours after treatment, the differences in the *B.tabaci* nymphal mortality among different isolates were not significant. Isolates I-1, I-2 and I-4

recorded highest nymphal mortality (12.50%), followed by I-7 (10.00%), I-3, I-5 and I-6 (all recorded 7.50% mortality), while it was 5.00% in control (Table 17).

Computation of the corrected nymphal mortality at 72 hours after spray revealed that there was no significant difference among the isolates. However, highest corrected nymphal mortality was recorded in isolates I-1, I-2 and I-4, all registered 7.78% mortality, followed by I-7 (5.00%), I-3, I-5 and I-6 (with 2.50% nymphal mortality), respectively (Table 17).

4.1.3.c . iv. At 96 hours after spraying

Perusal of the data in Table 17 revealed that at 96 hours after spray, the differences in the mean nymphal mortality among different isolates were non-significant. Among the isolates I-1 and I-4 were found to be most effective as they recorded highest nymphal mortality (25.00%), followed by isolates I-2 (20.00%), I-7(17.50%),I-3 (15.00%), I-5 and I-6 (both recorded 10.00% mortality), respectively. However, 7.50% nymphal mortality was recorded in control.

Perusal of the data in Table 17 revealed that at 96 hours after spray there was significant difference in the corrected nymphal mortality. Isolates I-1 and I-4 were found to be most effective as they recorded highest corrected nymphal mortality (18.61%), followed by I-2 (13.61%), I-7 (10.28%) and I-3 (8.33%), but all were at par with each other. The least effective isolates were I-5 and I-6, both recorded 2.78% mortality, however non-significant differences were observed between them.

4.1.3. c. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *B.tabaci* nymphal mortality among different isolates were significant. Among the isolates, I-1 recorded highest nymphal mortality (35.00%), followed by isolate I-4 (32.50%), I-2 and I-7 (both registered 25.00% mortality), but all were statistically at par with each other. The next effective isolate was I-6 (20.00%), followed by I-5 and I-3 (with 17.50% mortality), but there was non-significant difference among the isolates. However, nymphal mortality recorded in control was 10.00% (Table 17).

Perusal of the data in Table 17 revealed that at 120 hours after spray there was no significant difference in the corrected nymphal mortality. Isolate

I-1 was found to be highly effective as it recorded highest corrected nymphal mortality (27.78%), followed by isolate I-4 (25.00%), I-2 and I-7 (with 16.67% mortality), I-6 (11.11%), I-3 and I-5 (both registered 8.33% mortality), respectively.

4.1.3. d. Spore concentration : 1 x 10^8 spores ml^{-1}

4.1.3. d. i. At 24 hours after spraying

Differences in the *B.tabaci* nymphal mortality among different *B. bassiana* isolates tested were not significant. Among the isolates I-1 and I-4 recorded highest nymphal mortality (2.50%), while no mortality was registered in I-2, I-3, I-5 and I-6 including control (Table 18).

Computation of the corrected nymphal mortality at 24 hours after spray revealed that there was no significant difference among the isolates and followed the same trend as observed in nymphal mortality (Table 18).

4.1.3. d. ii. At 48 hours after spraying

Perusal of the data in Table 18 revealed that at 48 hours after spray, the differences in the mean *B.tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-1 and I-2, both recorded 7.50% mortality, followed by I-4 and I-7 with 5.00% nymphal mortality and I-3, I-5 and I-6 (registered 2.50% nymphal mortality), respectively. However, no nymphal mortality was recorded in control.

Computation of the corrected nymphal mortality at 48 hours after spray revealed that there was no significant difference among the isolates and the trend was similar as observed in nymphal mortality (Table 18).

4.1.3. d. iii. At 72 hours after spraying

Perusal of the data in Table 18 revealed that at 72 hours after spray, the differences in the mean nymphal mortality among different isolates were non-significant. Among the isolates, I-4 recorded highest nymphal mortality (17.50%), followed by isolates, I-1 and I-2 (both recorded 15.00% nymphal mortality), I-7 (12.50%), I-3 and I-5 with 7.50% mortality and lowest mortality (5.00%) in I-6 and control, respectively.

Perusal of the data in Table 18 revealed that at 72 hours after spray there was no significant difference in the corrected nymphal mortality. Isolate I-4 was found to be most effective as it recorded highest corrected nymphal mortality (13.33%), followed by I-1 and I-2 (both recorded 10.00% mortality), I-

7 (7.50%), I-3 and I-5 (both registered 2.50% mortality),respectively. However, no nymphal mortality was recorded in I-6.

4.1. 3. d. iv. At 96 hours after spraying

Perusal of the data in Table 18 revealed that at 96 hours after spray, the differences in the mean nymphal mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest nymphal mortality (35.00%), followed by isolates, I-1 and I-2 (both registered 30.00% mortality) and I-7 (22.50%), but all were statistically at par with each other. The next effective isolate was I-3 (20.00%), followed by I-6 (17.50%) and I-5 (15.00%), however non-significant differences were observed between them. Lowest nymphal mortality (7.50%) was recorded in control.

Perusal of the data in Table 18 revealed that at 96 hours after spray there was no significant difference in the corrected nymphal mortality. Isolate I-4 was found to be most effective as it recorded highest corrected nymphal mortality (29.17%), followed by isolates I-1 and I-2 (both registered 24.17% mortality), I-7 (15.83%), I-3 (13.33%), I-6 (10.28%) and I-5 (8.06%), respectively.

4.1.3. d. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *B.tabaci* nymphal mortality among different isolates were significant. Among the isolates, I-4 recorded highest nymphal mortality (42.50%), followed by isolates I-1 (40.00%), I-2 (35.00%) and I-7 (30.00%), but all were statistically at par with each other. The next effective isolates were I-3, I-5 and I-6, all of them registered 27.50% mortality, but they did not differ significantly from each other. While 10.00% morality was recorded in control (Table 18).

Perusal of the data in Table 18 revealed that at 120 hours after spray there was no significant difference in the corrected nymphal mortality. Isolate I-4 recorded highest corrected nymphal mortality (36.11%), followed by isolate I-1 (33.33%), I-2 (27.78%), I-7 (22.22%), I-3, I-5 and I-6 (all of them recorded 19.44% mortality), respectively.

4.1.3. e. Spore concentration : 1 x 10^9 spores ml^{-1}

4.1.3. e. i. At 24 hours after spraying

Differences in the *B. tabaci* nymphal mortality among different

B.bassiana isolates tested were not significant. Among the isolates, I-4 recorded highest nymphal mortality (7.50%), followed by I-1, I-2 and I-7 (all of them registered 5.00% mortality), I-3 and I-5, both recorded 2.50% mortality, respectively. While no nymphal mortality was recorded in I-6 and control (Table 19).

Computation of the corrected nymphal mortality at 24 hours after spray revealed that there was no significant difference among the isolates and exhibited similar trend as observed in nymphal mortality (Table 19).

4.1.3. e. ii. At 48 hours after spraying

Perusal of the data in Table 19 revealed that at 48 hours after spray, the differences in the mean *B.tabaci* nymphal mortality among different isolates were not significant. Among the isolates, I-4 was found to be most effective as it recorded highest nymphal mortality (12.50%), followed by isolates I-1,I-2 and I-7 (all of them recorded 10.00% mortality), I-3 and I-5, both recorded 7.50% mortality and lowest in I-6 (2.50%), respectively. While no nymphal mortality was recorded in control .

Computation of the corrected nymphal mortality at 48 hours after spray revealed that there was no significant difference among the isolates and followed similar trend as observed in nymphal mortality.

4.1.3. e. iii. At 72 hours after spraying

Perusal of the data in Table 19 revealed that at 72 hours after spray, the differences in the mean nymphal mortality among different isolates were significant. Among the isolates, I-4 recorded highest nymphal mortality (27.50%), followed by I-1 and I-2 (both registered 22.50% mortality) and I-7 (17.50%), however all these isolates were statistically at par with each other. The next effective isolates were I-3 and I-5 as they recorded 12.50% nymphal mortality followed by I-6 (7.50%) and control (5.00%), but all were at par with each other.

Perusal of the data in Table 19 revealed that at 72 hours after spray there was significant difference in the corrected nymphal mortality. Isolate I-4 was found to be most effective as it recorded highest corrected nymphal mortality (23.61%), followed by I-1 and I-2 (both recorded 18.06% mortality), I-7(12.78%), but they did not differ significantly from each other.The next

effective isolates were I-3 and I-5 (both registered 7.50% mortality), followed by I-6 (2.50%), but all these isolates were at par with each other.

4.1.3. e. iv. At 96 hours after spraying

Perusal of the data in Table 19 revealed that at 96 hours after spray, the differences in the mean nymphal mortality among different isolates were significant. Among the isolates, I-4 was found to be most effective as it recorded highest nymphal mortality (57.50%) and was significantly superior than all other isolates.The next effective isolate was isolate I-1 (40.00%), followed by I-2 (35.00%), I-7 (30.00%), I-3 (27.50%), I-5 and I-6 (both recorded 25.00% mortality), but all were statistically at par with each other. Mortality recorded in control was 7.50%.

Perusal of the data in Table 19 revealed that at 96 hours after spray there was significant difference in the corrected nymphal mortality. Isolate I-4 was found to be most effective as it recorded highest corrected nymphal mortality (53.89%), followed by I-1 (34.72%), but both were statistically at par with each other. The next effective isolate was I-2 (30.00%), followed by I-7 (24.17%), I-3 (21.11%), I-5 and I-6 (both recorded 18.61% mortality), respectively, but they did not differ significantly from each other.

4.1.3. e. v. At 120 hours after spraying

At 120 hours after treatment, the differences in the *B. tabaci* nymphal mortality among different isolates were significant. Among the isolates, I-4 recorded highest nymphal mortality (67.50%), followed by I-1 (57.50%), but there was no significant difference between both the isolates. The next effective isolate was I-2 (45.00%), followed by I-3 (40.00%), I-6 and I-7 (both registered 37.50% mortality) and I-5 (35.00%), but all were statistically at par with each other. However, nymphal mortality recorded in control was 10.00% (Table 19).

Perusal of the data in Table 19 revealed that at 120 hours after spray there was significant difference in the corrected nymphal mortality. Isolate I-4 registered 63.89% nymphal mortality, followed by I-1 (52.78%), but both were at par with each other. The next effective isolate was I-2 (38.89%), followed by I-3 (33.33%), I-6 and I-7 (both recorded 30.56% mortality) and I-5 (27.78%), respectively, but all were at par with each other.

4.1.4. Impact of *Beauveria bassiana* isolates and spore concentration on mortality of tomato insect pest complex

4.1.4.i *Helicoverpa armigera* (2nd instar larvae) (Table 20)

4.1.4. a. i. At 24 hours after spraying

Isolates

Perusal of data in Table 20.1 revealed that at 24 hours after spray, the differences in the *H.armigera* larval mortality among different *B. bassiana* isolates were significant. Among the different isolates, highest mortality was recorded in isolate I-4 (8.50%), followed by isolate I-1 (6.50%), but they differed significantly from each other.The next effective isolate was I-2 (4.00%), followed by I-3, I-5 and I-7, all of them registered 3.50% larval mortality, but were at par with each other. Whereas, the least effective isolate was I-6, as it recorded lowest larval mortality (2.00%).

Spore concentration

Different spore concentrations of *B. bassiana* evaluated for larval mortality at 24 hours after spray were found to be significant. Highest larval mortality (10.00%) was registered with spore concentration S-1 ($1x10^9$ spores ml^{-1}), followed by S-2 ($1x10^8$ spores ml^{-1}) (7.50%), S-3 ($1x10^7$ spores ml^{-1}) (3.57%) and S-4 ($1x10^6$ spores ml^{-1}) (1.07%), while lowest larval mortality of 0.36% was recorded in S-5 ($1x10^5$ spores ml^{-1}), and they differed significantly from each other (Table 20.1).

The results indicate that the *H.armigera* larval mortality is dependent on spore concentration.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 20: Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Helicoverpa armigera* (2nd instar larvae)

Table 20.1 At 24 hrs after treatment

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1 ($1x10^9$)	S_2 ($1x10^8$)	S_3^* ($1x10^7$)	S_4^* ($1x10^6$)	S_5^* ($1x10^5$)	Mean
I_1	12.50 (20.91)	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	2.50 (7.77)	6.50 (14.71)

I_2	7.50 (15.20)	5.00 (11.48)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	4.00 (11.54)
I_3	7.50 (15.20)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (10.78)
I_4	17.50 (24.92)	15.00 (22.91)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	8.50 (16.95)
I_5	7.50 (15.20)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (10.78)
I_6	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (8.13)
I_7	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (10.78)
Mean	10.00 (18.91)	7.50 (15.20)	3.57 (10.89)	1.07 (5.94)	0.36 (3.43)	-
	SEm±			**CD (P=0.05)**		
Isolates	0.30			0.84		
Spore concentration	0.21			0.60		
Isolate x Spore concentration	1.49			NS		

() = Figures in parentheses are arcsin transformed values

*() = Figures in parentheses are (x+0.5) arcsin transformed values

NS = Non-significant

4.1.4. b. i. At 48 hours after spraying

Isolates

Perusal of data in Table 20.2 revealed that at 48 hours after spray, the differences in the *H.armigera* larval mortality among different isolates were significant. Highest larval mortality was recorded in isolate I-4 (23.50%), followed by I-1 (14.50%), but both of them differed significantly from each other. The next effective isolate was I-2 (13.00%), followed by I-5 (12.50%), but these isolates were statistically at par with each other. The next effective isolate was I-3 (11.50%), followed by I-7 (7.00%) and they differed significantly from each other. While lowest mortality was recorded in I-6 (6.50%).

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 48 hours after spray were found to be significant. Highest larval mortality (22.50%) was registered with spore concentration S-1, followed by

S-2 (18.57%), S-3 (12.50%), S-4 (7.86%) and S-5 (1.79%), but they differed significantly from each other (Table 20.2).

The results indicate that the larval mortality was dependent on spore concentration and period of infection.

Interactions: Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 20.2 At 48 hrs after treatment

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5^*	Mean
I_1	20.00 (26.92)	20.00 (26.92)	12.50 (20.91)	12.50 (20.91)	5.00 (11.48)	14.50 (21.97)
I_2	22.50 (28.32)	20.00 (26.57)	15.00 (22.92)	7.50 (15.20)	0.00 (4.05)	13.00 (21.13)
I_3	22.50 (28.32)	17.50 (24.57)	10.00 (18.91)	10.00 (18.91)	0.00 (4.05)	11.50 (20.27)
I_4	42.50 (40.96)	35.00 (34.87)	20.00 (26.92)	12.50 (20.91)	7.50 (15.20)	23.50 (28.66)
I_5	22.50 (28.32)	17.50 (24.57)	15.00 (22.92)	7.50 (15.20)	0.00 (4.05)	12.50 (20.91)
I_6	12.50 (20.91)	10.00 (18.91)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	6.50 (14.77)
I_7	15.00 (22.92)	10.00 (18.91)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	7.00 (15.34)
Mean	22.50 (28.32)	18.57 (25.26)	12.50 (20.91)	7.86 (16.28)	1.79 (7.68)	-
	SEm±			CD (P=0.05)		
Isolates	0.23			0.64		
Spore concentration	0.16			0.46		
Isolate x Spore concentration	1.14			NS		

() = Figures in parentheses are arcsin transformed values

* () = Figures in parentheses are (x+0.5) arcsin transformed values

NS = Non-significant

4.1.4. c. i. At 72 hours after spraying

Isolates

At 72 hours after spraying, the differences in the *H.armigera* larval mortality among different isolates were significant. Among the isolates,

highest mortality was recorded in isolate I-4 (42.50%) and was significantly superior than all other isolates. The next effective isolate was I-1 (27.50%), followed by I-3 (27.00%), but both the isolates were statistically at par with each other. The next effective isolate was I-2 (26.00%), followed by I-5 (24.00%), but they differed significantly from each other.The next effective isolate was I-7 (14.00%), followed by I-6 (13.50%), however non-significant differences were observed between them (Table 20.3).

Spore concentration

Different spore concentrations of *B.bassiana* were evaluated for larval mortality at 72 hours after spray were found to be significant. Highest larval mortality (42.50%) was registered with spore concentration S-1, followed by S-2 (32.14%), S-3 (24.64%), S-4 (17.86%) and S-5 (6.79%), but they differed significantly from each other (Table 20.3).

The results indicate that the larval mortality was directly proportional to the spore concentration and the period of infection.

Interactions: Isolate x Spore concentration

The interaction of isolates and spore concentration had significant impact on the larval mortality. Isolate I-4 with S-1 spore concentration was found to be most effective against *H.armigera* 2nd instar larvae.

Table 20.3 At 72 hrs after treatment

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	52.50 (46.43)	40.00 (42.13)	27.50 (31.63)	7.50 (15.20)	5.00 (11.48)	27.50 (31.63)
I_2	45.00 (42.13)	32.50 (34.76)	27.50 (31.63)	22.50 (28.57)	2.50 (7.77)	26.00 (30.66)
I_3	42.50 (40.69)	30.00 (33.21)	27.50 (31.63)	22.50 (28.57)	12.50 (20.91)	27.00 (31.29)
I_4	70.00 (56.79)	55.00 (47.87)	40.00 (39.23)	30.00 (33.21)	17.50 (24.57)	42.50 (40.69)
I_5	37.50 (37.76)	30.00 (33.21)	25.00 (30.02)	22.50 (28.57)	5.00 (11.48)	24.00 (29.33)
I_6	25.00 (30.02)	20.00 (26.57)	10.00 (18.91)	10.00 (18.91)	2.50 (7.77)	13.50 (21.56)
I_7	25.00 (30.02)	17.50 (24.57)	15.00 (22.79)	10.00 (18.91)	2.50 (7.77)	14.00 (21.97)

Mean	42.50 (40.69)	32.14 (34.97)	24.64 (29.76)	17.86 (25.00)	6.79 (15.10)	-
	SEm±			**CD (P=0.05)**		
Isolates	0.28			0.79		
Spore concentration	0.20			0.57		
Isolate x Spore concentration	1.42			3.97		

() = Figures in parentheses are arcsin transformed values

4.1.4. d. i. At 96 hours after spraying

Isolates

Perusal of data in Table 20.4 revealed that at 96 hours after spray, the differences in the mean larval mortality of *H.armigera* among different isolates were significant. Significantly highest mortality was recorded in isolate I-4 (59.00%), followed by I-1 (55.00%), I-5 (45.50%), but there was no significant difference among these isolates. The next effective isolate was I-2 (40.50%), followed by I-3 (40.00%), but both were at par with each other. The next effective isolate was I-6 (30.00%), followed by I-7 (28.50%), but they did not differ significantly from each other.

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 96 hours after spray were found to be significant. Spore concentration S-1 recorded highest larval mortality (64.29%), followed by S-2 (51.43%), S-3 (43.57%), S-4 (36.07%) and S-5 (17.86%), but they differed significantly from each other (Table 20.4).

The results indicate that the larval mortality was dependent on spore concentration and period of infection.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had significant impact on the larval mortality. Highest larval mortality was registered by isolate I-4 with S-1 spore concentration.

Table 20.4 At 96 hrs after treatment

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	85.00 (67.21)	65.00 (52.24)	57.50 (49.31)	47.50 (43.57)	20.00 (26.57)	55.00 (49.95)
I_2	57.50 (49.31)	47.50 (43.57)	45.00 (42.13)	30.00 (33.21)	22.50 (28.32)	40.50 (39.53)
I_3	57.50 (49.31)	50.00 (45.00)	40.00 (39.23)	32.50 (34.76)	20.00 (26.57)	40.00 (39.28)
I_4	90.00 (71.57)	72.50 (58.37)	57.50 (49.31)	47.50 (43.57)	27.50 (31.63)	59.00 (52.55)
I_5	70.00 (56.79)	50.00 (45.00)	42.50 (40.69)	45.00 (42.13)	20.00 (26.57)	45.50 (42.56)
I_6	42.50 (40.69)	37.50 (37.76)	32.50 (34.76)	30.00 (33.21)	7.50 (15.20)	30.00 (33.21)
I_7	47.50 (43.57)	47.50 (43.57)	30.00 (33.21)	20.00 (26.57)	7.50 (15.20)	28.50 (32.13)
Mean	64.29 (56.75)	51.43 (46.02)	43.57 (41.48)	36.07 (36.84)	17.86 (24.92)	-
	SEm±			CD (P=0.05)		
Isolates	0.35			0.98		
Spore concentration	0.25			0.70		
Isolate x Spore concentration	1.75			4.90		

() = Figures in parentheses are arcsin transformed values

4.1.4. e. i. At 120 hours after spraying

Isolates

Perusal of data in Table 20.5 revealed that at 120 hours after spray, the differences in the *H.armigera* larval mortality among different isolates were significant. Highest larval mortality was registered in isolate I-4 (75.50%) and was significantly superior than all other isolates. The next effective isolate was I-1 (72.50%) and was significantly superior than I-5 (56.50%) and I-3 (56.00%), but the later two isolates were statistically at par with each other. The next effective isolate was I-2 (45.50%) and was significantly better than I-7 (43.00%) and I-6 (42.50), respectively. However, the latter two did not differ significantly from each other.

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 120 hours after spray were found to be significant. Highest larval mortality (77.50%) was registered with S-1, followed by S-2 (67.86%), S-3 (56.07%), S-4 (47.86%) and S-5 (30.36%), but they differed significantly from each other (Table 20.5).

The results indicate that the larval mortality was dependent both on spore concentration and period of infection.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 20.5 At 120 hr after treatment

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	95.00 (83.36)	82.50 (68.79)	72.50 (58.61)	65.00 (54.00)	47.50 (43.34)	72.50 (58.37)
I_2	62.50 (52.34)	57.50 (49.61)	47.50 (46.44)	37.50 (37.66)	22.50 (27.86)	45.50 (42.99)
I_3	77.50 (62.14)	72.50 (58.61)	60.00 (50.83)	42.50 (40.61)	27.50 (31.02)	56.00 (48.45)
I_4	100.00 (90.00)	90.00 (77.09)	72.50 (58.61)	65.00 (55.28)	50.00 (45.00)	75.50 (60.67)
I_5	80.00 (63.81)	65.00 (53.78)	57.50 (49.39)	47.50 (43.56)	32.50 (34.72)	56.50 (48.73)
I_6	65.00 (53.84)	50.00 (45.00)	42.50 (40.61)	42.50 (40.61)	12.50 (20.91)	42.50 (40.61)
I_7	62.50 (52.34)	57.50 (49.39)	40.00 (39.17)	35.00 (36.00)	20.00 (26.57)	43.00 (40.98)
Mean	77.50 (65.40)	67.86 (57.47)	56.07 (49.09)	47.86 (43.96)	30.36 (32.66)	-
	SEm±			CD (P=0.05)		
Isolates	0.37			1.04		
Spore concentration	0.26			0.74		
Isolate x Spore concentration	1.85			NS		

() = Figures in parentheses are arcsin transformed values

4.2.4.i *Tuta absoluta* (2nd instar larvae) (Table 21)

4.2.4. a. i. At 24 hours after spraying

Isolates

Perusal of data in Table 21.1 revealed that at 24 hours after spray, there was no significant difference in *T.absoluta* larval mortality. Among the different isolates, highest mortality was recorded in isolate I-4 (5.50%), followed by isolates I-3, I-6 and I-7, all of them registered 3.50% larval mortality, while lowest mortality (2.00%) was recorded in two isolates, I-1 and I-5, respectively.

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for *T.absoluta* larval mortality at 24 hours after spray were found to be significant. Highest larval mortality (8.93%) was registered with spore concentration S-1, followed by S-2 (5.36%) and S-3 (1.79%), respectively. Whereas, no larval mortality was recorded with spore concentration S-4 and S-5 (Table 21.1).

The results indicate that the larval mortality is dependent on spore concentration.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 21: Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Tuta absoluta* (2nd instar larvae)

Table 21.1 At 24 hrs after treatment

Isolates (I)	Spore concentration (S) (ml L^{-1})					
	S_1	S_2	S_3^*	S_4^*	S_5^*	Mean
I_1	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (7.03)
I_2	7.50 (15.20)	5.00 (11.48)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.50 (7.77)
I_3	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (9.25)
I_4	12.50 (19.20)	10.00 (18.91)	5.00 (11.48)	0.00 (4.05)	0.00 (4.05)	5.50 (11.88)

I_5	5.00 (11.48)	5.00 (11.48)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (7.03)
I_6	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (9.25)
I_7	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (9.25)
Mean	8.93 (17.07)	5.36 (12.01)	1.79 (6.71)	0.00 (4.05)	0.00 (4.05)	-
	SEm±			**CD (P=0.05)**		
Isolates	0.27			NS		
Spore concentration	0.19			0.54		
Isolate x Spore concentration	1.35			NS		

() = Figures in parentheses are arcsin transformed values

*() = Figures in parentheses are (x+0.5) arcsin transformed values

NS = Non-significant

4.2.4. b. i. At 48 hours after spraying

Isolates

Perusal of data in Table 21.2 revealed that at 48 hours after spray, the differences in the *T.absoluta* larval mortality among different isolates were significant. Highest larval mortality was registered with isolate I-4 (13.50%) and was significantly superior than all other isolates. The next effective isolates were I-3 and I-5 (both recorded 7.50% mortality), followed by I-6 (7.00%), but all were statistically at par with each other.The next effective isolate was I-2 (6.50%) and it differed significantly from I-1 and I-7 and both registered lowest larval mortality (5.50%).

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 48 hours after spray were found to be significant. Highest larval mortality (18.57%) was registered with spore concentration S-1, followed by S-2 (10.00%), S-3 (5.71%) and S-4 (3.21%), respectively. However, no mortality was recorded with spore concentration S-5 (Table 21.2).

The results indicate that the larval mortality was directly proportional to spore concentration and the period of infection.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 21.2 At 48 hrs after treatment

Isolates (I)	Spore concentration (S) (ml L^{-1})					
	S_1	S_2	S_3	S_4^*	S_5^*	Mean
I_1	17.50 (24.92)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	5.50 (11.20)
I_2	15.00 (21.20)	7.50 (15.20)	5.00 (11.48)	5.00 (11.48)	0.00 (4.05)	6.50 (12.68)
I_3	17.50 (24.92)	10.00 (18.91)	5.00 (11.48)	5.00 (11.48)	0.00 (4.05)	7.50 (15.20)
I_4	30.00 (31.63)	17.50 (24.92)	12.50 (20.91)	7.50 (15.20)	0.00 (4.05)	13.50 (19.29)
I_5	20.00 (26.57)	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	7.50 (15.20)
I_6	17.50 (24.92)	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	7.00 (13.08)
I_7	15.00 (22.79)	7.50 (15.20)	5.00 (11.48)	0.00 (4.05)	0.00 (4.05)	5.50 (11.20)
Mean	18.57 (25.24)	10.00 (18.91)	5.71 (12.30)	3.21 (8.83)	0.00 (4.05)	-
	SEm±			CD (P=0.05)		
Isolates	0.33			0.92		
Spore concentration	0.23			0.66		
Isolates x Spore concentration	1.64			NS		

() = Figures in parentheses are arcsin transformed values
*() = Figures in parentheses are (x+0.5) arcsin transformed values
NS = Non-significant

4.2.4. c. i. At 72 hours after spraying

Isolates

Among different isolates tested, the differences in the *T. absoluta* larval mortality at 72 hours after spray were significant. Highest mortality was recorded in isolate I-4 (23.50%), followed by I-2 (21.50%) and I-1 (17.50%), but no significant difference was observed between them.The next effective isolate was I-5 (15.50%), followed by I-3 (14.50%), but both the isolates were statistically at par with each other. The next effective isolate was I-6 (12.50%),followed by I-7 (11.00%), however non-significant difference was observed between them (Table 21.3).

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 72 hours after spray were found to be significant. Highest larval mortality (33.21%) was registered with spore concentration S-1, followed by S-2 (21.07%), S-3 (13.93%), S-4 (10.36%) and S-5 (4.29%) and they differed significantly from each other (Table 21.3).

The results indicate that the larval mortality was dependent on spore concentration and the period of infection .

Interactions : Isolates x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the larval mortality.

Table 21.3 At 72 hrs after treatment

Isolates (I)	Spore concentration (S) (ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	40.00 (39.23)	25.00 (30.02)	12.50 (20.91)	7.50 (15.20)	2.50 (7.77)	17.50 (24.73)
I_2	40.00 (39.23)	27.50 (36.57)	20.00 (26.57)	15.00 (22.79)	5.00 (11.48)	21.50 (27.62)
I_3	27.50 (31.63)	20.00 (26.57)	12.50 (20.91)	7.50 (15.20)	5.00 (11.48)	14.50 (22.38)
I_4	47.50 (43.57)	25.00 (30.02)	20.00 (26.57)	17.50 (24.73)	7.50 (15.20)	23.50 (29.00)
I_5	32.50 (34.76)	17.50 (24.73)	12.50 (20.91)	10.00 (18.91)	5.00 (11.48)	15.50 (23.18)
I_6	22.50 (28.32)	15.00 (22.79)	12.50 (20.91)	10.00 (18.91)	2.50 (7.77)	12.50 (20.91)
I_7	22.50 (28.32)	17.50 (24.73)	7.50 (15.20)	5.00 (11.48)	2.50 (7.77)	11.00 (19.32)
Mean	33.21 (35.19)	21.07 (27.32)	13.93 (21.91)	10.36 (18.77)	4.29 (11.95)	-
	SEm±			CD (P=0.05)		
Isolates	0.36			1.01		
Spore concentration	0.26			0.72		
Isolates x Spore concentration	1.79			NS		

() = Figures in parentheses are arcsin transformed values
NS = Non-significant

4.2.4. d. i. At 96 hours after spraying

Isolates

Perusal of data in Table 21.4 revealed that at 96 hours after spray, the differences in the *T.absoluta* larval mortality among different isolates were significant. Among the different isolates, highest mortality was recorded in isolate I-4 (44.00%) and was significantly superior than all other isolates. The next effective isolate was I-2 (33.00%), followed by I-5 (25.50%) and I-1 (25.00%), and they differed significantly from each other. The next effective isolate was I-7 (23.50%), followed by I-6 (23.00%) and I-3 (22.00%), but all were statistically at par with each other .

Spore concentration

Different spore concentrations of *B.bassiana* evaluated for larval mortality at 96 hours after spray were found to be significant. Highest larval mortality (48.21%) was registered with spore concentration S-1, followed by S-2 (35.71%) S-3 (28.21%), S-4 (18.21%) and S-5 (9.64%) and they differed significantly from each other (Table 21.4).

The results indicate that the larval mortality was dependent on spore concentration and period of infection.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had significant impact on the larval mortality. Highest mortality was registered by isolate I-4 with S-1 spore concentration.

Table 21.4 At 96 hrs after treatment

Isolates (I)	Spore concentration (S) (ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	55.00 (47.87)	37.50 (37.76)	25.00 (30.02)	7.50 (15.20)	5.00 (11.48)	25.00 (30.02)
I_2	55.00 (47.87)	42.50 (40.69)	37.50 (37.76)	20.00 (26.57)	10.00 (18.91)	33.00 (35.06)
I_3	32.50 (34.76)	27.50 (31.63)	22.50 (28.32)	15.00 (22.79)	12.50 (20.91)	22.00 (27.97)
I_4	75.00 (60.00)	55.00 (47.87)	42.50 (40.69)	32.50 (34.76)	15.00 (22.79)	44.00 (41.55)
I_5	45.00 (42.13)	32.50 (34.76)	25.00 (30.02)	15.00 (22.79)	10.00 (18.91)	25.50 (30.33)

I_6	37.50 (37.76)	27.50 (31.63)	22.50 (28.32)	20.00 (26.57)	7.50 (15.20)	23.00 (28.66)
I_7	37.50 (37.76)	32.50 (34.76)	22.50 (28.32)	17.50 (24.73)	7.50 (15.20)	23.50 (29.00)
Mean	48.21 (43.98)	35.71 (36.70)	28.21 (32.08)	18.21 (25.26)	9.64 (18.09)	-
	SEm±			**CD (P=0.05)**		
Isolates	0.28			0.79		
Spore concentration	0.20			0.57		
Isolates x Spore concentration	1.41			3.96		

() = Figures in parentheses are arcsin transformed values

4.2.4. e. i. At 120 hours after spraying

Isolates

Perusal of data in Table 21.5 revealed that at 120 hours after spray, the differences in the *T.absoluta* larval mortality among isolates were significant. Highest larval mortality was registered in isolate I-4 (54.50%), followed by I-2 (43.50%) and I-1 (38.50%), but they differed significantly from each other.The next effective isolate was I-3 (37.00%), followed by I-7 (36.50%), but both were statistically at par with each other. The least effective isolates were I-6 (34.50%) and I-5 (34.00%) and they did not differ significantly from each other (Table 21.5).

Spore concentration

Different spore concentrations of *B. bassiana* evaluated for larval mortality at 120 hours after spray were found to be significant. Highest larval mortality (61.07%) was registered with spore concentration S-1, followed by S-2 (49.29%), S-3 (38.21%), S-4 (31.07%) and S-5 (19.29%), and they differed significantly from each other (Table 21.5).

The results indicate that the larval mortality is dependent on spore concentration.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had significant impact on the larval mortality. Highest larval mortality was registered by isolate I-4 with S-1 spore concentration.

Table 21.5 At 120 hrs after treatment

Isolates (I)	Spore concentration (S) (ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	62.50 (52.24)	47.50 (43.57)	37.50 (37.76)	32.50 (34.76)	12.50 (20.91)	38.50 (38.35)
I_2	62.50 (52.24)	60.00 (50.77)	45.00 (42.13)	30.00 (33.21)	20.00 (26.57)	43.50 (41.27)
I_3	52.50 (46.43)	45.00 (42.13)	37.50 (37.76)	27.50 (31.63)	22.50 (28.32)	37.00 (37.46)
I_4	80.00 (63.43)	65.00 (53.73)	47.50 (43.57)	45.00 (42.13)	35.00 (36.27)	54.50 (47.58)
I_5	60.00 (50.77)	40.00 (39.23)	32.50 (34.76)	22.50 (28.32)	15.00 (22.79)	34.00 (35.67)
I_6	52.50 (46.43)	40.00 (39.23)	35.00 (36.27)	32.50 (34.76)	12.50 (20.91)	34.50 (35.97)
I_7	57.50 (49.31)	47.50 (43.57)	32.50 (34.76)	27.50 (31.63)	17.50 (24.73)	36.50 (37.17)
Mean	61.07 (51.40)	49.29 (44.59)	38.21 (38.18)	31.07 (33.88)	19.29 (26.05)	
	SEm±			CD (P=0.05)		
Isolates	0.20			0.56		
Spore concentration	0.14			0.40		
Isolates x Spore concentration	1.00			2.81		

() = Figures in parentheses are arcsin transformed values

4.3.4.i *Bemisia tabaci* (3rd instar nymph)

4.3.4. a. i. At 24 hours after spraying (Table 22.1)

Isolates

Perusal of data in Table 22.1 revealed that at 24 hours after spray, the differences in the *B.tabaci* nymphal mortality among different isolates were non-significant. Among the isolates, highest mortality was recorded in isolate I-4 (2.00%), followed by isolates I-1 and I-2 (both registered 1.50% mortality), I-7 (1.00%), I-3 and I-5 (both recorded 0.50% mortality),while no mortality was recorded in I-6.

Spore concentration

Different spore concentrations of *B. bassiana* evaluated for nymphal mortality at 24 hours after spray were found to be significant. Highest nymphal

mortality (3.93%) was registered with spore concentration S-1, followed by S-2 (1.07%), but they differed significantly from each other. While no nymphal mortality was recorded with spore concentration S-3, S-4 and S-5 (Table 22.1).

The results indicate that the nymphal mortality was dependent on spore concentration and period of infection .

Interactions : Isolate x Spore concentration

The interaction of isolate and spore concentration had no significant impact on the nymphal mortality.

Table 22: Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Bemisia tabaci* (3rd instar nymph)

Table 22.1 At 24 hrs after spraying

Isolates (I)	Spore concentration (S) (spores ml L^{-1})*					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	1.50 (6.28)
I_2	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	1.50 (6.28)
I_3	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.50 (4.80)
I_4	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (7.03)
I_5	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.50 (4.80)
I_6	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
I_7	5.00 (11.48)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	1.00 (5.54)
Mean	3.93 (12.15)	1.07 (7.20)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	-
	SEm±			CD (P=0.05)		
Isolates	0.20			NS		
Spore concentration	0.14			0.40		
Isolates x Spore concentration	0.99			NS		

() = Figures in parentheses are (x+0.5) arcsin transformed values
NS = Non-significant

4.3.4. b. i. At 48 hours after spraying

Isolates

Perusal of data in Table 22.2 revealed that at 48 hours after spray, the differences in the *B.tabaci* nymphal mortality among different isolates were significant. Highest nymphal mortality was recorded in isolates I-1, I-2 and I-4, all of them registered 4.00% mortality and were at par with each other.The next effective isolate was I-7 (3.50%) and was significantly superior than I-3 and I-5, as both recorded 2.00% mortality. Whereas, the least effective isolate was I-6 as it recorded 0.50% nymphal mortality.

Spore concentrations

Different spore concentrations of *B. bassiana* evaluated for nymphal mortality at 48 hours after spray were found to be significant. Highest nymphal mortality was registered with spore concentration S-1(8.57%), followed by S-2 (4.29%), S-3 (1.43%) and they differed significantly from each other. Whereas, no mortality was registered with spore concentrations S-4 and S-5, while non-significant difference was observed between them (Table 22.2) .

The results indicate that the nymphal mortality was dependent on spore concentration and period of infection .

Interactions: Isolate x Spore concentration

The interaction of isolates and spore concentration had no significant impact on nymphal mortality.

Table 22.2 At 48 hrs after spraying

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2^*	S_3	S_4^*	S_5^*	Mean
I_1	10.00 (18.91)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	4.00 (11.54)
I_2	10.00 (18.91)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	4.00 (11.54)
I_3	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (7.03)
I_4	12.50 (20.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	4.00 (11.54)
I_5	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	2.00 (7.03)
I_6	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.50 (4.80)

I_7	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	3.50 (9.25)
Mean	8.57 (16.54)	4.29 (10.42)	1.43 (6.18)	0.00 (4.05)	0.00 (4.05)	-
	SEm±			**CD (P=0.05)**		
Isolates	0.23			0.66		
Spore concentration	0.17			0.47		
Isolates x Spore concentration	1.17			NS		

() = Figures in parentheses are arcsin transformed values
*() = Figures in parentheses are (x+0.5) arcsin transformed values
NS = Non-significant

4.3.4. c. i. At 72 hours after spraying

Isolates

Perusal of data in Table 22.3 revealed that at 72 hours after spray, the differences in the *B.tabaci* nymphal mortality among different isolates were significant. Among the isolates, significantly highest mortality was recorded in isolate I-4 (14.00%) and was significantly superior than all other isolates. The next effective isolate was I-1 (13.00%), followed by I-2 (12.50%), but both were at par with each other. The next effective isolate was I-7(9.50%) and was significantly better then I-3 (6.00%), I-5 and I-6 (both registered 5.50% mortality), however the later three did not differ significantly from each other.

Spore concentrations

Different spore concentration of *B.bassiana* evaluated for nymphal mortality at 72 hours after spray were found to be significant. Spore concentration S-1 recorded highest nymphal mortality (17.50%), followed by S-2 (11.43%), S-3 (8.93%), S-4 (5.71%) and S-5 (3.93%), but they differed significantly from each other (Table 22.3).

The results indicate that the nymphal mortality was dependent on spore concentration and period of infection.

Interactions: Isolates x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the nymphal mortality.

Table 22.3 At 72 hrs after spraying

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	22.50 (28.32)	15.00 (22.79)	12.50 (20.91)	10.00 (18.91)	5.00 (11.48)	13.00 (21.13)
I_2	22.50 (28.32)	15.00 (22.79)	12.50 (20.91)	7.50 (15.20)	5.00 (11.48)	12.50 (20.91)
I_3	12.50 (20.91)	7.50 (15.20)	5.00 (11.48)	2.50 (7.77)	2.50 (7.77)	6.00 (14.18)
I_4	27.50 (31.63)	17.50 (24.73)	12.50 (20.91)	7.50 (15.20)	5.00 (11.48)	14.00 (21.97)
I_5	12.50 (20.91)	7.50 (15.20)	5.00 (11.48)	2.50 (7.77)	2.50 (7.77)	6.00 (14.18)
I_6	7.50 (15.20)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.50 (13.56)
I_7	17.50 (24.73)	12.50 (20.91)	10.00 (18.91)	5.00 (11.48)	2.50 (7.77)	9.50 (17.95)
Mean	17.50 (24.73)	11.43 (19.76)	8.93 (17.39)	5.71 (13.83)	3.93 (11.43)	-
	SEm±			CD (P=0.05)		
Isolates	0.40			1.11		
Spore concentration	0.28			0.79		
Isolates x Spore concentration	1.98			NS		

() = Figures in parentheses are arcsin transformed values
NS = Non-significant

4.3.4. d. i. At 96 hours after spraying

Isolates

Perusal of data in Table 22.4 revealed that at 96 hours after spray, the differences in *B.tabaci* nymphal mortality among different isolates were significant. Among the isolates, highest mortality was recorded in isolate I-4 (28.50%) and was superior than all the other isolates. This was followed by I-1 (25.50%), I-2 (22.00%), I-7 (18.50%) and I-3 (16.00%), but they differed significantly from each other. The least effective isolates were I-6 (13.00%) and I-5 (12.50%), and were statistically at par with each other.

Spore concentrations

Different spore concentrations of *B.bassiana* evaluated for nymphal mortality at 96 hours after spray were found to be significant. Highest nymphal

mortality (34.29%) was registered with spore concentration S-1 followed by S-2 (24.29%), S-3 (17.50%), S-4 (12.86%) and S-5 (8.21%), but they differed significantly from each other (Table 22.4).

The results indicate that the nymphal mortality was dependent on spore concentration and period of infection.

Interactions : Isolates x Spore concentration

The interaction of isolates and spore concentration had no significant impact on the nymphal mortality.

Table 22.4 At 96 hrs after spraying

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	40.00 (39.23)	30.00 (33.21)	25.00 (30.02)	20.00 (26.57)	12.50 (20.91)	25.50 (30.33)
I_2	35.00 (36.27)	30.00 (33.21)	20.00 (26.57)	15.00 (22.79)	10.00 (18.91)	22.00 (27.97)
I_3	27.50 (31.63)	20.00 (26.57)	15.00 (22.56)	10.00 (18.91)	7.50 (15.20)	16.00 (23.58)
I_4	57.50 (49.31)	35.00 (36.27)	25.00 (30.02)	17.50 (24.73)	7.50 (15.20)	28.50 (32.27)
I_5	25.00 (30.02)	15.00 (22.79)	10.00 (18.91)	7.50 (15.20)	5.00 (11.48)	12.50 (20.91)
I_6	25.00 (30.02)	17.50 (24.73)	10.00 (18.91)	7.50 (15.20)	5.00 (11.48)	13.00 (21.13)
I_7	30.00 (33.21)	22.50 (28.32)	17.50 (24.73)	12.50 (20.91)	10.00 (18.91)	18.50 (25.47)
Mean	34.29 (35.84)	24.29 (29.53)	17.50 (24.73)	12.86 (21.01)	8.21 (16.65)	-
	SEm±			CD (P=0.05)		
Isolates	0.38			1.07		
Spore concentration	0.27			0.77		
Isolates x Spore concentration	1.92			NS		

() = Figures in parentheses are arcsin transformed values
NS = Non-significant

4.3.4. e. i. At 120 hours after spraying

Isolates

Perusal of data in Table 22.5 revealed that at 120 hours after spray, the differences in the *B. tabaci* nymphal mortality among different isolates

were significant. Highest nymphal mortality was recorded in isolate I-4 (36.00%) and was significantly superior than all other isolates. This was followed by I-1 (35.00%), I-2 (27.50%), I-7 (25.00%) and I-3 (24.00%), but they differed significantly from each other. The least effective isolates were I-6 (22.50%) and I-5 (22.00%), and were statistically at par with each other.

Spore concentrations

Different spore concentrations of *B.bassiana* evaluated for nymphal mortality at 120 hours after spray were found to be significant. Highest nymphal mortality (45.71%) was registered with spore concentration S-1, followed by S-2 (32.86%), S-3 (25.71%), S-4 (19.29%) and S-5 (13.57%), but they differed significantly from each other (Table 22.5).

The results indicate that the nymphal mortality was dependent on spore concentration and the period of infection.

Interactions : Isolate x Spore concentration

The interaction of isolates and spore concentration had significant impact on the nymphal mortality. Highest nymphal mortality was registered by isolate I-4 with S-1 spore concentration.

Table 22.5 At 120 hrs after spraying

Isolates (I)	Spore concentration (S) (spores ml L^{-1})					
	S_1	S_2	S_3	S_4	S_5	Mean
I_1	57.50 (49.31)	40.00 (39.23)	35.00 (36.27)	27.50 (31.63)	15.00 (22.79)	35.00 (36.27)
I_2	45.00 (42.13)	35.00 (36.27)	25.00 (30.02)	17.50 (24.73)	15.00 (22.79)	27.50 (31.63)
I_3	40.00 (39.23)	27.50 (31.63)	22.50 (28.32)	17.50 (24.73)	12.50 (20.91)	24.00 (29.33)
I_4	67.50 (55.24)	42.50 (40.69)	32.50 (34.76)	25.00 (29.72)	12.50 (20.91)	36.00 (36.87)
I_5	35.00 (36.27)	27.50 (31.63)	20.00 (26.57)	15.00 (22.92)	12.50 (20.91)	22.00 (27.97)
I_6	37.50 (37.76)	27.50 (31.63)	20.00 (26.57)	15.00 (22.79)	12.50 (20.91)	22.50 (28.32)
I_7	37.50 (37.76)	30.00 (33.21)	25.00 (30.02)	17.50 (24.73)	15.00 (22.79)	25.00 (30.02)
Mean	45.71 (42.54)	32.86 (34.97)	25.71 (30.47)	19.29 (26.05)	13.57 (21.62)	-

	SEm±	CD (P=0.05)
Isolates	0.17	0.48
Spore concentration	0.12	0.34
Isolates x Spore concentration	0.85	2.39

() = Figures in parentheses are arcsin transformed values

4.4 Relative susceptibility of insect pest complex of tomato to *B.bassiana*

Bioassays performed with seven fungal isolates of *B.bassiana* revealed that isolate I-4 registered highest mortality against all the tested tomato insect pests at highest spore concentration S-1 ($1x10^9$ spores ml^{-1}) at 120 hours after treatment and to further confirm and to conclude the results, studies on median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were carried out .

4.4.1 Median lethal concentration (LC_{50}) and Median lethal time (LT_{50}) at 120 hours after treatment

4.4.1.a *Helicoverpa armigera*

The data are presented in Tables 23 and 24 and depicted in Figures 2a, 2b and 3.

The bioassay studies revealed that *H.armigera* 2nd instar larvae were susceptible to all the fungal isolates tested. On subjecting the data to probit analysis, the LC_{50} values revealed that isolate I-4 followed by isolate I-1 registered higher virulence with the lowest LC_{50} values of 2.02 $x10^5$ and 2.74 $x10^5$ spores ml^{-1}, respectively (Table 23 and Figure 2a-Isolate 1 and 4). The lowest and highest fiducial limits for I-4 was 5.24 x 10^4 and 7.76 x 10^5 spores ml^{-1}, whereas for I-1 it was 5.10 x 10^4 and 1.47 x 10^6 spores ml^{-1}, respectively.The x^2 values calculated were found to be non-significant which confirmed the homogeneity of the test population (Table 23).

Time mortality response of *H.armigera* 2nd instar larvae to different fungal isolates indicated that isolate I-4 required less time to kill the larvae as compared to the other isolates. At spore concentration of S-1 ($1x10^9$ spores ml^{-1}), the LT_{50} value for I-4 was 54.47 hours (Figure 3), followed by I-

Table 24 : Time mortality response of *H.armigera* to different isolates of *Beauveria bassiana* at 1x10^9 spores ml^{-1}

Isolates (I)	Heterogeneity (x^2)	Regression equation	LT_{50} (hr)	Fiducial limits	
				Lower	Upper
I-1	6.81	y = 3.363 - 4.652x	66.12	60.00	72.85
I-2	1.97	y = 3.643 - 4.404x	92.76	79.67	108.01
I-3	0.80	y = 4.302 - 4.814x	85.58	76.18	96.13
I-4	7.15	y = 4.815 - 5.866x	54.47	46.56	63.72
I-5	2.00	y = 4.922 - 5.147x	84.95	77.51	93.11
I-6	0.50	y = 3.818 - 4.323x	107.50	94.69	122.03
I-7	2.25	y = 1.389 - 3.097x	109.88	92.89	129.97

x^2 table value =7.81

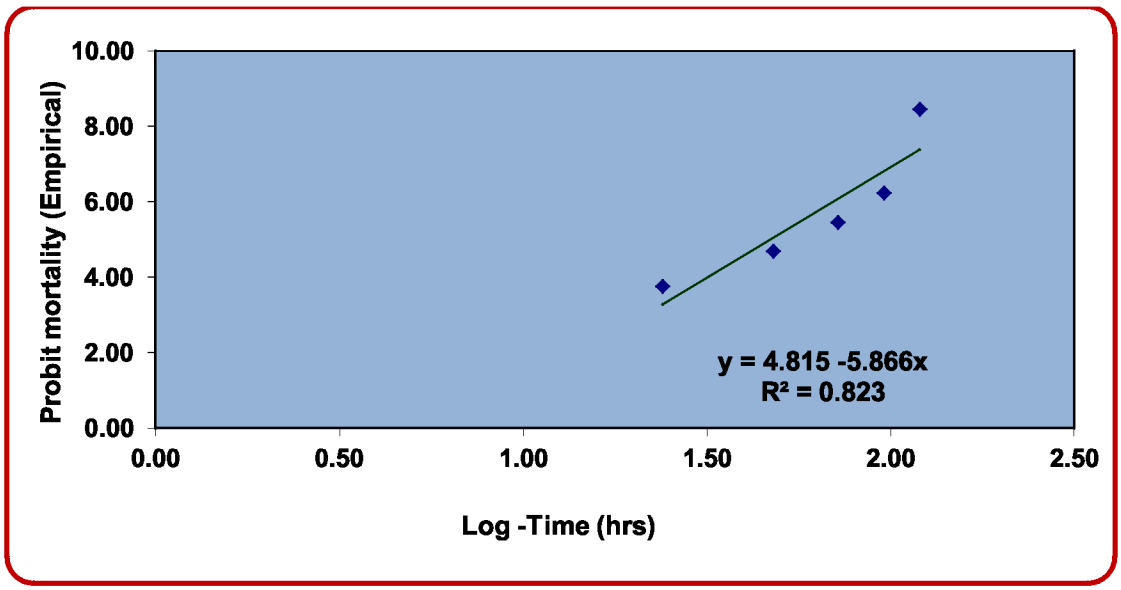

Figure 3 :Time mortality response of *H. armigera* to *B. bassiana* isolate I-4 at 1x10^9 spores ml^{-1}

Table 26 : Time mortality response of *T.absoluta* to different isolates of *Beauveria bassiana* at 1x10^9 spores ml^{-1}

Isolates (I)	Heterogeneity (x^2)	Regression equation	LT_{50} (hr)	Fiducial limits	
				Lower	Upper
I-1	1.07	y = 3.779 - 4.428x	96.97	84.37	111.46
I-2	1.52	y = 3.921 - 4.489x	97.37	85.41	110.99
I-3	1.28	y = 0.465 - 2.531x	140.23	103.83	189.39
I-4	1.45	y = 1.544 - 3.524x	72.45	63.99	82.02
I-5	0.85	y = 3.279 - 4.100x	108.34	90.69	129.42
I-6	1.36	y = 0.573 - 2.586x	136.41	103.47	179.83
I-7	2.39	y = 0.914 - 2.773x	126.44	101.08	158.17

x^2 table value =7.81

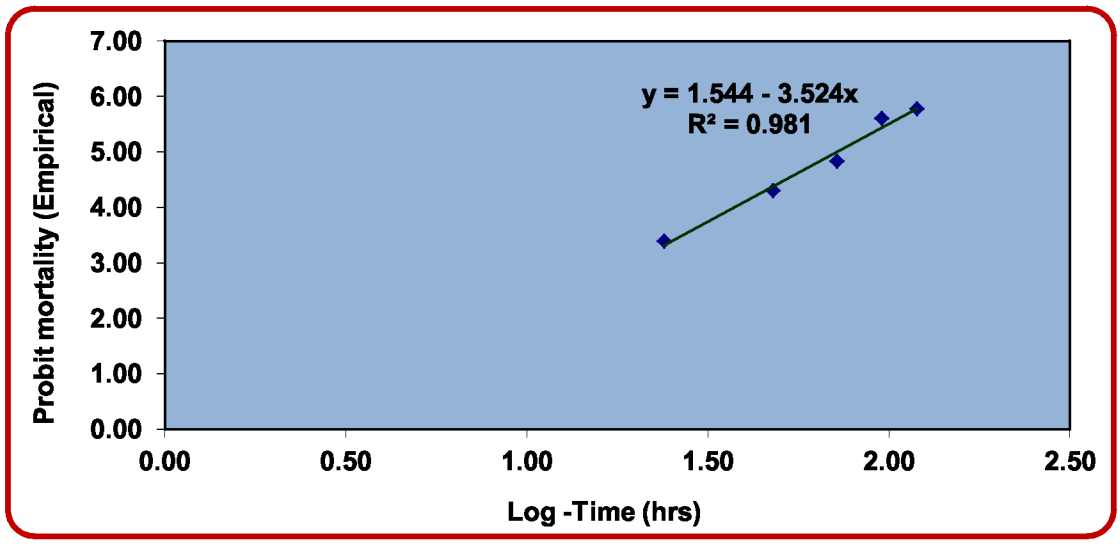

Figure 5 Time mortality response of *Tuta absoluta* to *B.bassiana* isolate I-4 at 1x10^9 spores ml^{-1}

1 (66.12 hours), whereas the LT_{50} values for isolates I-2, I-3, I-5, I-6 and I-7 were higher and it ranged between 84.95 and 109.88 hours (Table 24).

4.4.1.b *Tuta absoluta*

The data are presented in Tables 25 and 26 and depicted in Figures 4a, 4b and 5.

The bioassay results showed that *T.absoluta* 2nd instar larvae were susceptible to all the fungal isolates tested. Probit analysis of the data revealed that the LC_{50} values of isolate I-4 followed by isolate I-1 recorded higher virulence with the lowest LC_{50} values of 1.15 $x10^7$ and 2.00 $x10^8$ spores ml^{-1},respectively (Table 25 and Figure 4a-Isolate 1 and 4). The lowest and highest fiducial limits for I-4 was 3.63 x 10^6 and 3.65 x 10^7 spores ml^{-1}, whereas for I-1 it was 4.57 x 10^7 and 8.71 x 10^8 spores ml^{-1}, respectively. The x^2 values calculated were found to be non-significant which confirmed the homogeneity of the test population (Table 25).

Time mortality response of *T.absoluta* 2nd instar larvae to different fungal isolates indicated that isolate I-4 required less time to kill the larvae as compared to other isolates. At the spore concentration of S-1 ($1x10^9$ spores ml^{-1}), LT_{50} value for I-4 was 72.45 hours (Figure 5), followed by I-1 (96.97 hours), whereas LT_{50} values for other isolates I-2, I-3, I-5, I-6 and I-7 were higher and it ranged between 97.37 and 140.23 hours (Table 26).

4.4.1.c *Bemisia tabaci*

The data are presented in Tables 27and 28 and depicted in Figures 6a, 6b and 7.

The bioassay studies revealed that *B.tabaci* 3rd instar nymphs were susceptible to all the fungal isolates tested. On subjecting the data to probit analysis, the LC_{50} values revealed that isolate I-4 followed by isolate I-1 registered higher virulence with the lowest LC_{50} values 2.25 $x10^8$ and 6.22 x 10^8 spores ml^{-1}, respectively (Table 27 and Figure 6a - Isolate 1 and 4). The lowest and highest fiducial limits for I-4 was 6.25 x 10^7 and 8.06 x 10^7 spores ml^{-1}, whereas for I-1 it was 8.25 x 10^7 and 4.68 x 10^9 spores ml^{-1},

Table 28 : Time mortality response of *B.tabaci* to different isolates of *Beauveria bassiana* at 1x10^9 spores ml^{-1}

Isolates (I)	Heterogeneity (x^2)	Regression equation	LT_{50} (hr)	Fiducial limits	
				Lower	Upper
S-1	0.23	y = 3.711 - 4.185x	115.05	100.01	132.34
S-2	0.38	y = 3.075 - 3.776x	134.59	107.69	168.21
S-3	1.09	y = 3.180 - 3.618x	145.78	115.38	184.20
S-4	0.94	y = 4.309 - 4.660x	97.85	88.08	108.70
S-5	0.87	y = 2.982 - 3.488x	154.50	117.77	202.69
S-6	3.80	y = 2.905 - 3.323x	136.77	113.17	165.29
S-7	0.21	y = 2.570 - 3.424x	156.97	115.72	212.92

x^2 table value =7.81

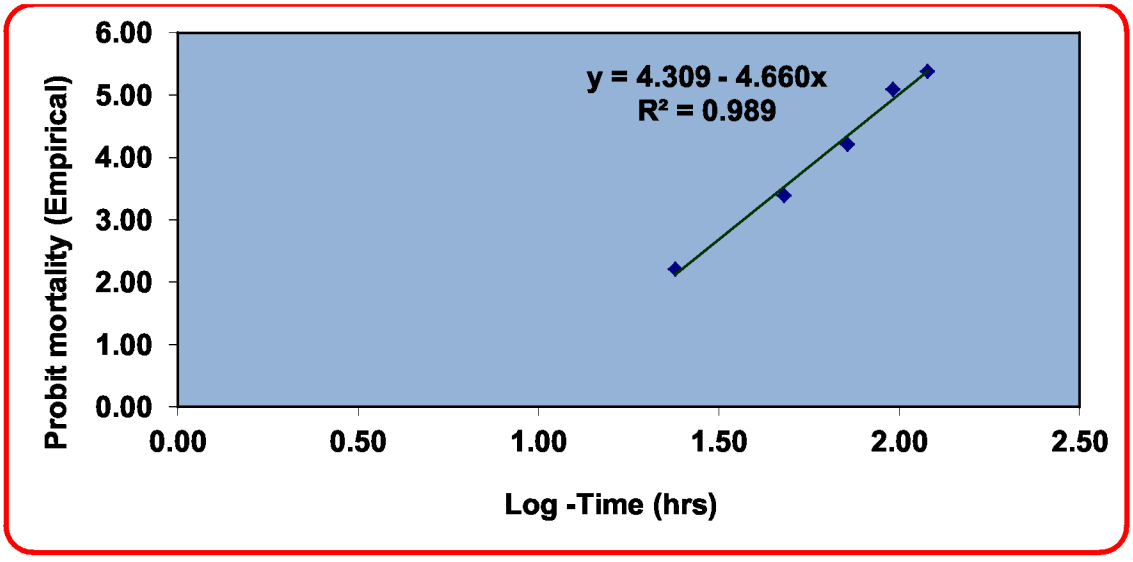

Figure 7. Time mortality response of *Bemisia tabaci* to *B. bassiana* isolate I-4 at 1x10^9 spores ml^{-1}

respectively. The x^2 values calculated were found to be non-significant which confirmed the homogeneity of the test population.

Time mortality response of *B.tabaci* 3rd instar nymphs to different fungal isolates indicated that isolate I-4 required less time to kill the nymphs as compared to the other isolates. At spore concentration of S-1 ($1x10^9$ spores ml^{-1}), the LT_{50} value for I-4 was 97.85 hours (Figure 7), followed by I- 1 (115.05 hours), whereas the LT_{50} values for isolates I-2, I-3, I-5, I-6 and I-7 were higher and it ranged between 134.59 and 156.97 hours (Table 28).

4.2 Identification and standardization of economic substrate for mass production of the virulent isolate of *Beauveria bassiana*

For mass production of *B.bassiana* an experiment was undertaken with nine different treatments which included six solid substrates and three liquid media's for standardization of a suitable economic medium for growth and sporulation of fungus that could be taken as base for formulation preparations. During the period of study, the maximum and minimum temperature of the laboratory were 31 ± 2^0C and 25.57 ± 3^0C, respectively. While morning and evening relative humidity were 72.42 ± 2.35% and 52.81 ± 4.50% , respectively. The observations on spore load were recorded on 10th, 20th and 30th days after inoculation and the data presented in Table 29 and depicted in Figure 8.

4.2.1 Spore count at different days after inoculation

4.2.1.a. Ten days after inoculation

Among the different substrates/media evaluated there was significant difference. Highest conidial count of (6.33 x 10^9 spores ml^{-1}) was recorded on rice (T_5), which was significantly superior than all other substrates. This was followed by chickpea (T_2) (5.00 x 10^9 spores ml^{-1}), sorghum (T_3) (3.00 x 10^9 spores ml^{-1}) and PDB (T_7) ($2.67x10^9$ spores ml^{-1}), but they did not differ significantly from each other. The next two substrates *i.e.* ragi (T_4) and CDB (T_8) recorded 1.67 x 10^9 spores ml^{-1} and were at par with each other. Maize (T_1) was the next substrate which registered 1.33 x 10^9 spores ml^{-1}, followed by SDB (T_9)

(1.00 x 10^9 spores ml^{-1}), but did not differ significantly from each other and least spore count was recorded on neem cake (T_6) (0.33 x 10^9 spores ml^{-1}).

4.2.1.b. Twenty days after inoculation

Among the different substrates/media evaluated, there was significant difference. Highest conidial count of 8.33 x 10^9 spores ml^{-1} was recorded on rice (T_5) and was significantly superior than all other substrates. This was followed by PDB (T_7) (7.33 x 10^9 spores ml^{-1}), chickpea (T_2) (5.67 x 10^9 spores ml^{-1}) and sorghum (T_3) (4.67 x 10^9 spores ml^{-1}), but they differed significantly from each other. The next substrate was CDB (T_8) which recorded 7.67 x 10^9 spores ml^{-1}, followed by ragi (T_4) (3.33 x 10^9 spores
ml^{-1}), but both were at par with each other.The next two substrates maize (T_1) and SDB (T_9) were at par with each other and both registered spore load of 3.00 x 10^9 spores ml^{-1}. Whereas least spore count was recorded on neem cake (T_6) (1.00 x 10^9 spores ml^{-1}).

4.2.1.c. Thirty days after inoculation

Among the different substrates/media evaluated, there was significant difference. Highest conidia was observed on rice (T_5) (11.67 x 10^9 spores ml^{-1}), followed by PDB (T_7) (11.00 x 10^9 spores ml^{-1}), were significantly superior than all the other substrates, but both were at par with each other. The next substrate was chickpea (T_2) (8.67 x 10^9 spores ml^{-1}) and was significantly better than CDB (T_8) (7.67 x 10^9 spores ml^{-1}), sorghum (T_3) (6.67 x 10^9 spores ml^{-1}) and ragi (T_4) (6.00 x 10^9 spores ml^{-1}), but the latter three were at par with each other. The next group of substrates *i.e.* SDB (T_9) and maize (T_1) were at par with each other with spore load of 4.67 x 10^9 spores ml^{-1} and 4.33 x 10^9 spores ml^{-1}, respectively. However, lowest spore count was recorded on neem cake (T_6) (1.33 x 10^9 spores ml^{-1}).

4.2.1.d. Mean

Among the different substrates/media evaluated, there was significant difference. Highest conidial count was recorded on rice (T_5) (8.78 x 10^9 spores ml^{-1}), followed by PDB (T_7) (7.44 x 10^9 spores ml^{-1}), chickpea (T_2) (6.44 x 10^9 spores ml^{-1}), sorghum (T_3) (4.78 x 10^9 spores ml^{-1}), CDB (T_8)(4.33 x 10^9 spores

ml^{-1}), ragi (T_4)(3.67 x 10^9 spores ml^{-1}), maize (T_1) (2.89 x 10^9 spores ml^{-1}) and SDB (T_9) (2.89 x 10^9 spores ml^{-1}), but all were at par with each other. The least spore count was recorded in neem cake (T_6) (0.89 x 10^9 spores ml^{-1}).

4.2.2 Rate of increase in growth

Rate of increase in growth of *B.bassiana* was calculated and the data presented Table 29 and depicted in Figure 8.

4.2.2.a. 10 to 20 days after inoculation

The rate of increase in growth of *B.bassiana* on different substrates/media from 10^{th} to 20^{th} day after inoculation was found to be non-significant. However, the rate of increase in growth of fungus ranged from 72.22% (SDB,T_9) to 23.15% (rice, T_5) .

4.2.2.b. 20 to 30 days after inoculation

The rate of increase in growth of *B. bassiana* on different substrates/ media from 20^{th} to 30^{th} day after inoculation were found to be non-significant. Highest rate of increase in growth of fungus was recorded in CDB (T_8) (50.00%), while it was lowest in maize (T_1) (26.76%).

4.2.3 Economics of mass production of *Beauveria bassiana* on/in different substrates

Cost of production of 1x10^9 spores ml^{-1} were calculated for all substrates/media and the data presented in Table 30. The cost of production of *B.bassiana* on different substrates / media was found to be significant. Minimum production cost was recorded in PDB (T_7) (Rs.0.58), followed by rice (T_5) (Rs.1.01), but both were at par with each other. The next substrate was sorghum (T_3) (Rs.1.57), followed by CDB (T_8) (Rs.1.92), but they did not differ significantly from each other. The next substrate was ragi (T_4) (Rs.2.00) and was significantly superior than neem cake (T_6) (Rs.3.09) and maize (T_1) (Rs.3.17), however, the latter two were at par with each other. Higher cost of production was recorded on SDB (T_9) (Rs.3.70) and chickpea (T_2) (Rs.3.86), but they did not differ significantly from each other.

Table 30 : Economics of *Beauveria bassiana* production on different substrates

Treatment code	Substrates	Mean spore count (1x 10^9 spores ml^{-1})	Cost of substrate per 100g (Rs)	Production cost of *B.bassiana* 1x10^9 spores ml^{-1} (Rs)
I Solid substrates				
T_1	Maize	2.89(1.81)	5=50	3=17
T_2	Chickpea	6.44(2.62)	10=00	3=86
T_3	Sorghum	4.78(2.27)	3=50	1=57
T_4	Ragi	3.67(1.99)	4=20	2=22
T_5	Rice	8.78(3.02)	3=00	1=01
T_6	Neem cake	0.89(1.16)	3=50	3=09
II Liquid (artificial) media				
T_7	PDB	7.00(2.66)	1=44	0=58
T_8	CDB	4.33(2.12)	3=80	1=92
T_9	SDB	2.89(1.79)	6=20	3=70
	SEm±	0.17	-	0.21
	CD at 5%	1.36	-	0.44

() = Figures in parentheses are square root transformed values

4.3 To study the compatibility of virulent isolate of *B.bassiana* with various additives

An experiment was carried out under *in-vitro* conditions to screen additives at various concentrations (0.1%, 0.5% and 1.00%) with virulent isolate of *B.bassiana* to identify the most compatible additive that could be further utilized for formulation preparation. Data on growth performance of *B.bassiana* at ten days after inoculation in different additives are presented in Table 31 and depicted in Figure 9 and Plate 11 a, b, c.

4.3.1 Impact of additives on growth and development of *B.bassiana*

4.3.1.1. Radial growth

4.3.1.1.a. At 0.1% concentration

The results revealed that the differences in the mean growth of *B.bassiana* in different additives at 0.1% concentration was significant. Control registered maximum growth of 83.95 mm. However, among the additives, CMC (T_{12}) recorded maximum growth (73.25 mm), followed by Kaolite (T_{10}) (66.90 mm), Silica gel (T_{11}) (65.20 mm), Sunflower oil (T_9) (64.15 mm) and Glycerol (T_5) (52.23 mm), respectively and they differed significantly from each other. Whereas the growth of *B.bassiana* in the remaining additives was below 50 mm and lowest was recorded in Pongamia oil (T_8) (26.03 mm).

4.3.1.1.b. At 0.5% concentration

The results revealed that the differences in the mean growth of *B.bassiana* in different additives at 0.5% concentration was significant. Control registered maximum growth of 83.95 mm. However, among the additives, CMC (T_{12}) recorded maximum growth (76.93 mm), followed by Kaolite (T_{10}) (73.83 mm), Silica gel (T_{11}) (68.33 mm) and Sunflower oil (T_9) (55.45 mm), respectively, but they differed significantly from each other. The next effective additive was Glycerol (T_5) (52.93 mm), followed by Tween-80 (T_4) (51.49 mm), but both were statistically at par with each other. Whereas, the growth of *B.bassiana* in the remaining additives was below 50 mm and lowest was recorded in Triton -X (T_6) (23.69 mm).

4.3.1.1.c. At 1.0 % concentration

The results revealed that the differences in the mean growth of *B.bassiana* in different additives at 1.0% concentration was significant. Control registered maximum growth of 83.95 mm. However, among the additives, CMC (T_{12}) recorded maximum growth (81.29 mm), followed by Kaolite (T_{10}) (66.48 mm) and Silica gel (T_{11}) (62.07 mm), respectively, but they differed significantly from each other. The next effective additive was Glycerol (T_5) (50.32 mm), followed by Tween-80 (T_4) (49.03 mm), but both were statistically at par with each other. Whereas, the growth of *B.bassiana* in the remaining additives was below 50 mm and lowest was recorded in Pongamia oil (T_8) (12.94 mm).

4.3.1.1.d. Mean

The results revealed that the differences in the mean growth of *B.bassiana* among different additives was significant. Control registered maximum growth of 83.95 mm. However, among the additives, CMC (T_{12}) recorded maximum growth (77.16 mm), followed by Kaolite (T_{10}) (69.07 mm), Silica gel (T_{11}) (65.20 mm) and Sunflower oil (T_9) (55.13 mm), respectively, but they differed significantly from each other. The next effective additive was Glycerol (T_5) (51.83 mm), followed by Tween-80 (T_4) (50.20 mm), but both were at par with each other. Whereas, the growth of *B.bassiana* in the remaining additives was below 50 mm and lowest was recorded in Pongamia oil (T_8) (20.98 mm).

4.3.1.2. Growth inhibition

The data on effects of additives on the growth inhibition of *B. bassiana*, ten days after inoculation are presented in Table 31 and depicted in Figure 9.

4.3.1.2.a. At 0.1% concentration

The results revealed that there was significant difference in the percent growth inhibition of *B. bassiana* among different additives at 0.1% concentration. Treatment CMC (T_{12}) showed minimum growth inhibition (12.69%) and was significantly superior than the other additives. This was followed by Kaolite (T_{10}) (20.28%), Silica gel (T_{11}) (22.25%), but were at par with each other. The next effective additive was Sunflower oil (T_9)

(23.48%), but did not differ significantly with T_{11}. While the remaining additives inhibited more than 35% growth of *B. bassiana*, and maximum inhibition was recorded in Pongamia oil (T_8) (69.02%).

4.3.1.2.b. At 0.5% concentration

The results revealed that there was significant difference in percent growth inhibition of *B.bassiana* among different additives at 0.5% concentration. Treatment CMC (T_{12}) showed minimum growth inhibition (8.28%), followed by Kaolite (T_{10}) (11.97%) and Silica gel (T_{11}) (18.52%), but they differed significantly with each other. The next effective additive was Sunflower oil (T_9) (33.98%), followed by Glycerol (T_5) (36.87%), but they did not differ significantly from each other. While the remaining additives inhibited more than 35% growth of *B.bassiana* and maximum inhibition was recorded in Triton-X (T_6) (71.73%).

4.3.1.2.c. At 1.0 % concentration

The results revealed that there was significant difference in percent growth inhibition of *B.bassiana* among different additives at 1.0% concentration. Treatment CMC (T_{12}) showed minimum growth inhibition (3.13%), followed by Kaolite (T_{10}) (20.69%) and Silica gel (T_{11}) (25.98%), but they differed significantly with each other. The next effective additive was Glycerol (T_5) (40.08%), followed by Tween-80 (T_9) (41.58%), but they were statistically at par with each other. While the remaining additives inhibited more than 45% growth of *B.bassiana* and maximum inhibition was recorded in Pongamia oil (T_8) (84.54%).

4.3.2.d. Mean

The results revealed that there was significant difference in percent growth inhibition of *B.bassiana* among different additives. Treatment CMC (T_{12}) showed minimum growth inhibition (8.03%), followed by Kaolite (T_{10}) (17.65%) and Silica gel (T_{11}) (22.25%), but they differed significantly from each other. The next effective additive was Sunflower oil (T_9) (34.29%), followed by Glycerol (T_5) (38.22%), but they were at par with each other. While the remaining additives inhibited more than 35% growth of *B.bassiana* and maximum inhibition was recorded in Pongamia oil (T_8) (74.98%).

4.3.1.3. Mean spore count

Data on sporulation of *B. bassiana* in media treated with additives are presented in Table 31 and depicted in Figure 9.

4.3.1.3 a. At 0.1% concentration

Perusal of the data revealed that differences in the mean spore count of *B.bassiana* at 0.1% concentration was significant. Highest mean spore count was recorded in Control (T_{13}) (5.33 x 10^9 spores ml^{-1}), followed by CMC (T_{12}) (4.67 x 10^9 spores ml^{-1}), but were at par with each other. The next effective additive was Tween-80 (T_4) (4.00 x 10^9 spores ml^{-1}). This was followed by Glycerol (T_5), Neem oil (T_7) (both recorded 3.00 x 10^9 spores ml^{-1}), Pongamia oil (T_8), Silica gel (T_{11}) and Kaolite (T_{10}) (all three registered 2.67 x 10^9 spores ml^{-1}) and Tween-60 (T_3) (2.33 x 10^9 spores ml^{-1}), respectively but they did not differ significantly from each other. The mean spore count recorded in the remaining additives was less than 2 x 10^9 spores ml^{-1}.

4.3.1. 2.b. At 0.5% concentration

Perusal of the data revealed that differences in the mean spore count of *B.bassiana* at 0.5% concentration was significant. Highest mean spore count was recorded in CMC (T_{12}) (5.67 x 10^9 spores ml^{-1}), followed by Control (T_{13}) (5.33 x 10^9 spores ml^{-1}), but were at par with each other. The next effective additive was Tween-80 (T_4) (4.33 x 10^9 spores ml^{-1}), followed by Kaolite (T_{10}) (3.67x10^9 spores ml^{-1}), but they did not differ significantly from each other. The next group of additives were Glycerol (T_5) and Silica gel (T_{11}) (both registered 3.33 x 10^9 spores ml^{-1}), followed by Neem oil (T_7) (3.00 x 10^9 spores ml^{-1}), respectively, but were at par with each other. The mean spore count recorded in the remaining additives was less than 2.50 x 10^9 spores ml^{-1}.

4.3.2.c. At 1.0 % concentration

Perusal of the data revealed that differences in the mean spore count of *B.bassiana* at 1.0% concentration was significant. Highest mean spore count was recorded in Control (T_{13}) (4.67 x 10^9 spores ml^{-1}), followed by CMC (T_{12}) (3.67 x 10^9 spores ml^{-1}), Kaolite (T_{10}) (2.33 x 10^9 spores ml^{-1}),

but they differed significantly from each other. Spore count recorded in the remaining additives was less than 1.50 x 10^9 spores ml^{-1}.

4.3.1.2.d. Mean

Perusal of the data revealed that differences in the mean spore count of *B.bassiana* was significant. Highest mean spore count was recorded in Control (T_{13}) (5.22 x10^9 spores ml^{-1}), followed by CMC (T_{12}) (4.67 x 10^9 spores ml^{-1}), but they did not differ significantly from each other. The next effective additive was Tween-80 (T_4) (3.44 x 10^9 spores ml^{-1}), followed by Kaolite (T_{10}) (2.89 x 10^9 spores ml^{-1}) and Glycerol (T_5) (2.78 x10^9 spores ml^{-1}), but they were at par with each other. Spore count recorded in the remaining additives was less than 2.50 x 10^9 spores ml^{-1}.

4.3.3.e. Impact of additives and its concentrations on *B. bassiana*

Perusal of data in Table 31 revealed that the additives, concentrations and their interaction had significant impact on the radial growth and growth inhibition of *B.bassiana*. Further, additives and concentrations had significant effect on spore load, but there interaction was found to be non-significant.

4.4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulations against insect-pest complex on tomato under protected conditions

4.4.a Shelf life studies of *B. bassiana* formulations

The shelf life of a microbial pesticide is an important factor for insect control. Virulent isolates with prolonged shelf life are very helpful that can be attained by preparing different formulations. Three formulations *i.e.* wettable powder, talc based and aqueous formulation of *B.bassiana* were prepared and stored under ambient conditions for a period of 13 months. Observations on spore count and spore viability at concentrations of 1x10^8 and 1x10^9 spores g^{-1}/ ml^{-1} from all the three formulations were recorded at monthly interval. The data are presented in Tables 32 and 33 and Figures 10,11 and 12.

4.4.a.i. Effect of storage period of *B.bassiana* on spore count and viability at 1 x 10^8 spores g^{-1}/ ml^{-1} (Figures 10,11 and 12)

4.4.a.i.1. Wettable Powder formulation

a) Spore count

Perusal of data in Table 32 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month, T_1), the spore count was highest (8.22 x 10^8 spores g^{-1}). There was a gradual decrease in the spore count *viz.* 8.02, 7.95, 7.61 and 7.25 x 10^8 spores ml^{-1} after 1(T_2), 2 (T_3), 3 (T_4) and 4 months (T_5) of storage, however all were at par with T_1, but significantly superior than the remaining storage periods (*i.e.* T_6 toT_{13}).The spore count continued to decrease and was 7.00, 6.67 and 6.04 x 10^8 spores g^{-1} after 5 (T_6), 6 (T_7) and 7 months (T_8) of storage, respectively, but they did not differ significantly from each other. After 7 months of storage, there was a significant decrease in the spore count and was found to be minimum after 12 months of storage (0.50 x 10^8 spores g^{-1}), while no spores were observed after 13 months of storage.

Data presented in Table 32 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage (T_2), the reduction in the spore count was minimum (0.88%), followed by 2 month of storage (T_3) (1.69%) and both were at par with each other. After 2 months of storage, there was gradual increase in the reduction 1) and 5 (T_6) months of storage, respectively but non-significant differences were observed between them. After 5 months of storage, there was a significant increase in the reduction of spore count and was found to be maximum after 12 months of storage (92.46%).

b) Spore viability

Perusal of data in Table 32 revealed that the differences in the spore viability over storage period was significant. Highest viable spores (7.67 x 10^8 spores g^{-1}) was recorded on the initial day of storage (T_1). With the increase in the storage period, there was a gradual decrease in the spore viability and it was 7.33, 7.18, 6.67, 6.33, 6.00 and 5.67 x 10^8 spores g^{-1} after 1 (T_2), 2 (T_3), 3 (T_4), 4 (T_5), 5 (T_6) and 6 months (T_7) of storage, respectively but all were at par with each other. After 7 months of storage, the spore viability was 4.67 x

10^8 spores g^1 there after a significant decrease in the spore viability was observed and was found to be minimum (0.67 x 10^8 spores g^{-1}) after 10 months of storage. While no viable spores were observed after 11 months of storage.

Data presented in Table 32 revealed that the differences in the reduction in the spore viability over storage period was significant. No reduction in spore viability was observed after 1 month of storage. However after 2 months (T_3) (13.10%), there was a decrease in the spore viability and was 13.10%, 17.26%, 12.50% and 17.26% after 3, 4, 5 and 6 months of storage, respectively, but all were at par with each other. After 7 months of storage, the spore reduction was 38.69% and it was found to be maximum (90.48%) after 10 months of storage.

4.4.a.i.2. Talc formulation

a) Spore count

Perusal of data in Table 32 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month, T_1), the spore count was highest (6.29 x10^8 spores g^{-1}). There was a gradual decrease in the spore count *viz.* 5.95 and 5.23 x 10^8 spores g^{-1} after 1(T_2) and 2 months of storage (T_3), but both were at par with T_1, but significantly superior than the rest of the storage periods (*i.e.* T_4 to T_7). The spore count continued to decrease and was 4.22 x 10^8 spores g^{-1} after 3 months of storage (T_4). After 4 months of storage (T_5),there was significant decrease in the spore count and was found to be minimum after 7 months of storage (T_8) (0.67 x 10^8 spores g^{-1}), while no spores were observed after 8 months of storage (T_9).

Data presented in Table 32 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage (T_2), the reduction in the spore count was minimum (3.69%), followed by 2 (T_3) and 3 months of storage (T_4) with 15.28% and 31.71% reduction in spore count, but they did not differ significantly from each. There was a gradual reduction in the spore count *viz.* 51.71% and 62.77% after 4 (T_5) and 5 months of storage (T_6), respectively but were at par

with each other. After 5 months of storage (T_6), there was a significant increase in the reduction of spore count and was found to be maximum after 7 months of storage (T_8) (89.36%).

b) Spore viability

Perusal of data in Table 32 revealed that the differences in the spore viability over storage period was significant. Highest viable spores (5.67 x 10^8 spores g^{-1}) was recorded at initial day of storage (T_1), followed by 1 month of storage (T_2) (5.33 x 10^8 spores g^{-1}), but both were at par with each other. With the increase in the storage period, there was a gradual decrease in the spore viability after 2 months of storage (T_3) (4.67 x10^8 spores g^{-1}) and was found to be minimum after 5 months of storage (T_6) (0.67 x 10^8 spores g^{-1}). While no viable spores were observed after 6 months of storage (T_7).

Data presented in Table 32 revealed that the differences in the reduction in the spore viability over storage period was significant. Reduction in spore viability after 1 month of storage (T_2) was 5.56%. However, after 2 months (T_3) (17.78%), there was an increase in the reduction in the spore viability and it was found to be maximum (70.00%) after 5 months of storage (T_6).

4.4.a.i.3. Aqueous formulation

a) Spore count

Perusal of data in Table 32 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month,T_1), the spore count was highest (7.96 x 10^8 spores ml^{-1}). There was a gradual decrease in the spore count *viz.* 7.83, 7.43, 7.34 and 7.26 x 10^8 spores ml^{-1} after 1 (T_2), 2 (T_3), 3 (T_4) and 4 months of storage (T_5), however they were statistically significant with T_1, but significantly superior than the remaining storage period (*i.e.*T_6 toT_{11}).The spore count continued to decrease and was 6.11 and 5.63 x 10^8 spores ml^{-1} after 5 (T_6) and 6 months of storage (T_7), respectively, but they did not differ significantly from each other. After 7 months of storage (T_8) there was a significant decrease in the spore count and was found to be minimum after 10 months of storage (T_{11}) (1.11 x 10^8 spores ml^{-1}), while no viable spores were observed after 11

months of storage (T_{12}).

Data presented in Table 32 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage (T_2) , the reduction in the spore was minimum (1.01%), followed by 2 (T_3) and 3 months of storage (T_4)(5.47% and 6.59%, respectively), but were at par with each other. Spore reduction after 4 months of storage (T_5) was 7.45%. After 5 months of storage (T_6), there was a gradual increase in the reduction of spore count and was found to be maximum after 10 months of storage (T_{11}) (85.88%).

b) Spore viability

Perusal of data in Table 32 revealed that the differences in the spore viability over storage period was significant. Highest viable spores (7.67 x 10^8 spores ml^{-1}) was recorded at initial day of storage (T_1). With the increase in the storage period, there was a gradual decrease in the spore viability and it was 7.33, 7.00 and 7.00 x 10^8 spores ml^{-1} after 1(T_2), 2 (T_3) and 3 months of storage (T_4), respectively but all were at par with each other. After 4 months of storage (T_5), the spore viability was 6.33 x 10^8 spores ml^{-1} and it decreased significantly and was found to be minimum (0.33 x 10^8 spores ml^{-1}) after 8 months of storage (T_9). While no viable spores were observed after 9 months of storage (T_{10}).

Data presented in Table 32 revealed that the differences in the reduction in the spore viability over storage period was significant. Reduction in spore viability after 1 month of storage (T_2) was 4.17%. However, after 2 months (T_3) (8.33%), there was an increase in reduction in the spore viability and was 8.33% and 16.67% after 3 (T_4) and 4 months of storage (T_5), respectively, but both were at par with each other. After 5 months of storage (T_6), the spore reduction was 25.60% and it was found to be maximum (95.83%) after 8 months of storage (T_9).

4.4.a.ii. Effect of storage period of *B. bassiana* on spore count and viability at 1 x 10^9 spores g^{-1}/ml^{-1}

4.4.a.ii.1. Wettable Powder formulation

a) Spore count

Perusal of data in Table 33 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month, T_1), the spore count was highest (7.65 x10^9 spores g^{-1}). There was a gradual decrease in the spore count *viz.* 6.82, 6.57 and 5.78 x 10^9 spores g^{-1} after 1 (T_2), 2 (T_3) and 3 months of storage (T_4), respectively but all were at par with T_1, but significantly superior than the rest of the storage periods (*i.e.* T_5 toT_{10}).The spore count continued to decrease and was 5.24 and 4.21 x 10^9 spores g^{-1} after 4 (T_5) and 5 months of storage (T_6), respectively, but they did not differ significantly from each other. After 6 months of storage (T_7), there was a significant decrease in the spore count and was found to be minimum after 9 months of storage (T_{10}) (0. 99 x 10^9 spores g^{-1}), while no spores were observed after 10 months of storage (T_{11}).

Data presented in Table 33 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage (T_2), the reduction in the spore count was minimum (0.46%) followed by 2 months of storage (T_3) (4.46%) and both were at par with each other. After 2 months of storage, there was a gradual reduction in the spore count and was 13.16% and 16.98% after 3 (T_4) and 4 months of storage (T_5), respectively but non-significant differences were observed between them. After 5 months of storage (T_6), there was a significant increase in the reduction of spore count and was found to be maximum after 9 months of storage (T_{10}) (98.36%).

b) Spore viability

Perusal of data in Table 33 revealed that the differences in the spore viability over storage period was significant. Highest viable spores (7.05 x 10^9 spores g^{-1}) was recorded at initial day of storage (T_1). With increase in the storage period, there was a gradual decrease in the spore viability and was 6.62, 6.62 and 5.51 x 10^9 spores g^{-1} after 1(T_2), 2(T_3) and 3 months of storage (T_4), respectively but were at par with each other. After 4 (T_5) and 5 months of storage (T_6), the spore viability was 4.67 and 4.24x10^9 spores g^{-1}, respectively

and both were at par with each other. After 6 months of storage (T_7), the spore viability was 2.77 x 10^9 spores g^{-1} and it decreased significantly and was

found to be minimum (0.67 x 10^9 spores g^{-1}) after 9 months of storage (T_{10}), while no viable spores were observed after 10 months of storage (T_{11}).

Data presented in Table 33 revealed that the differences in the reduction in the spore viability over storage period was significant. There was no reduction in spore viability after 1 month of storage (T_2). However, after 2 months (T_3) there was an increase in the reduction of spore viability and it was 16.03%, followed by 3 months of storage (T_4) (20.62%), but both were at par with each other. Further, there was an increase in the reduction of spore viability and was 24.18%, 46.32% and 69.77% after 4(T_5), 5(T_6) and 6 months of storage (T_7), but they differed significantly from each other. After 7 months of storage (T_8), the spore reduction was 74.14% and was found to be maximum after 9 months of storage (T_{10}) (89.94%).

4.4.a.ii.2. Talc formulation

a) Spore count

Perusal of data in Table 33 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month, T_1), the spore count was highest (5.8 x10^9 spores g^{-1}). There was a gradual decrease in the spore count *viz.* 5.31 and 4.28 x 10^9 spores g^{-1} after 1(T_2) and 2 months of storage (T_3), respectively but both were at par with T_1, but significantly superior than the remaining storage periods (*i.e.* T_4 toT_7). After 3 months of storage (T_4), there was a significant decrease in the spore count and was minimum after 6 months of storage (T_7) (0.41 x 10^9 spores g^{-1}), while no spores were observed after 7 months of storage (T_8).

Data presented in Table 33 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage (T_2), the reduction in the spore was minimum (7.13%), followed by 2 months of storage (T_3)(18.10%) but both were at par with each other. After 3 months of storage (T_4), there was a significant increase in the reduction of spore count and was found to be maximum after 5 months of storage (T_6) (89.36%).

b) Spore viability

Perusal of data in Table 33 revealed that the differences in the spore

viability over storage period was significant. Highest viable spores (5.41 x 10^9 spores g^{-1}) was recorded at initial day of storage (T_1). With the increase in the storage period, there was a gradual decrease in the spore viability 4.85 and 4.09 x 10^9 spores g^{-1} after 1 (T_2) and 2 months of storage (T_3) respectively, but all were at par with each other. After 3 months of storage (T_4), the spore viability was 2.50 x 10^9 spores g^{-1}, it decreased significantly and was found to be minimum (0.33 x 10^9 spores g^{-1}) after 5 months of storage (T_6). While no viable spores were observed after 6 months of storage (T_7).

Data presented in Table 33 revealed that the differences in the reduction in the spore viability over storage period was significant. Reduction in spore viability after 1 month of storage (T_2) was 15.30%. However, after 2 months (T_3) (20.71%), there was an increase in the reduction in the spore viability and was found to be maximum after 5 months of storage (T_6) (82.11%).

4.4.a.ii.3. Aqueous formulation

a) Spore count

Perusal of data in Table 33 revealed that the differences in the spore count over storage period was significant. Initially, during the start of the experiment (0 month,T_1), the spore count was highest (6.99 x10^9 spores ml^{-1}). There was a gradual decrease in the spore count after 1 (T_2) and 2 months after storage (T_3) with 6.82 and 5.51x10^9 spores ml^{-1}, respectively, but all were at par with T_1, but significantly superior than the rest of the storage periods (*i.e.* T_4 toT_8). The spore count continued to decrease and was 3.95 and 3.38 x 10^9 spores ml^{-1} after 3 (T_4) and 4 months of storage (T_5), respectively but they did not differ significantly from each other. After 4 months of storage, there was a significant decrease in the spore count and was found to be minimum after 7 months of storage(T_8) (0.33 x 10^9 spores ml^{-1}). While no spore count was recorded after 8 months of storage (T_9) .

Data presented in Table 33 revealed that the differences in the reduction in the spore count over storage period was significant. After 1 month of storage, the reduction in the spore count was minimum (T_2) (7.05%), followed by 2 months of storage (T_3)(12.78%) and both were at par with each other. After 3 months of storage (T_4), there was a significant increase in the

reduction in spore count and was found to be maximum after 7 months of storage (T_8) (97.08%).

b) Spore viability

Perusal of data in Table 33 revealed that the differences in the spore viability over storage period was significant. Highest viable spores (6.74 x 10^9 spores ml^{-1}) was recorded at initial day of storage followed by 1 month of storage (T_2) (6.18 x 10^9 spores ml^{-1}), but both were at par with each other. There was a gradual decrease in the spore viability after 2 (T_3), 3 (T_4) and 4 months of storage (T_5) (5.33, 3.78 and 3.18 x 10^9 spores ml^{-1}, respectively), but they differed significantly from each other. After 5 months of storage (T_6) (1.33 x 10^9 spores ml^{-1}), there was a significant decrease in the spore viability and was found to be minimum (0.67 x 10^9 spores ml^{-1}) after 7 months of storage (T_8), however no viable spores were observed after 8 months of storage (T_9).

Data presented in Table 33 revealed that the differences in the reduction in the spore viability over storage period was significant. Reduction in spore viability after 1 month of storage (T_2) was 16.57%, followed by 2 months (T_3) (27.93%), but both were at par with each other. However, after 3 months of storage (T_4) (43.84%), there was an increase in the reduction in the spore viability and was 62.95% after 4 months of storage (T_5), but both were at par with each other. After 5 months of storage (T_6), the spore reduction was 72.61% and was found to be maximum after 6 months of storage (T_7) (87.37%).

4.4.a.iii. Effect of storage period on the virulence of *B.bassiana*

The shelf life of a microbial pesticide and its virulence are important factor for insect control. The wettable, talc and aqueous formulations of *B. bassiana* were stored in air tight bottles at room temperature for 360 days. Virulence studies were carried out against 2nd instar larvae of *Helicoverpa armigera* under *in-vitro* conditions at three months interval.

Observations on insect mortality during each spray was recorded at 24, 48, 72, 96 and 120 hours after treatment. However, maximum mortality of the

test insect was observed at 120 hours after spray, analysed data of the same is presented in Table 34.

Period of storage

0 days

Bioefficacy studies of freshly prepared *B.bassiana* formulations at $1x10^8$ spores ml^{-1} against *H.armigera* larvae revealed that the differences in the larval mortality was found to be significant. Among the treatments, WP formulation (T_1) was most effective with highest larval mortality (95.00%), followed by aqueous formulation (T_3) (90.00%), but both the treatments were at par with each other. The next effective treatment was talc formulation (T_2) (80.00%), while in control (T_4) mortality recorded was 5.00%.

Mortality of *H.armigera* larvae with freshly prepared *B.bassiana* formulations at $1x10^9$ spores ml^{-1} concentration was found to be significant. Among the treatments, WP formulation (T_1) was most effective with hundred percent larval mortality, followed by aqueous formulation (T_3) (90.00%), but they did not differ significantly from each. The next effective treatment was talc formulation (T_2) (80.00%), while control (T_4) recorded a mortality of 5.00%.

90 days

Evaluation of 90 days old *B.bassiana* formulations at $1x10^8$ spores ml^{-1} against *H.armigera* larvae revealed that the differences in the larval mortality was found to be significant. Among the treatments, WP formulation (T_1) was most effective with highest larval mortality (80.00%), followed by aqueous formulation (T_3) (77.50%), but both were at par with each other. The next effective treatment was talc formulation (T_2) (60.00%), while in control (T_4) recorded was 7.50% mortality .

Mortality of *H.armigera* larvae with 90 days old *B.bassiana* formulations at $1x10^9$ spores ml^{-1} concentration was found to be significant. Among the treatments, WP formulation (T_1) was most effective as it recorded 82.50% larval mortality, followed by aqueous formulation (T_3) (80.00%), but there was no significant difference among the treatments. The next effective treatment was talc formulation (T_2) (62.50%), while lowest mortality of 7.50% was recorded in control (T_4).

180 days

Bioefficacy studies of 180 days old *B.bassiana* formulations at $1x10^8$ spores ml^{-1} against *H.armigera* larvae revealed that the differences in the larval mortality was found to be significant. Among the treatments, WP formulation (T_1) was most effective with highest larval mortality (70.00%), followed by aqueous formulation (T_3) (47.50%) and talc formulation (T_2) (32.50%), respectively, but all were significantly different from each other, while in control (T_4) mortality recorded was 10.00%.

Mortality of *H.armigera* larvae with 180 days old *B.bassiana* formulations at $1x10^9$ spores ml^{-1} concentration was found to be significant. Among the treatments, WP formulation (T_1) recorded highest larval mortality (72.50%), followed by aqueous formulation (T_3) (62.50%), but both the treatments did not differ significantly from each other. Talc was the least effective formulation (T_2) (37.50%), while control (T_4) recorded 10.00% mortality.

270 days

Evaluation of 270 days old *B.bassiana* formulations at $1x10^8$ spores ml^{-1} against *H.armigera* larvae revealed that the differences in the larval mortality was found to be significant. Among the treatments, WP formulation (T_1) was most effective with highest larval mortality (47.50%), followed by aqueous formulation (T_3) (22.50%), but both were significantly different from each other. Lowest larval mortality was recorded in control (T_4) (5.00%) and talc formulation (T_2) with 0.00% mortality.

Mortality of *H.armigera* larvae with 270 days old *B.bassiana* formulations at $1x10^9$ spores ml^{-1} concentration was found to be significant. Among the treatments, WP formulation (T_1) was most effective with highest larval mortality (50.00%), followed by aqueous formulation (T_3) (25.00%), but they differed significantly from each other. Lowest larval mortality was recorded both in control (T_4) (5.00%) and talc formulation (T_2) with 0.00% mortality .

360 days

Bioefficacy studies of 360 days old *B.bassiana* formulations at $1x10^8$ spores ml^{-1} and $1x10^9$ spores ml^{-1} against *H.armigera* larvae revealed that the differences in the larval mortality at both spore concentrations were found to be significant. But, no larval mortality was recorded in any of the tested formulations and concentrations as no viable spores were observed after 360 days of storage, while under natural conditions control recorded a mortality of 7.50%.

4.4.b Evaluate the effective formulations against insect - pest complex on tomato under protected conditions

The data on bioefficacy of different *B.bassiana* formulations against insect-pest complex on tomato under protected conditions are presented in Tables 35 to 39.

4.4.b.1. (i) Tomato fruit borer, *Helicoverpa armigera*

Data presented in Table 35

Pre- treatment

Differences in the *H.armigera* mean larval population per plant among different treatments were found to be not significant during 2016-17 and 2017-18, indicating more or less uniform distribution of the pest in the experimental plots.

Mean of five sprayings

a) Three days after spray

During 2016-17, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, Wettable Powder (WP) formulation @ $2x10^9$ spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.38 larvae / plant), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1.52 larvae / plant), but there was non-significant difference among these treatments. The next effective treatment was WP formulation @ $2x10^8$ spores g^{-1} (T_1) (1.54 larvae / plant), followed by aqueous formulation @ $2x10^8$ spores ml^{-1} (T_3) (1.62 larvae / plant), but were at par with each other. However, highest larval population was recorded in control (T_5) (2.29 larvae / plant).

During 2017-18 , the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.78 larvae/ plant), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1.90 larvae/ plant), but they did not differ significantly from each other. The next effective treatment was WP formulation @ 2 $x10^8$ spores g^{-1} (T_1) (2.13 larvae/ plant), followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.16 larvae / plant), but were at par with each other. While highest larval population was observed in control (T_5) (3.19 larvae / plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.58 larvae/ plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.71 larvae / plant), but non-significant difference was observed between them. The next effective treatment was WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.83 larvae / plant), followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.89 larvae / plant), but were at par with each other. Whereas, highest mean larval population was observed in control (T_5) (2.74 larvae / plant).

b) Seven days after spray

During 2016-17, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.10 larvae / plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.29 larvae / plant), WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.42 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.56 larvae / plant), but there was significant difference among the treatments. However, highest larval population was observed in control (T_5) (2.47 larvae / plant).

During 2017-18 , the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.49 larvae / plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.65 larvae / plant), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.79 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.93 larvae / plant), but significant differences were observed between them treatments. However, highest larval population was recorded in control (T_5) (3.15 larvae / plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.29 larvae/ plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.47 larvae / plant), WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.60 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.74 larvae / plant), but they differed significantly from each other. Whereas, highest mean larval population was observed in control (T_5) (2.81 larvae / plant).

c) Ten days after spray

During 2016-17, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval mortality (1.13 larvae / plant). The next effective treatment was aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (1.40 larvae / plant), followed by WP formulation @ 2x10^8 spores g^{-1} (T_1) (1.49 larvae / plant), but both were at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.71 larvae / plant). However, highest larval population was recorded in control (T_5) (2.56 larvae / plant).

During 2017-18, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded

lowest larval population (1.51 larvae/ plant). The next effective treatment was aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1.77 larvae / plant), followed by WP formulation @ $2x10^8$ spores g^{-1} (T_1) (1.85 larvae / plant), but both were at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.99 larvae / plant). However, highest larval population was observed in control (T_5) (3.20 larvae / plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.32 larvae/ plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.59 larvae / plant), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.67 larvae / plant), but both were statistically at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) with mean larval population of 1.85 larvae / plant. While, highest mean larval population was recorded in control (T_5) (2.81 larvae / plant).

d) Overall mean

Perusal of the overall mean data of first year (2016-17), presented in Table 35 revealed that there was significant difference in the mean larval population among the treatments. Treatment, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.79 larvae/ plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.91 larvae / plant), WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (2.09 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.10 larvae / plant), but non-significant differences were observed between them. While, highest mean larval population was recorded in control (T_5) (2.77 larvae/ plant).

Perusal of the overall mean data of second year (2017-18), presented in Table 35 revealed that there was significant difference in the mean larval population among the treatments. Treatment, WP formulation @ 2 x 10^9

spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.78 larvae/ plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.90 larvae / plant), but both differed significantly from each other. The next effective treatment was WP formulation @ 2 x10^8 spores g^{-1} (T_1) (2.13 larvae / plant), followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.16 larvae / plant), but both were at par with each other. However, highest mean larval population was observed in control (T_5) (3.19 larvae/ plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.79 larvae / plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.90 larvae / plant), but they did not differ significantly from each other. The next effective treatment was WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (2.11 larvae / plant) followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.13 larvae / plant), but were at par with each other. However, highest larval population was recorded in control (T_5) (2.98 larvae / plant).

4.4.b.1. (ii) South American tomato leaf miner, *Tuta absoluta*

Data presented in Table 36

Pre- treatment

Differences in the *T.absoluta* mean larval population per plant among different treatments were found to be not significant during 2016-17 and 2017-18, indicating more or less uniform distribution of the pest in the experimental plots.

Mean of five sprayings

a) Three days after spray

During 2016-17, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) was most effective as it registered lowest larval population (1.29 larvae / plant). The next effective treatment was

aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.78 larvae / plant), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.96 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.05 larvae / plant), but all were at par with each other, while highest larval population was observed in control (T_5) (3.11 larvae / plant).

During 2017-18 , the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.61 larvae / plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.84 larvae / plant), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.91 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.97 larvae / plant), but all were at par with each other. However, highest larval population was recorded in control (T_5) (2.66 larvae/ plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it registered lowest larval population (1.45 larvae / plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.81 larvae / plant), followed by WP formulation @ 2 x10^8 spores g^{-1} (T_1) (1.93 larvae / plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.01 larvae / plant), respectively but they did not differ significantly from each other. While highest larval population was observed in control (T_5) (2.87 larvae / plant).

b) Seven days after spray

During 2016-17, differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.28 larvae/ plant). The next effective treatment was WP formulation @ 2x10^8 spores g^{-1} (T_1) (1.50 larvae / plant), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.55 larvae/ plant) and aqueous

formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.82 larvae / plant), respectively but all were at par with each other. However, highest larval population was observed in control (T_5) (3.11 larvae/ plant).

During 2017-18, differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) recorded lowest larval population (1.35 larvae/ plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.60 larvae / plant) and WP formulation @ 2x10^8 spores g^{-1} (T_1) (1.77 larvae / plant), but non-significant differences were observed among between them. The next effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.81 larvae / plant), followed by control (T_5) (3.10 larvae/ plant), but they differed significantly from each other.

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it registered lowest larval population (1.24 larvae/ plant). The next effective treatment was aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (1.57 larvae / plant), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.61 larvae/ plant) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.82 larvae / plant), respectively, but were at par with each other. While highest larval population was in observed control (T_5) (3.06 larvae / plant).

c) Ten days after spray

During 2016-17, the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) was most effective as it registered lowest larval population (1.36 larvae/ plant), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (1.77 larvae / plant), WP formulation @ 2x10^8 spores g^{-1} (T_1) (1.82 larvae / plant), but all were at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3)

(2.05 larvae / plant). However, highest larval population was recorded in control (T_5) (4.39 larvae / plant).

During 2017-18 , the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.49 larvae/plant). The next effective treatment was aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1.81 larvae / plant), followed by WP formulation @ $2x10^8$ spores g^{-1} (T_1) (2.00 larvae / plant) and aqueous formulation @ $2x10^8$ spores ml^{-1} (T_3) (2.03 larvae / plant), but were at par with each other. While highest larval population was observed in control (T_5) (3.55 larvae / plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.42 larvae/ plant), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1.79 larvae / plant), but both were at par with each other. The next effective treatment was WP formulation @ $2x10^8$ spores g^{-1} (T_1) (1.91 larvae / plant), followed by aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (2.04 larvae / plant), but they did not differ significantly from each other. While highest larval population was recorded in control (T_5) (3.97 larvae / plant).

d) Overall mean

Perusal of the overall mean data of first year (2016-17), presented in Table 36 revealed that there was significant difference in the mean larval population among the treatments. Treatment, WP formulation @ 2×10^9 spores g^{-1} (T_2) recorded lowest larval population (1.29 larvae / plant), followed by aqueous formulation @ 2×10^9 spores ml^{-1} (T_4) (1.67 larvae /plant) and WP formulation @ 2×10^8 spores g^{-1} (T_1) (1.69 larvae / plant), but all were at par with each other. The least effective treatment was aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (1.87 larvae / plant). While, highest larval population was recorded in control (T_5) (3.07 larvae / plant).

Perusal of the overall mean data of second year (2017-18), presented in Table 36 revealed that there was significant difference in the mean larval population among the treatments. Treatment, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it recorded lowest larval population (1.49 larvae/ plant), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (1.70 larvae / plant) and WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.80 larvae / plant), but all were at par with each other. Aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) was the least effective formulation (1.89 larvae / plant), while highest larval population was recorded in control (T_5) (2.81 larvae / plant).

Pooled

Pooled data of both the years revealed that the differences in the mean larval population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) recorded lowest larval population (1.39 larvae / plant), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (1.68 larvae / plant) and WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (1.74 larvae/ plant), but all were at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (1.88 larvae / plant). However, highest mean larval population was observed in control (T_5) (2.94 larvae / plant).

4.4.b.1. (iii) Whitefly, *Bemisia tabaci*

The data is presented in Table 37

Pre- treatment

Differences in the *B.tabaci* mean nymphal population per 27cm^2 leaf area among different treatments were found to be not significant during 2016-17 and 2017-18, indicating more or less uniform distribution of the pest in the experimental plots.

Mean of five sprayings

a) Three days after spray

During 2016-17, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) was most effective as it

registered lowest nymphal population (26.50 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (26.91 nymphs / 27cm^2 leaf area), WP formulation @ 2x10^8 spores g^{-1} (T_1) (28.25 nymphs / 27cm^2 leaf area) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (28.96 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. While, highest nymphal population was recorded in control (T_5) (30.87 nymphs / 27cm^2 leaf area).

During 2017-18, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) was most effective as it recorded lowest nymphal population (26.22 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2 x10^9 spores g^{-1} (T_2) (26.37 nymphs / 27cm^2 leaf area) and WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (27.69 nymphs / 27cm^2 leaf area), but all were at par with each other. The nymphal population recorded in aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) was 28.92 nymphs/ 27cm^2 leaf area and it was highest in control (T_5) (30.61 nymphs/ 27cm^2 leaf area), but both were at par with each other.

Pooled

Pooled data of both the years revealed that the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as it registered lowest nymphal population (26.44 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (27.57 nymphs / 27cm^2 leaf area) and WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (27.97 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. Nymphal population recorded in aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) was 28.94 nymphs / 27cm^2 leaf area and it was highest in control (T_5) (30.74 nymphs / 27cm^2 leaf area), but both were at par with each other.

b) Seven days after spray

During 2017-18, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} (T_2) was most effective as

it registered with lowest nymphal population (25.88 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (27.69 nymphs / 27cm^2 leaf area) and WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (27.69 nymphs / 27cm^2 leaf area), but all were at par with each other. The least effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (28.24 nymphs / 27cm^2 leaf area). However, highest nymphal population was recorded in control (T_5) (30.71 nymphs / 27cm^2 leaf area).

During 2017-18, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) as it recorded lowest nymphal population (24.80 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2x10^8 spores g^{-1} (T_1) (26.76 nymphs / 27cm^2 leaf area) and aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (26.77 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. Nymphal population recorded in aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) was 27.75 nymphs / 27cm^2 leaf area while it was highest in control (T_5) (30.52 nymphs / 27cm^2 leaf area), but both differed significantly from each other.

Pooled

Pooled data of both the years revealed that the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2x10^9 spores g^{-1} (T_2) recorded lowest nymphal population (27.22 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2x10^8 spores g^{-1} (T_1) (27.22 nymphs / 27cm^2 leaf area) and aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (27.23 nymphs / 27cm^2 leaf area), but there was no significant difference among these treatments. Nymphal population recorded in aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) was 27.99 nymphs / 27cm^2 leaf area, followed by control (T_5) (30.62 nymphs / 27cm^2 leaf area), but both were at par with each other.

c) Ten days after spray

During 2016-17, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x10^9 spores g^{-1} (T_2) was most effective as

it recorded lowest nymphal population (25.85 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (26.06 nymphs / 27cm^2 leaf area), but both were at par with each other. The next effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (27.79 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (28.57 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. However, highest mean nymphal population was observed in control (T_5) (31.14 nymphs / 27cm^2 leaf area).

During 2017-18, the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x10^9 spores g^{-1} (T_2) was most effective as it recorded lowest nymphal population (25.37 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (25.92 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. The next effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (27.30 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2 x 10^8 spores g^{-1} (T_1) (27.86 nymphs / 27cm^2 leaf area), but both were at par with each other. While, highest mean nymphal population was recorded in control (T_5) (30.96 nymphs / 27cm^2 leaf area).

Pooled

Pooled data of both the years revealed that the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2 x10^9 spores g^{-1} (T_2) was most effective as it recorded lowest nymphal population (25.61 nymphs / 27cm^2 leaf area), followed by aqueous formulation @ 2x10^9 spores ml^{-1}(T_4) (26.06 nymphs / 27cm^2 leaf area), but they did not differ significantly from each other. The next effective treatment was aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (27.54 nymphs / 27cm^2 leaf area), followed by WP formulation @ 2 x10^8 spores g^{-1} (T_1) (27.99 nymphs / 27cm^2 leaf area), but both were at par with each other. However, highest mean nymphal population was recorded in control (T_5) (31.05 nymphs / 27 cm^2 leaf area).

d) Overall mean

During first year (2016-17), the differences in the overall mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} (T_2) was most effective as it recorded lowest nymphal population (27.34 nymphs / $27cm^2$ leaf area), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (28.03 nymphs / $27cm^2$ leaf area), but both were at par with each other. The next effective treatments were aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (28.79 nymphs / $27cm^2$ leaf area) and WP formulation @ $2x10^8$ spores g^{-1} (T_1) (29.00 nymphs / $27cm^2$ leaf area), but they did not differ significantly from each other. Highest nymphal population was recorded in control (T_5) (31.39 nymphs / $27cm^2$ leaf area).

During second year (2017-18), the differences in the overall mean nymphal population among different treatments were found to be significant. Treatment, WP formulation @ 2×10^9 spores g^{-1} (T_2) recorded lowest nymphal population (26.59 nymphs / $27cm^2$ leaf area), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (27.67 nymphs / $27cm^2$ leaf area), but both were at par with each other. The next effective treatment was WP formulation @ 2×10^8 spores g^{-1} (T_1) (28.35 nymphs / 27 cm^2 leaf area), followed by aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (28.46 nymphs / $27cm^2$ leaf area), but non-significant differences were observed between them. Highest nymphal population was recorded in control (T_5) (30.59 nymphs / $27cm^2$ leaf area).

Pooled

Pooled data of both the years revealed that the differences in the mean nymphal population among different treatments were found to be significant. Among the treatments, WP formulation @ 2×10^9 spores g^{-1} (T_2) was most effective as it recorded lowest nymphal population (26.52 nymphs / $27cm^2$ leaf area), followed by aqueous formulation @ 2×10^9 spores ml^{-1} (T_4) (27.72 nymphs / $27cm^2$ leaf area), but both were at par with each other. The next effective treatment was WP formulation @ 2×10^8 spores g^{-1} (T_1) (28.33 nymphs / $27cm^2$ leaf area), followed by aqueous formulation @ 2 x

10^8 spores ml^{-1} (T_3) (28.56 nymphs / 27cm^2 leaf area), but non-significant difference was observed between them. However, highest nymphal population was recorded in control (T_5) (30.78 nymphs / 27cm^2 leaf area).

4.4.b.2. Efficacy of *B.bassiana* formulations on tomato fruit damage

Data on fruit damage due to *H.armigera* and *T.absoluta* are presented in Table 38 and depicted in Plate 12 a,b.

4.4.b.2.1. Fruit damage

4.4.b.2.1.(i) *Helicoverpa armigera* (Plate 12 b (i))

Data on fruit damage due to *H.armigera* and *T.absoluta* are presented in Table 38.

During 2016-17, all the treatments significantly reduced the fruit damage by *H.armigera* as compared to control (13.18%). Among the treatments, WP formulation @ $2x10^9$ spores ml^{-1} (T_2) was found to be most effective as it recorded lowest fruit damage (2.41%), followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.56%), aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (3.12%) and WP formulation @ $2x10^8$ spores ml^{-1} (T_1) (3.22%), respectively but all the treatments were statistically at par with each other.

During 2017-18, all the treatments significantly reduced the fruit damage by *H.armigera* as compared to control (7.27%). Among the treatments, WP formulation @ $2x10^9$ spores ml^{-1} (T_2) recorded lowest fruit damage (1.52%), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (2.32 %), WP formulation @ $2x10^8$ spores ml^{-1} (T_1) (3.26 %) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (3.29%) respectively , but all the treatments were statistically at par with each other.

Pooled

All the treatments significantly reduced the fruit damage by *H.armigera* as compared to control (10.23%). Among the treatments, WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) recorded lowest fruit damage (1.96%), followed by aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (2.92%), aqueous formulation @ 2 x 10^9 spores ml^{-1}(T_4) (2.72%) and WP formulation @ 2 x 10^8 spores ml^{-1}

(T_1) (3.24%) respectively , but all the treatments were statistically at par with each other.

4.4.b.2.1.(ii) *Tuta absoluta* (Plate 12 b (ii), Plate 13)

During 2016-17, all the treatments significantly reduced the fruit damage by *T.absoluta* as compared to control (84.97%). Among the treatments, WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) was found to most effective as it recorded lowest fruit damage (26.65%), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (T_4) (29.15%) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (33.85 %), but all were at par with each other. The fruit damage recorded in WP formulation @ 2 x 10^8 spores ml^{-1} (T_1) (38.72%), was significantly inferior to all other treatments but was significantly superior to control.

During 2017-18, all the treatments significantly reduced the fruit damage by *T.absoluta* as compared to control (81.63%). Among the treatments, WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) recorded lowest fruit damage (24.68%), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (27.76%) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (29.71%), but all were statistically at par with each other. The fruit damage in WP formulation @ 2 x 10^8 spores ml^{-1} (T_1) (36.19%) was significantly inferior to all other treatments, but was significantly superior to control.

Pooled

All the treatments significantly reduced the fruit damage by *T.absoluta* as compared to control (83.30%). Among the treatments, WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) recorded lowest fruit damage (25.67%), followed by aqueous formulation @ 2 x10^9 spores ml^{-1} (T_4) (28.46 %) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) (31.78%), but all were at par with each other. The fruit damage recorded in WP formulation @ 2x10^8 spores ml^{-1} (T_1) was 37.45%, was significantly inferior to all other treatments, but was significantly superior to control.

4.5.Marketable fruit yield

During 2016-17, the marketable fruit yield of each plot was recorded and converted into t/ha. All the treatments registered significantly higher

marketable fruit yields as compared to the control (T_5) (37.94 t/ha). The highest fruit yield was recorded in WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) (60.45 t/ha), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (59.03 t/ha), but both the treatments were statistically at par with each other. The next effective treatments were, WP formulation @ 2x10^8 spores ml^{-1} (T_1) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) and they recorded fruit yield of 53.34 t/ha and 50.50 t/ha, respectively and both of them did not differ significantly from each other.

During 2017-18, all the treatments registered significantly higher marketable fruit yield as compared to the control (T_5) (38.25 t/ha). The highest fruit yield was recorded in WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) (62.51 t/ha), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (60.42 t/ha), but both the treatments were statistically at par with each other. The next effective treatments were WP formulation @ 2x10^8 spores ml^{-1} (T_1) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) and they recorded fruit yield of 56.95 t/ha and 54.17 t/ha, respectively, but non-significant differences were observed between them.

Pooled

All the treatments registered significantly higher marketable fruit yields as compared to the control (T_5) (38.10 t/ha). The highest fruit yield was recorded in WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) (61.48 t/ha), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (59.73 t/ha), but both the treatments were statistically at par with each other.The next effective treatments were WP formulation @ 2x10^8 spores ml^{-1} (T_1) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (T_3) and they recorded fruit yield of 55.15 t/ha and 52.34 t/ha, respectively, but non-significant differences were observed between them.

4.6. Economics of Treatments

4.6. (i) Increase in yield over control (Table 39)

Among the different treatments, highest increase in yield over control was registered in WP formulation @ 2 x 10^9 spores ml^{-1} (T_2) (23.39 t/ha). This was followed by aqueous formulation @ 2x10^9 spores ml^{-1} (T_4) (21.63 t/ha),

WP formulation @ $2x10^8$ spores ml^{-1} (T_1) (17.05 t/ha) and aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (14.24 t/ha), respectively.

4.6. (ii) Net profit (Table 39)

Among the different treatments, highest net profit was registered in case of WP formulation @ 2×10^9 spores ml^{-1} (T_2) (Rs 76946/ha), followed by aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (Rs 70239/ha), WP formulation @ $2x10^8$ spores ml^{-1} (T_1) (Rs 55473/ha) and aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (Rs 44533/ha), respectively.

4.6. (iii) Cost Benefit ratio (Table 39)

Among the different treatments, highest cost benefit ratio was obtained in WP formulation @ $2x10^9$ spores ml^{-1}(T_2) (1:4.99), followed by WP formulation @ $2x10^8$ spores ml^{-1} (T_1) (1:4.67), aqueous formulation @ $2x10^9$ spores ml^{-1} (T_4) (1:4.62) and aqueous formulation @ 2×10^8 spores ml^{-1} (T_3) (1:3.80), respectively.

DISCUSSION

The present investigation entitled **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"** was conducted to identify virulent *B. bassiana* isolate/s and to process the same in order to develop effective formulation for the management of tomato insect-pest complex. The findings are discussed in this chapter.

5.1 Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay

The virulence of fungal isolate and its application in appropriate dosage are predominant factors in determining the susceptibility of the insect pests. Hence, in the present study, seven isolates of *B.bassiana* were screened with five different spore concentrations ($1x10^5$ to $1x10^9$ spores ml^{-1}) against tomato insect pest complex *viz.* Fruit borer, *Helicoverpa armigera*; South American tomato leaf miner, *Tuta absoluta* and Whitefly, *Bemisia tabaci*.

a. *Helicoverpa armigera*

All the isolates of *B.bassiana* were found to be pathogenic against *H.armigera* second instar larvae. The present findings confirms the findings of Rathod (2002), Sridevi et al. (2004), Tyagi et al. (2010) and Qayyum et al. (2015).They also claimed pathogenicity of *B.bassiana* against *H.armigera*.

In the present study, out of the seven isolates of *B.bassiana* tested with five spore concentrations, isolate I-4 was identified as most virulent at highest spore concentration (1×10^9 spores ml^{-1}). All the isolates registered a gradual increase in the *H. armigera* larval mortality with increase in spore concentration and duration after application. The present findings are in agreement with the findings of Dhembare and Siddique (2004), Vinod and Chowdhry (2004), Gundannavar et al. (2006), Rijal et al. (2008), Prasad et al. (2010), Karkar et al. (2014), Mishra and Simon (2012), Prabhukarthikeya et al.

(2013) and Pandey and Das (2016). They also reported that *H.armigera* larval mortality increased with increase in the spore concentration.

At 24 hours after spray, the differences in the larval mortality among different *B. bassiana* isolates at spore concentration of $1x10^5$, $1x10^6$ and $1x 10^7$ spores ml^{-1} were not significant. However, it was found to be significant at spore concentration of $1x10^8$ and $1x 10^9$ spores ml^{-1}. At spore concentration of $1x10^8$ spores ml^{-1}, isolate I-4 recorded highest larval mortality (15.00%), followed by I-1 (10.00%), I-3 and I-5 (both recorded 7.50%), but all were at par with each other. Moreover, at highest spore concentration ($1 x 10^9$ spores ml^{-1}), there was an increase in the larval mortality with maximum mortality registered by isolate I-4 (17.50%), followed by I-1(12.50%), I-7(10.00%) and I-2, I-3, I-5 and I-6 (all registered 7.50% larval mortality), but all were at par with each other. While no mortality was recorded in control.

There was a gradual increase in the larval mortality and the most effective isolate I-4 at concentration of $1x10^9$ spores ml^{-1} registered highest mortality *i.e.* 42.50%, 70.00% and 90.00% after 48, 72 and 96 hours of spray, respectively. However, it recorded 100% larval mortality at 120 hours after spray. Isolate I-4 was immediately followed by I-1 and it recorded 22.50%, 52.50%, 85.00% and 95.00% after 48, 72, 96 and 120 hours of spray, respectively. The present findings are partially in accordance with the findings of Quesada et al. (2006).They reported 100% larval mortality after 96 hours of treatment. The delay in attaining the maximum mortality (100%) in the present studies might be attributed to the variation in the virulence of the *B. bassiana* isolate and spore concentration.

In the present investigation, the interaction of isolate and spore concentration was found to be significant with the most virulent isolate (I-4) at highest spore concentration ($1x10^9$ spores ml^{-1}) at 72 and 96 hours after spray.

Further, toxicity was determined against *H. armigera* by using probit analysis where median lethal concentration (LC_{50}, LC_{95}) and median lethal time (LT_{50}) values were also computed. The results indicated good fit as expressed by x^2 for all the isolates. Isolate I-4 at highest concentration of

$1x10^9$ spores ml^{-1} recorded highest mortality (100.00%) with lowest LC_{50}, LC_{95} and LT_{50} values (2.02×10^5 spores ml^{-1}, 5.19×10^8 spores ml^{-1} and 54.47 hours, respectively). This was followed by isolate I-1 ($1x10^9$ spores ml^{-1}) which recorded 2.74×10^5, 9.47×10^9 spores ml^{-1} and 66.21 hours of LC_{50}, LC_{95} and LT_{50} values, respectively. The present findings are in conformity with the findings of Prabhukarthikeya et al. (2013). They also recorded maximum *H. armigera* larval mortality of 73.33% with the LT_{50} value of 4.59 days (110.16 hours) at highest concentration of $1x10^8$ conidia ml^{-1}. It can be inferred that the larval mortality was directly proportional to the spore concentration of the virulent isolate and time period after application.

b. *Tuta absoluta*

All the isolates of *B.bassiana* were pathogenic and had great potential to control *T. absoluta* larvae. The present findings corroborates the findings of Shalaby et al. (2013). They also reported that *B. bassiana* has potential effect both on egg and neonate larvae of *T. absoluta*. In addition, *B. bassiana* has epiphytic and endophytic activity against *T.absoluta*, with highest mortality rates coupled with reduced larval longevity (Klieber and Reineke 2016).

In the present study, out of the seven isolates of *B.bassiana* tested with five spore concentrations, isolate I-4 was identified as most virulent at highest spore concentration ($1x10^9$ spores ml^{-1}). All the isolates registered a gradual increase in the *T.absoluta* larval mortality with increase in the spore concentration and duration after application. The present findings confirms the findings of Tadele and Emana (2017), as they also claimed the killing potentiality of *B. bassiana* against *T. absoluta* at higher spore concentration.

At 24 hours after spray, the differences in the larval mortality among different *B.bassiana* isolates at spore concentration of $1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ spores ml^{-1} were non-significant. However, it was found to be significant at highest spore concentration of $1x10^9$ spores ml^{-1} and it recorded maximum larval mortality(12.50%), followed by I-3, 1-6 and I-7(all registered 10.00%), I-1 and I-2 (both recorded 7.50%), but all were at par with each other. While no mortality was recorded in control.

There was a gradual increase in the larval mortality and the virulent isolate I-4 at 1 x 10^9 spores ml^{-1} recorded highest larval mortality *i.e.* 30.00%, 47.50% and 75.00% after 48, 72 and 96 hours of spray, respectively. However, it recorded highest mortality (80.00%) at 120 hours after spray. Isolate I-4 was immediately followed by I-1 and it recorded 40.00%, 55.00% and 62.50% mortality after 72, 96 and 120 hours of spray, respectively. The present findings are in contradictory with the findings of Tadele and Emana (2017), as they recorded 95.83% larval mortality of *T. absoluta* at 2.5 x 10^9 conidia ml^{-1}. Lower mortality in the present studies registered by most virulent isolate I-4, might be comparatively less virulent than the *B.bassiana* isolate evaluated by them.

In the present investigation, the interaction of isolate and spore concentration was found to be significant with the most virulent isolate (I-4) at highest spore concentration (1x10^9 spores ml^{-1}) at 96 and 120 hours after spray.

Computation of probit analysis exhibited good fit as expressed by x^2 for all the isolates. Isolate I-4 recorded highest mortality (80.00%) with lowest LC_{50}, LC_{95} and LT_{50} values (1.15 x 10^7 spores ml^{-1}, 3.78 x 10^{11} spores ml^{-1} and 72.45 hours, respectively). This was followed by isolate I-1 (1 x10^9 spores ml^{-1}) with LC_{50}, LC_{95} and LT_{50} values of 4.57 x 10^7, 8.71 x 10^8 spores ml^{-1} and 96.97 hours, respectively. The present findings are in conformity with the findings of Sabbour (2014). He also recorded maximum *T. absoluta* larval mortality (68.00%) with LC_{50} value of 129.4x10^4 spores ml^{-1}. It can be inferred that the larval mortality was directly proportional to the spore concentration of the virulent isolate and duration of infection.

c. *Bemisia tabaci*

All the isolates of *B.bassiana* were pathogenic against *B.tabaci* (third instar nymphs). The present findings were in conformity with the findings of Vincentini et al. (2001), James et al. (2003), Torrado-leon et al. (2006) and Al-Deghairi (2008). They also reported that *B.tabaci* nymphs are highly vulnerable to *B. bassiana* infection.

In the present study, out of the seven *B.bassiana* isolates tested with five spore concentrations, isolate I-4 exhibited highest virulency at maximum spore concentration of 1 x 10^9 spores ml^{-1}. All the isolates recorded gradual increase in the nymphal mortality of *B.tabaci* with increase in the spore concentration and time period after infection. The present findings were in accordance with the findings of Candido et al. (2006) and Al-Deghairi (2008). They also reported that nymphal mortality increases with time and spore concentration.

At 24 hours after spray, differences in the nymphal mortality among different *B. bassiana* isolates at all the tested spore concentrations *i.e.* 1x10^5 to 1x10^9 spores ml^{-1} were not significant. However, at highest spore concentration (1x10^9 spores ml^{-1}), isolate I-4 recorded highest nymphal mortality (7.50%), followed by I-1, I-2 and I-7 (all registered 5.00% mortality), I-3 and I-5 (both recorded 2.50% mortality), while no mortality was recorded in I-6 and control.

There was a gradual increase in the nymphal mortality and the virulent isolate I-4 at 1x10^9 spores ml^{-1} registered highest mortality *i.e.* 12.50%, 27.50% and 57.50% after 48, 72 and 96 hours of spray, respectively. However, it recorded highest nymphal mortality (67.50%) at 120 hours after spray. Isolate I-4 was immediately followed by I-1 and it recorded 10.00%, 22.50%, 40.00% and 57.50% after 48, 72, 96 and 120 hours of spray, respectively. The present findings are in agreement with the findings of Ramazeame (2012) and Ramos et al. (2000). They reported *B.tabaci* mortality which ranged from 72 to 98.33% and 62.71% after 72 and 166 hours after spray, respectively. Further, Kuang et al. (2005) and Zafar et al. (2016) reported nymphal mortality of 88.82% and 84.88% to 86.81% after 166 hours of spray with *B.bassiana* @ 1x10^8 conidia ml^{-1}, respectively. The variation in attaining the maximum mortality at different duration after spray might be attributed to the variation in the virulency and spore load of *B. bassiana* isolate.

In the present investigation, the interaction of isolate and spore concentration was found to be significant with the most virulent isolate (I-4) at highest spore concentration (1x10^9 spores ml^{-1}) at 120 hours after spray.

Computation of probit analysis indicated good fit as expressed by x^2 for all the isolates. Isolate I-4 at $1x10^9$ spores ml^{-1} recorded highest mortality (67.50%) with lowest LC_{50}, LC_{95} and LT_{50} values (2.25×10^8 ,1.14×10^{12} spores ml^{-1} and 97.85 hours, respectively). This was followed by isolate I-1 (1×10^9 spores ml^{-1}) with LC_{50}, LC_{95} and LT_{50} values of 6.22×10^8, 6.41×10^{13} spores ml^{-1} and 115.05 hours, respectively. The present findings are in agreement with the findings of Quesada et al.(2006), Aref et al. (2009), Al alawi et al. (2014) and Zafar et al. (2016).They also recorded highest mortality with LC_{50} values of 6.2×10^5 ,4.2×10^5, 9.33×10^5 and 2.70×10^7 spores ml^{-1}, respectively. The present results for LT_{50} values are in contradiction with the findings of Kuang et al. (2005). As they recorded LT_{50} value of 6.24 days (149.76 hours). This increase in the time period for attaining maximum mortality might be due to less virulency of *B.bassiana* isolate evaluated by them in comparison to isolate I-4 included in the present study.

In the present investigation, the reduced mortality of insect pests at lower spore concentration of the fungus might have occurred due to inadequate number of fungal propagules that adhered with the cuticle and or penetrated into the insects body, but did not prove to be fatal.

The present findings indicate that the *B. bassiana* based biopesticide can be used as a broad spectrum insecticide against a myriad of insect pests and corroborates the findings of Uma Devi et al. (2008).

5.2 Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*

Large-scale and easy availability of the microbial is a primary requirement of bio-control programme. Major obstacle in mass multiplication of *B. bassiana* is inadequate information about the availability of local substrates that are economically cheap which promotes faster growth of the fungus. In the present investigation, different solid substrates (*viz.* grains of maize, chickpea, sorghum, ragi and rice and neem cake) and liquid medias (*viz.* Potato Dextrose Broth (PDB), Czapek Dox Broth (CDB) and Sabouraud Dextrose Broth (SDB)) were evaluated for mass multiplication of *B.bassiana*.

In the present study, at ten days after inoculation, highest conidial count was recorded on rice (6.33 x 10^9 spores ml^{-1}), which was significantly superior than all other substrates. This was followed by chickpea (5.00 x 10^9 spores ml^{-1}), sorghum (3.00 x 10^9 spores ml^{-1}) and PDB (2.67x10^9 spores ml^{-1}), but they did not differ significantly from each other. While lowest spore count was recorded on neem cake (0.33 x 10^9 spores ml^{-1}).

Observations at twenty days after inoculation revealed that significantly highest spore load was recorded on rice (8.33 x 10^9 spores ml^{-1}), followed by PDB (7.33 x 10^9 spores ml^{-1}), chickpea (5.67 x 10^9 spores ml^{-1}) and sorghum (4.67 x 10^9 spores ml^{-1}), respectively. While lowest spore count was recorded on neem cake (1.00 x 10^9 spores ml^{-1}). Similar trend was observed at thirty days after inoculation.

On overall basis, among the different substrates evaluated, highest conidial count of *B.bassiana* was recorded on solid substrate rice (8.78 x 10^9 cfu ml^{-1}), followed by liquid media PDB (7.44 x 10^9 cfu ml^{-1}) and both emerged as the most potential medium for mass production, while neem cake was adjudged as the most inferior substrate (0.89 x 10^9 cfu ml^{-1}). These findings confirms the findings of Ibrahim and Low (1993), Sharma et al. (2002), Padmanabhan et al. (2009), Kalidas (2010), Karanja et al. (2010), Kumar et al. (2011), Suasa-ard et al. (2011), Mar and Lumyong (2012), Yadav et al. (2013), Kaur and Joshi (2014) and Babul et al. (2016). They also reported that rice was the best solid substrate for spore production with higher spore viability. Rice being a rich source of carbon, having adequate quantity of nitrogen, might have fulfilled the nutritive requirement of the fungus, which resulted in its luxuriant growth and spore production (Rajnikanth et al. 2011).

In the present investigation, PDB emerged as the best liquid media for mass culturing of *B.bassiana*. Presence of dextrose, an important nutrient of the fungus, might have stimulated the growth which significantly enhanced the conidial production (Siwach and Jaipal, 2004). The present finding deviates from the findings of Mondal and Bhattacharya (2004).They stated that the performance of PDB is intermittent among the substrates they evaluated.

Based on the local availability of the substrates, several authors have evaluated different substrates for *B.bassiana* mass production. In the present

investigation, growth on sorghum was found satisfactory with intermediate conidial count after rice, PDB and chickpea. These findings were in conformity with the findings of Pandey and Kanaujia (2005).Though the same substrates were not used for *B.bassiana* mass production by these authors, but they recorded intermediate performance of *B.bassiana* on sorghum. However, the present findings contradicts the findings of Rajanikanth et al.(2010), Rajnikanth et al.(2011) and Gangwar (2013). They claimed sorghum to be the best media as it registered maximum conidial counts among the substrates evaluated.

In the present study, rate of increase in growth of *B.bassiana* on different substrates/media from 10^{th} to 20^{th} day and from 20^{th} to 30^{th} day after inoculation were found to be non-significant.

However, no information is available in the literature on the impact of substrates on growth and development of fungus over period of time.

In the present findings, rice and PDB were identified as the best solid substrate and liquid media and were found to be economical as the cost of production was Rs.0.58 and Rs.1.01 per $1x10^9$ spores ml^{-1}, respectively. However, no information is available in the literature on the economics of mass multiplication of *B. bassiana*.

It can be inferred from the present findings that in addition to rice and PDB, *B. bassiana* is able to grow on a variety of substrates / media and looking to its availability and cost they can be further promoted for the mass multiplication of the fungus.

5.3 Compatibility of *B. bassiana* with various additives

Formulation of the myco-insecticide must be compatible with the additive agent, which must maintain an adequate shelf-life and virulency then only it can be recognised as an effective and successful microbial insecticide (Derakhshan et al. 2008). In this context, effect of twelve additives (*viz.* Tween-20, Tween-40, Tween-60, Tween-80, Triton-X, Glycerol, Kaolite, Silica gel, Sunflower oil, Neem oil, Pongamia oil and Carboxyl Methyl Cellulose (CMC)) were evaluated at three concentrations (0.1, 0.5 and 1.0%) on the mycelial growth and sporulation of *B. bassiana* under *in-vitro* conditions.

Differences in the radial growth of *B.bassiana* in different additives at 0.1% concentration was significant. Control recorded maximum radial growth (83.95 mm). However, among the additives, CMC recorded maximum growth (73.25 mm), followed by Kaolite (66.90 mm), Silica gel (65.20 mm), Sunflower oil (64.15 mm) and Glycerol (52.23 mm), respectively. Whereas, the growth of *B.bassiana* in the remaining additives was below 50 mm, with lowest growth in Pongamia oil (26.03mm). Similar trend of radial growth was registered with additives at 0.5% and 1.00% concentration. Impact of additives at 0.1%, 0.5% and 1.00% concentration on the growth of *B.bassiana* over control ranged from 12.74% (CMC) to 68.99% (Pongamia oil), 8.36% (CMC) to 71.45% (Pongamia oil) and 3.16% (CMC) to 84.58% (Pongamia oil), respectively.

However, the differences in the overall mean radial growth of *B.bassiana* in different concentrations of additives were found to be significant. Maximum radial growth (83.95 mm) was registered in control. Among the additives, CMC recorded maximum growth (77.16 mm), followed by Kaolite (69.07 mm), Silica gel (65.20 mm) and Sunflower oil (55.13 mm), respectively. Whereas, the growth of *B. bassiana* in the remaining additives was below 50 mm and lowest was recorded in Pongamia oil (20.98 mm). Reduction in radial growth ranged from 8.08% (CMC) to 75.00% (Pongamia oil) over control. In the present findings, maximum growth in CMC might be due to the release of cellulolytic enzymes by *B.bassiana* on CMC which resulted in its degradation to carbon, an important nutrient source of the fungus (Petlamul et al. 2017).

Differences in the growth inhibition of *B.bassiana* in different additives at 0.1% concentration was significant. CMC showed minimum growth inhibition (12.69%) and was significantly superior than the other additives. This was followed by Kaolite (20.28%), Silica gel (22.25%), but were at par with each other. While the remaining additives inhibited more than 35% growth of *B. bassiana*. Similar pattern of observations were recorded at 0.5% and 1.00% concentration, but with increased growth inhibition of 35% and 45% than the effective additive (CMC), respectively.

Differences in the overall mean growth inhibition of *B.bassiana* in additives of different concentrations were found to be significant. Additive CMC showed minimum growth inhibition (8.03%), followed by Kaolite (17.65%) and Silica gel (22.25%), but they differed significantly from each other. While the remaining additives inhibited more than 35% growth of *B.bassiana* and maximum inhibition was recorded in Pongamia oil (74.98%). The variation or negative impact of additives on the growth and development of microorganisms might be probably due to increase in cell permeability which leads to the leakage of amino acid through the inner membrane, that causes inhibition of catalase system, decrease in fungal adherence and inhibition of germ tube and mycelial formation (Tanuja et al. (2010).

Differences in the spore count of *B.bassiana* in different additives at 0.1% concentration was significant. recorded Highest spore count was recorded in control (5.33 x 10^9 spores ml^{-1}), followed by CMC (4.67 x 10^9 spores ml^{-1}) and Tween-80 (4.00 x 10^9 spores ml^{-1}). The mean spore count recorded in the remaining additives was less than 2 x 10^9 spores ml^{-1}. Similar trend was observed at 0.5% concentration.

However, differences in the spore count of *B.bassiana* in different additives at 1.00% concentration was significant. Highest spore count was recorded in Control (4.67 x 10^9 spores ml^{-1}), followed by CMC (3.67 x 10^9 spores ml^{-1}), Kaolite (2.33 x 10^9 spores ml^{-1}) and Glycerol (2.00 x 10^9 spores ml^{-1}), respectively. Spore count recorded in the remaining additives was less than 1.50 x 10^9 spores ml^{-1}.

Differences in the overall mean spore count of *B.bassiana* in additives at different concentrations was found to be significant. Highest mean spore count was recorded in control (5.22 x10^9 spores ml^{-1}), followed by CMC (4.67 x 10^9 spores ml^{-1}) and Tween-80 (3.44 x 10^9 spores ml^{-1}). Spore count recorded in the remaining additives was less than 3.50 x 10^9 spores ml^{-1}.

In the present findings CMC supported a higher population of viable spores of *B.bassiana* and it confirms the findings of Chakravarty and Kalita (2011) and Vidhyasekaran and Muthamilan (1995). They also reported that use of CMC as an additive in different formulations of the fungus

Pseudomonas fluorescens, maintained higher viable spore population of more than 10^6 cfu g^{-1} even after 120 and 240 days after storage, respectively.

Additives at different concentrations and their interaction had significant impact on the radial growth and growth inhibition of *B.bassiana*. Further, both of them exhibited significant effect on the spore population, but there interaction was found to be non-significant. No reports are available in the literature on the interaction of additives at different concentrations.

From the present findings, it can be concluded that the additive Carboxy Methyl Cellulose (CMC) proved to be relatively less toxic to *B.bassiana* than the other additives, as it recorded highest mean radial growth with least growth inhibition and the spore population was only 10% less than the control.

5.4 Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulation/s against insect-pest complex on tomato under protected conditions

5.4.a Shelf life studies of different *B. bassiana* formulations

Formulation is mandatory for spore application in the field and also to enhance the efficacy of the product. Further, the shelf life of a microbial pesticide is an important factor for insect control. In the present study, three formulations *i.e.* wettable powder, talc and aqueous formulation of *B.bassiana* were prepared and stored under ambient conditions for a period of 13 months. Spore count and spore viability of all the three formulations at concentration of $1x10^8$ and $1x10^9$ spores g^{-1}/ml^{-1} were recorded at monthly interval.The results are discussed below:

5.4.a.i. Effect of storage period of *B.bassiana* on spore count and viability (1×10^8 and 1×10^9 spores g^{-1}/ml^{-1})

Wettable Powder formulation (WP)

In the present findings, during the start of the experiment WP formulation at $1x10^8$ spores g^{-1} recorded highest spore count and viable spores (8.22 and 7.67 x 10^8 spores g^{-1}, respectively). There was a significant

reduction in both spore count and spore viability after 7 months of storage and was found to be minimum after 12 and 10 months of storage *i.e.* 0.50 and 0.67 x 10^8 spores g^{-1}, respectively.

During the start of the experiment WP formulation at 1x10^9 spores g^{-1} recorded highest spore count and viability (7.65 and 7.05 x 10^9 spores g^{-1}, respectively). There was a significant reduction both in the spore count and spore viability after 6 months of storage and was found to be minimum after 9 months of storage *i.e.* 0. 99 and 0.67 x 10^9 spores g^{-1}, respectively.

Talc formulation

In the present investigation, during the start of the experiment talc formulation at 1x10^8 spores g^{-1} recorded highest spore count and spore viability (6.29 and 5.67 x 10^8 spores g^{-1}, respectively). There was a significant reduction both in the spore count and spore viability after 4 and 2 months of storage, respectively. However, it was found to be minimum after 7 and 5 months of storage *i.e.* 0.67 and 0.67 x 10^8 spores g^{-1}, respectively.

During the start of the experiment talc formulation at 1x10^9 spores g^{-1} recorded highest spore count and spore viability (5.80 and 5.41 x 10^9 spores g^{-1}, respectively). There was a significant reduction both in the spore count and spore viability after 3 months of storage and it was found to be minimum after 6 and 5 months of storage *i.e.* 0.41 and 0.33 x 10^9 spores g^{-1}, respectively.

Aqueous formulation

In the present findings, during the start of the experiment aqueous formulation at 1x10^8 spores ml^{-1} recorded highest spore count and spore viability (7.96 and 7.67 x 10^8 spores ml^{-1}, respectively). There was a significant reduction both in the spore count and spore viability after 7 and 4 months of storage, respectively. However, it was found to be minimum after 10 and 8 months of storage *i.e.* 1.11 and 0.33 x 10^8 spores ml^{-1}, respectively.

During the start of the experiment aqueous formulation at 1x10^9 spores ml^{-1} recorded highest spore count and spore viability (6.99 and 6.74 x 10^9 spores ml^{-1}, respectively). There was a significant reduction both in the spore

count and spore viability after 4 and 5 months of storage, respectively. However, it was found to be minimum after 7 months of storage *i.e.*0.33 and 0.67 x 10^9 spores ml^-, respectively.

In the present findings it is observed that in WP formulation at 1x10^8 and 1x10^9 spores g^{-1} there was no significant reduction in the spore viability up to 7 months (210 days) and 6 months (180 days) of storage, respectively. Talc formulation at both the concentrations registered spore viability upto to 2 months (60 days) and 3 months (90 days) of storage, respectively. While, aqueous formulations at 1x10^8 and 1x10^9 spores ml^{-1} retained spore viability up to 4 months (120 days) and 5 months (150 days) of storage, respectively. It can be said that the spore viability is dependent on the storage period and temperature. The present findings are in conformity with the findings of Simková (2009) and Kaur and Joshi (2014), as they also reported that the storage period and temperature had significant impact on the spore viability.

In the present investigation, the effective storage period for WP, talc and aqueous formulations at both the concentrations is 6, 2 and 4 months with 40.83%, 26.17% and 42.24% reduction in the spore viability, respectively. The present findings contradicts the findings of Alves et al.(1996), Šimková (2009) and Mola and Afkari (2012).They reported that oil and powdered formulations of *B.bassiana*, completely lost the spore viability after 8 months of storage under normal conditions. The variation in the effective storage period under normal conditions may be attributed to the temperature prevailing during the storage period.

It can be concluded that since the fungal spores are living organisms, their viability diminishes with time, depending on the environmental conditions. The variation in the shelf life over period of storage might be due to temperature which may be responsible for depletion of nutrients and moisture in the medium resulting in desiccation of conidia, having detrimental effect on spore count and viability. However, it can be minimised to a considerable extent by adding certain additives and by providing controlled storage conditions.

5.4.a.ii. Effect of storage period on the virulence of *B.bassiana*

Virulence studies were carried out with the stored three formulations of *B.bassiana* at two concentrations ($1x10^8$ and $1x10^9$ spores g^{-1}/ ml^{-1}) against 2nd instar larvae of *H. armigera* under *in-vitro* conditions.

During the start of the experiment, freshly prepared formulations (0 days of storage), at both the tested concentrations, WP formulation registered highest larval mortality (95.00% and 100%), followed by aqueous and talc formulation, (both registered 90.00% and 80.00%), respectively. There was a gradual reduction in the larval mortality with increase in the storage period.

Mortality recorded by the application of 90 days old/stored formulation of WP at $1x10^8$ and $1x10^9$ spores g^{-1} was 80 and 82.50%, while for 180 days old formulation it was 70% and 72.50%, respectively. After 180 days the spore viability reduced drastically as was evident by the larval mortality which was 50% less than the mortality recorded by application of freshly prepared formulation (0 days storage).

Mortality recorded by the application of 90 days old / stored talc formulation at $1x10^8$ and $1x10^9$ spores g^{-1} was 60 and 62.50%, respectively. After 90 days the spore viability decreased gradually and the larval mortality was about 40% less than that recorded by the application of freshly prepared formulation / zero days storage.

Mortality recorded by the application of 90 days old / stored aqueous formulation at $1x10^8$ and $1x10^9$ spores g^{-1} was 77.50 and 80%, respectively. After 120 days the spore viability reduced drastically and the larval mortality was 40% less than that recorded by the application of freshly prepared formulation / zero days storage.

The present findings, revealed that the effective storage period under normal conditions for WP and aqueous formulation was 6 months and 4 months, while it was 3 months for talc formulation. After the effective storage period there was a sudden decrease in the virulence. This may be attributed to the reduction in the spore density and germination due to prolonged storage period under ambient conditions. The present findings confirms the findings of Puzari et al. (2003). They also reported that with the increase in

age/storage period, the viability and infectivity of the fungus decreased significantly.

5.4.b. Evaluate the effective formulations against insect - pest complex on tomato under protected conditions

The investigation on bioefficacy of different *B.bassiana* formulations at concentration of $2x10^8$ and $2x10^9$ spores g^{-1}/ ml^{-1} against insect-pest complex (*viz. H.armigera*, *T.absoluta* and *B.tabaci*) on tomato under protected conditions were carried out during *kharif* 2016-17 and 2017-18 and the results are discussed below :

Pre-treatment observations of all the tested insects were non-significant. Three days after spray, differences in the mean larval/nymphal population of *H. armigera, T. absoluta* and *B. tabaci* during both the years, among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} was most effective as it recorded lowest larval/nymphal population followed by aqueous formulation @ $2x10^9$ spores ml^{-1}. Similar trend of the effective treatments was observed after seven and ten days of treatment.

Efficacy of *B. bassiana* formulations on tomato fruit damage

During both the years of study, all the treatments significantly reduced the fruit damage by *H.armigera* and *T.absoluta* as compared to control. Among the treatments, WP formulation @ 2 x 10^9 spores g^{-1} recorded lowest fruit damage followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} and aqueous formulation @ 2 x 10^8 spores ml^{-1}.

Marketable fruit yield

All the treatments registered significantly higher marketable fruit yields as compared to the control. The highest marketable fruit yield was recorded in WP formulation @ 2 x 10^9 spores g^{-1}, followed by aqueous formulation @ $2x10^9$ spores ml^{-1} .

It is observed that WP formulation @ 2 x 10^9 spores g^{-1}, followed aqueous formulation @ 2 x 10^9 spores ml^{-1}, WP formulation @ 2 x 10^8 spores g^{-1} and aqueous formulation@ 2 x 10^8 spores ml^{-1} proved effective

not only in reducing the damage due to insect pest complex, but also recorded higher marketable fruit yields (61.48 , 59.73, 55.15 and 52.34 t/ha, respectively).

The WP and aqueous formulations of *B.bassiana* under protected conditions proved their superiority over control in reducing the pest population and fruit damage with increase in the marketable fruit yield. Several workers have also reported similar findings, that application of different formulations of entomopathogenic fungi effectively reduced the damage due to insect pests in tomato and other crops with increased fruit yield (Forschler and Nordin ,1989 ; Zhang et al. 1992 ; Nankinga and Moore , 2000 ; Orozcosatho et al. 2000 ; Sood et al. 2001; Ramarethinam et al. 2002 ; Phukon et al. 2014 and Prithiva et al. 2017).

Economics of Treatments

Net profit

Maximum net profit was obtained in case of WP formulation @ 2 x 10^9 spores ml^{-1} (Rs 76946/ha), followed by aqueous formulation @ 2x10^9 spores ml^{-1} (Rs 70239/ha), WP formulation @ 2x10^8 spores ml^{-1} (Rs 55473/ha) and aqueous formulation @ 2 x 10^8 spores ml^{-1} (Rs 44533/ha), respectively.

Cost Benefit ratio

Taking into account the cost of inputs, the cost benefit ratio of WP formulation @ 2 x 10^9 spores ml^{-1} was maximum (1:4.99), followed by WP formulation @ 2 x 10^8 spores ml^{-1} (1:4.67), aqueous formulation @ 2 x 10^9 spores ml^{-1} (1:4.62) and minimum in aqueous formulation @ 2 x 10^8 spores ml^{-1} (1:3.80), respectively.

However, no information is available in the literature on the economics of different formulations of *B. bassiana* on tomato crop.

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

The present investigation entitled **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"** were carried in the Bio-control laboratory (Insect Pathology laboratory) during *Kharif* seasons of 2016-17 and 2017-18 at Division of Entomology and Nematology, ICAR-Indian Institute of Horticulture Research (IIHR), Bengaluru. The research was conducted with the following objectives:

1. Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci)* through laboratory bioassay.
2. Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana.*
3. Compatibility of virulent isolate of *B. bassiana* with various additives.
4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulation/s against insect-pest complex on tomato under protected conditions.

6.1 Summary

6.1.1 Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay

In-vitro screening with seven isolates of *B.bassiana* at five concentrations (*i.e.* $1x10^5$ to $1x10^9$ spores ml^{-1}) against insect pests of tomato revealed that at 120 hours after treatment, isolate I-4 was most virulent and registered highest mortality of *H.armigera*, *T.absoluta* and *B.tabaci* (100.00, 80.00 and 67.50%, respectively). This was followed by

isolate I-1 which recorded mortality of 95.00, 62.50 and 57.50% against *H.armigera*, *T.absoluta* and *B.tabaci* , respectively.

Insect mortality was dose dependent *i.e.*, highest spore concentration ($1x10^9$ spores ml^{-1}) registered highest mortality as compared to the lower concentrations ($1x10^5$, $1x10^6$, $1x10^7$ and $1x10^8$ spores ml^{-1}).

There was a gradual increase in the insect mortality with time *i.e.*, lowest mortality was recorded after 24 hours of spray and it gradually increased with increase in the time period and attained maximum mortality of the test insects at 120 hours after spray.

The interaction of isolate and spore concentration was found to be significant with the most virulent isolate (I-4) at highest spore concentration ($1x10^9$ spores ml^{-1}) against *H.armigera* , *T.absoluta* and *B.tabaci* at 72 and 96 hours; 96 and 120 hours and 120 hours after spray , respectively.

Probit analysis also showed good fit as expressed by x^2 for all the isolates.

***H.armigera* (2nd instar larvae)**

Isolate I-4 recorded highest larval mortality with lowest median lethal concentration (LC_{50} and LC_{95}) and median lethal time (LT_{50}) values of 2.02 x 10^5, 5.19 x 10^8 spores $ml^{-1}$and 54.47 hours, respectively. This was followed by isolate I-1 which recorded LC_{50}, LC_{95} and LT_{50} values of 2.74 x 10^5 , 9.47 x 10^9 spores ml^{-1} and 66.21 hours, respectively.

***T. absoluta* (2nd instar larvae)**

Isolate I-4 recorded highest larval mortality with lowest LC_{50}, LC_{95} and LT_{50} values of 1.15 x 10^7 spores ml^{-1}, 3.78 x 10^{11} spores ml^{-1} and 72.45 hours, respectively. This was followed by isolate I-1 with LC_{50}, LC_{95} and LT_{50} values of 4.57 x 10^7 , 8.71 x 10^8 spores ml^{-1} and 96.97 hours, respectively.

***B. tabaci* (3rd instar nymph)**

Isolate I-4 recorded highest nymphal mortality with lowest LC_{50}, LC_{95} and LT_{50} values of 2.25 x 10^8,1.14 x10^{12} spores ml^{-1} and 97.85 hours, respectively. This was followed by isolate I-1 with LC_{50}, LC_{95} and LT_{50} values of 6.22 x 10^8, 6.41x 10^{13} spores ml^{-1} and 115.05 hours, respectively.

6.1.2 Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*

Among the different substrates/media evaluated for mass production of *B.bassiana*, highest conidial count was recorded on rice followed by Potato Dextrose Broth (PDB) and both emerged as the most potential medium with lowest production cost of Rs.0.58 and Rs.1.01 per $1x10^9$ spores ml^{-1}, while neem cake was the most inferior substrate.

However,highest rate of increase in growth of the fungus was recorded in Sabouraud Dextrose Broth (SDB), followed by Czapex Dox Broth (CDB).

6.1.3 Compatibility of virulent isolate of *B. bassiana* with various additives

The studies on effect of additives at three concentrations (*i.e.* 0.1, 0.5 and 1.00%) on the mycelial growth of *B. bassiana* under *in-vitro* conditions revealed that Carboxyl Methyl Cellulose (CMC) at 1.00% concentration was found to be most compatible and recorded maximum radial growth with least growth inhibition followed by Kaolite, Silica gel and Sunflower oil. While the remaining additives inhibited more than 35% growth of *B.bassiana* and maximum inhibition was recorded in Pongamia oil.

Highest mean spore count was recorded in control followed by CMC (4.67×10^9 spores ml^{-1}), Tween-80 (3.44×10^9 spores ml^{-1}), Kaolite (2.89×10^9 spores ml^{-1}) and Glycerol (2.78×10^9 spores ml^{-1}) at 0.5% concentration, whereas in the remaining additives the spore count was less than 2.50×10^9 spores ml^{-1}.

Additives at different concentrations and their interaction had significant impact on the radial growth and growth inhibition of *B.bassiana*. However, additives at different concentrations had significant effect on spore load, but there interaction was found to be non-significant.

The present investigation, revealed that using CMC or Tween-80 or Kaolite or Glycerol at 0.5% concentration as an additive in the formulation can help in retaining the spore count with least inhibition of fungal growth that will ultimately be helpful in increasing the shelf life of the formulation.

6.1.4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulation/s against insect-pest complex on tomato under protected conditions

6.1.4.a. Shelf life studies of *B. bassiana* formulations

The shelf life of a microbial pesticide is an important factor for insect control. To enhance the myco-pesticide efficacy, appropriate formulation is required which stabilizes the productivity, distribution and storage of the fungus. In the present study, solid (WP and talc) and aqueous formulations of *B.bassiana* were stored for 13 months under ambient conditions. Spore count and spore viability of all the three formulations at $1x10^8$ and $1x10^9$ spores g^{-1}/ml^{-1} concentration was recorded at monthly interval.

The effective period of storage for Wettable Powder (WP) formulation was 6 months, followed by aqueous formulation (4 months) and minimum in talc formulation (3 months), respectively.

Effect of storage period on the virulence of *B.bassiana*

Virulence studies under *in-vitro* conditions were carried out at three months interval with stored formulations of *B. bassiana* (*i.e.* wettable, talc and aqueous) against 2nd instar larvae of *H. armigera* at concentration of $1x10^8$ and $1x10^9$ spores g^{-1} / ml^{-1}.

During the start of the experiment, highest larval mortality with freshly prepared formulations (0 days of storage), at both the concentrations was registered in WP formulation followed by aqueous and talc formulation, respectively. With increase in the storage period, there was a gradual reduction in the larval mortality from 90, 120 and 180 days onwards in talc, aqueous and WP formulations, respectively.

6.1.4.b. Evaluate the effective formulation/s against insect-pest complex on tomato under protected conditions

Differences in the mean population of *H. armigera, T. absoluta* larvae and *B. tabaci* nymphs during both the years, among different treatments were found to be significant. Among the treatments, WP formulation @ $2x10^9$ spores g^{-1} was most effective as it recorded lowest larval/nymphal

population followed by aqueous formulation @ $2x10^9$ spores ml^{-1}. The efficacy trend of the effective treatments remained same after seven and ten days of treatment .

Wettable powder formulation @ 2 x 10^9 spores g^{-1}, followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} proved to be most effective not only in reducing the fruit damage due to insect pest complex, but also recorded higher marketable fruit yields.

Highest cost benefit ratio was obtained with WP formulation @ 2 x 10^9 spores ml^{-1} (1:4.99), followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} (1:4.67), respectively.

The WP and aqueous formulations of *B.bassiana* under protected conditions proved their superiority over control in reducing the pest population and fruit damage with increase in the marketable fruit yield.

In the pest management program by reducing the chemical usage and by incorporating the microbial insecticides will promote environmental friendly farming with higher economic returns.

6.2 Conclusion

- Bioassay studies with seven isolates of *B.bassiana* against insect pests of tomato revealed isolate I-4 to be most virulent with highest mortality of the tested insects *i.e., H.armigera, T.absoluta* and *B.tabaci* followed by isolate I-1 and I-5.
- Mortality was dose dependent *i.e.*, highest spore concentration of $1x10^9$ spores ml^{-1} showed highest mean mortality as compared to the lower concentrations ($1x10^5$ to $1x10^8$ spores ml^{-1}).
- Mortality was directly proportional to the duration of infection. There was a gradual increase in the mortality with increase in the time *i.e.,* at 24 hours after treatment, it was minimum and maximum mortality was attained at 120 hours after treatment.
- Highest spores were harvested in rice and PDB with lowest production cost.

- Carboxy Methyl Cellulose followed by Tween-80, Kaolite and Silica gel at 0.5% concentration were the best additive with minimum detrimental effect on growth, spore count and viability and can be used in WP and aqueous formulations.
- Effective storage period under normal conditions for Wettable powder, aqueous and talc formulation was 6, 4 and 3 months, respectively .
- WP formulation @ 2 x 10^9 spores g^{-1} was most effective as it recorded lowest fruit damage with highest marketable fruit yield, followed by aqueous formulation @ 2 x 10^9 spores ml^{-1} .
- Highest cost benefit ratio was obtained with WP formulation @ 2 x 10^9 spores ml^{-1}, followed by aqueous formulation @ 2 x 10^9 spores ml^{-1}, respectively.

Therefore, on the basis of the above facts it can be concluded that *B.bassiana* isolate I-4 is the most virulent isolate which can be mass produced on an economical solid substrate rice or liquid media PDB. The effective storage period under normal conditions for WP formulation is 6 months followed 4 months of aqueous and 3 months of talc formulation.

WP formulation of *B.bassiana* virulent isolate I-4 at 1x10^9 spores g^{-1} has been found to be most effective in reducing the fruit damage with highest marketable fruit yield as well as cost benefit ratio followed by aqueous formulation at 1x10^9 spores ml^{-1}.

6.3 Suggestions for further work

Studies on screening of *B.bassiana* against tomato insect pest complex gave an indication that isolate I-4 was found to be most virulent. Hence, using this virulent isolate in combination with other entomopathogenic fungus, can be carried out in order to enhance its efficacy under the field conditions.

Susceptibility of different instars of insects to the fungal infection has to be taken up, that may be a critical factor for determining the time of fungal application.

Detail studies on economically cheap and easily available substrates, agricultures wastes and byproducts alone and their combinations with nutrient and various incubation temperature should be tested for mass production of

entomopathogenic fungus *B.bassiana*.

Formulation of mycoinsecticides must be compatible with the agent, that enhance its performance and ideally it must maintain an adequate shelf-life of the microbial agent for its successful utilization. In the present studies, there is some indication of the additives that could be used to increase the shelf life of the product, hence indepth studies should be carried out.

Studies on biological and physical properties of the formulation must be carried out at varied storage temperatures, so that the shelf life should be of at least 1 year to retain the spore viability which is a pre-requisite for registration of microbial pesticides under Central Insecticide Board act.

Higher costs of plant protection inputs and the hazardous effects of the synthetic insecticides need to be replaced with new alternatives of pest control strategies. The usage of microbial formulation could be one of the alternative for promoting organic cultivation of tomato.This may also affect the socio-economic status of the farmers with increased profits.

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APPENDIX - I

Weekly meteorological data of Meteorological Observatory, Division of Plant Physiology and Biochemistry, ICAR-IIHR, Bengaluru , Karnataka (2016-17)

SW	Date		Temperature (°C)		Relative Humidity (%)		Wind velocity (Km/hr)	Evaporation (mm)	Rainfall (mm)	Rainy days (no.)
	From	To	Max.	Min.	Morning	Evening				
22	27/05/2016	02/06/2016	34.85	21.57	76.57	38.42	3.94	4.28	4.14	2
23	03/06/2016	09/06/2016	33.14	20.85	79.14	33.42	5.07	3.95	5.42	2
24	10/06/2016	16/06/2016	31.71	20.00	77.57	38.71	6.25	2.94	1.07	2
25	17/06/2016	23/06/2016	29.42	19.71	82.42	41.71	4.75	3.22	6.77	5
26	24/06/2016	30/06/2016	28.57	19.14	83.14	46.57	7.41	2.22	2.07	3
27	01/07/2016	07/07/2016	28.57	20.28	78.71	53.42	8.54	3.02	0.54	2
28	08/07/2016	14/07/2016	29.57	20.28	76.57	48.85	9.55	3.61	0.00	0
29	15/07/2016	21/07/2016	29.28	21.85	75.42	49.42	4.08	4.10	3.42	2
30	22/07/2016	28/07/2016	29.71	20.57	80.57	46.57	3.70	3.27	13.62	4
31	29/07/2016	04/08/2016	28.00	19.57	78.57	55.71	7.72	2.98	7.28	3
32	05/08/2016	11/08/2016	28.14	22.28	77.57	56.57	6.14	4.50	0.00	0
33	12/08/2016	18/08/2016	27.71	21.57	76.57	57.00	4.71	3.84	0.00	0
34	19/08/2016	25/08/2016	28.14	21.00	79.71	58.14	3.14	3.08	1.57	3
35	26/08/2016	01/09/2016	25.85	20.14	83.14	60.57	4.12	2.71	1.14	2
36	02/09/2016	08/09/2016	25.85	20.28	78.28	55.71	3.28	2.25	1.07	2
37	09/09/2016	15/09/2016	25.00	20.28	78.57	57.42	3.67	2.57	4.21	2
38	16/09/2016	22/09/2016	25.42	21.57	77.42	51.28	5.22	3.45	1.42	4
39	23/09/2016	29/09/2016	25.14	21.71	83.28	58.85	3.64	2.61	0.57	2
40	30/09/2016	06/10/2016	27.28	21.85	71.28	48.00	3.04	4.25	0.00	0
41	07/10/2016	13/10/2016	28.00	22.42	78.42	51.42	3.10	4.38	0.88	2

APPENDIX - II

Weekly meteorological data of Meteorological Observatory, Division of Plant Physiology and Biochemistry, ICAR-IIHR, Bengaluru , Karnataka (2017-18)

SW	Date		Temperature (°C)		Relative Humidity (%)		Wind velocity (Km/hr)	Evaporation (mm)	Rainfall (mm)	Rainy days (no.)
	From	To	Max.	Min.	Morning	Evening				
21	22/05/2017	04/06/2017	25.71	20.57	81.29	57.71	1.01	2.34	12.31	4
22	05/06/2017	11/06/2017	25.29	20.43	77.86	55.43	1.83	5.00	0.00	0
23	12/06/2017	18/06/2017	26.14	20.57	79.00	64.00	2.94	3.61	0.55	1
24	22/05/2017	04/06/2017	26.86	20.29	75.14	70.29	1.47	2.17	2.43	3
25	19/06/2017	25/06/2017	27.14	20.57	82.00	68.71	2.15	2.43	5.45	5
26	26/06/2017	02/07/2017	27.29	20.86	70.86	57.00	3.86	4.74	0.00	0
27	03/07/2017	09/07/2017	28.00	20.57	80.43	63.00	1.29	2.11	3.36	4
28	10/07/2017	16/07/2017	25.57	20.29	74.57	62.43	2.67	4.13	0.36	1
29	17/07/2017	23/07/2017	25.71	20.14	75.29	65.00	4.05	3.70	0.41	3
30	24/07/2017	04/06/2017	27.29	20.00	73.57	54.00	1.85	4.00	1.60	3
31	31/07/2017	06/08/2017	26.43	21.14	71.43	60.71	1.50	3.57	0.54	3
32	07/08/2017	13/08/2017	24.86	20.64	81.29	63.86	0.54	2.43	14.47	3
33	14/08/2017	20/08/2017	23.93	20.21	86.43	61.29	0.98	2.64	3.47	5
34	21/08/2017	27/08/2017	24.57	20.71	87.57	68.29	1.26	1.70	12.41	6
35	28/08/2017	03/09/2017	23.57	20.79	88.71	57.29	1.41	2.19	13.01	5
36	04/09/2017	10/09/2017	23.57	20.79	88.71	57.29	1.41	2.19	13.01	7
37	11/09/2017	17/09/2017	25.14	20.36	89.29	65.29	0.24	1.63	20.81	3
38	18/09/2017	24/09/2017	24.79	19.00	79.43	60.57	2.12	3.29	0.21	1
39	25/09/2017	01/10/2017	24.43	20.07	90.14	73.00	0.30	1.26	20.44	7

APPENDIX III

1. Screening isolates of *Beauveria bassiana* for identification of most virulent isolate against tomato insect pest complex (*Helicoverpa armigera, Tuta absoluta* and *Bemisia tabaci*) through laboratory bioassay

ANOVA 1. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	48.26	6.89	1.00	2.42
Error	24	165.45	6.89		
Total	31	213.70			
SEm±	1.31	SEd	1.86	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	544.59	77.80	4.84*	2.42
Error	24	386.04	16.09		
Total	31	930.63			
SEm±	2.01	SEd	2.84	CD	5.85

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1231.66	175.95	3.25*	2.42
Error	24	1300.96	54.21		
Total	31	2532.62			
SEm±	3.68	SEd	5.21	CD	10.74

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1556.06	222.29	4.27*	2.42
Error	24	1249.13	52.05		
Total	31	2805.09			
SEm±	3.61	SEd		CD	10.53

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3048.77	435.54	7.67*	2.42
Error	24	1362.68	56.78		
Total	31	4411.46			
SEm±	3.77	SEd	5.33	CD	11.00

*Significant at 5% level of significance

ANOVA 2. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	47.27	7.88	1.00	2.57
Error	21	165.45	7.88		
Total	27	212.72			
SEm±	1.40	SEd	1.98	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	519.97	86.66	4.71*	2.57
Error	21	386.04	18.38		
Total	27	906.01			
SEm±	2.14	SEd	3.03	CD	6.30

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1458.74	243.12	10.80*	2.57
Error	21	472.77	22.51		
Total	27	1931.51			
SEm±	2.15	SEd	3.36	CD	6.32

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	2095.44	349.24	4.56*	2.57
Error	21	1607.09	76.53		
Total	27	3702.53			
SEm±	3.82	SEd	6.19	CD	11.24

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	3058.68	509.78	6.89*	2.57
Error	21	1553.39	73.97		
Total	27	4612.07			
SEm±	4.36	SEd	6.08	CD	12.82

* Significant at 5% level of significance

ANOVA 3. Virulence of *Beauveria bassiana* isolates (1x 10^6spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	103.40	14.77	0.71	2.42
Error	24	496.34	20.68		
Total	31	599.74			
SEm±	2.27	SEd	3.22	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1093.00	156.14	4.06*	2.42
Error	24	923.57	38.48		
Total	31	2016.57			
SEm±	3.10	SEd	4.39	CD	9.05

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2058.55	294.08	11.73*	2.42
Error	24	601.62	25.07		
Total	31	2660.17			
SEm±	2.50	SEd	3.54	CD	7.31

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3311.13	473.02	16.74*	2.42
Error	24	678.23	28.26		
Total	31	3989.36			
SEm±	2.66	SEd	3.76	CD	7.76

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	4179.02	597.00	17.87*	2.42
Error	24	801.86	33.41		
Total	31	4980.88			
SEm±	2.78	SEd	4.09	CD	8.11

* Significant at 5% level of significance

ANOVA 4. Virulence of *Beauveria bassiana* isolates (1x 10^6 spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	94.54	15.76	0.67	2.57
Error	21	496.34	23.64		
Total	27	590.88			
SEm±	2.43	SEd	3.44	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	695.95	115.99	2.64	2.57
Error	21	926.57	43.98		
Total	27	1619.53			
SEm±	3.32	SEd	4.69	CD	9.75

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1450.56	241.76	3.80*	2.57
Error	21	1334.55	63.55		
Total	27	2785.11			
SEm±	3.39	SEd	5.64	CD	9.96

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1448.70	241.45	3.19*	2.57
Error	21	1589.72	75.70		
Total	27	3038.41			
SEm±	3.30	SEd	6.15	CD	9.69

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1604.59	267.43	4.66*	2.57
Error	21	1204.81	57.37		
Total	27	2809.39			
SEm±	3.33	SEd	5.36	CD	9.80

* Significant at 5% level of significance

ANOVA 5. Virulence of *Beauveria bassiana* isolates (1x 10^7spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	413.62	59.09	1.29	2.42
Error	24	1102.97	45.96		
Total	31	1516.59			
SEm±	3.39	SEd		CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1361.38	194.48	7.59*	2.42
Error	24	614.83	25.62		
Total	31	1976.20			
SEm±	2.53	SEd	3.58	CD	7.39

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2739.55	391.36	14.00*	2.42
Error	24	671.36	27.96		
Total	31	3410.62			
SEm±	2.64	SEd	3.74	CD	7.72

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	4139.23	591.32	15.26*	2.42
Error	24	930.10	38.75		
Total	31	5069.33			
SEm±	3.11	SEd	4.40	CD	9.09

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	5539.22	731.32	18.69*	2.42
Error	24	1016.08	42.34		
Total	31	6555.31			
SEm±	3.25	SEd	4.60	CD	9.50

* Significant at 5% level of significance

ANOVA 6. Virulence of *Beauveria bassiana* isolates (1x 10^7spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	315.52	52.59	1.00	2.57
Error	21	1103.22	52.53		
Total	27	1418.74			
SEm±	3.62	SEd	5.13	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	426.14	71.02	2.44	2.57
Error	21	611.19	29.10		
Total	27	1037.34			
SEm±	2.70	SEd	3.81	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	760.50	126.75	4.09*	2.57
Error	21	650.30	30.98		
Total	27	1410.79			
SEm±	3.49	SEd	3.93	CD	10.26

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1497.12	249.52	4.68*	2.57
Error	21	1120.14	53.34		
Total	27	2617.27			
SEm±	2.92	SEd	5.16	CD	8.58

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1905.55	317.59	5.43*	2.57
Error	21	1228.83	58.52		
Total	27	3134.38			
SEm±	3.54	SEd	5..41	CD	10.42

* Significant at 5% level of significance

ANOVA 7. Virulence of *Beauveria bassiana* isolates (1x 10^{8} spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1014.81	144.97	3.47*	2.42
Error	24	1001.76	41.74		
Total	31	2016.57			
SEm±	3.23	SEd	4.57	CD	9.43

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2375.83	339.40	4.78*	2.42
Error	24	1702.82	70.95		
Total	31	4078.64			
SEm±	4.21	SEd	5.96	CD	12.29

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3944.26	563.47	15.01*	2.42
Error	24	901.00	37.54		
Total	31	4845.26			
SEm±	3.06	SEd	4.33	CD	8.94

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	5797.13	828.16	10.61*	2.42
Error	24	1873.99	78.08		
Total	31	7671.12			
SEm±	4.42	SEd	6.25	CD	12.90

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	9563.43	1366.20	14.31*	2.42
Error	24	2291.67	95.49		
Total	31	11855.11			
SEm±	4.89	SEd	6.91	CD	14.26

* Significant at 5% level of significance

ANOVA 8. Virulence of *Beauveria bassiana* isolates (1x 10^{8} spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	617.77	102.96	2.16	2.57
Error	21	1001.76	47.70		
Total	27	1619.53			
SEm±	3.45	SEd	4.88	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	847.83	847.83	5.39*	2.57
Error	21	550.25	550.25		
Total	27	1398.08	1398.08		
SEm±	2.56	SEd	3.62	CD	7.53

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1672.15	278.69	8.50*	2.57
Error	21	688.38	32.78		
Total	27	2360.54			
SEm±	2.99	SEd	4.05	CD	8.81

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	2254.00	375.67	4.35*	2.57
Error	21	1812.50	86.31		
Total	27	4066.50			
SEm±	4.35	SEd	6.57	CD	13.66

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	3725.45	620.91	5.49*	2.57
Error	21	2374.34	113.06		
Total	27	6099.79			
SEm±	5.32	SEd	7.52	CD	15.64

* Significant at 5% level of significance

ANOVA 9. Virulence of *Beauveria bassiana* isolates (1x 10^9spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	997.04	142.43	2.52*	2.42
Error	24	1355.25	56.47		
Total	31	2352.29			
SEm±	3.76	SEd	5.31	CD	10.97

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2968.47	424.07	6.07*	2.42
Error	24	1676.53	69.86		
Total	31	4656.00			
SEm±	4.18	SEd	5.91	CD	12.20

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	6094.55	870.65	19.59*	2.42
Error	24	1066.84	44.45		
Total	31	7161.39			
SEm±	3.33	SEd	4.71	CD	9.73

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	11849.31	1692.76	15.75*	2.42
Error	24	2579.77	107.49		
Total	31	14429.08			
SEm±	5.18	SEd	7.33	CD	15.13

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	14481.18	2068.74	39.99*	2.42
Error	24	1241.60	51.73		
Total	31	15722.79			
SEm±	3.60	SEd	5.09	CD	10.50

* Significant at 5% level of significance

ANOVA 10. Virulence of *Beauveria bassiana* isolates (1x 10^9spores ml^{-1}) to *Helicoverpa armigera* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	334.37	55.73	1.51	2.57
Error	21	777.25	37.01		
Total	27	1111.62			
SEm±	3.04	SEd	4.30	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	989.64	164.94	2.87*	2.57
Error	21	1205.80	57.42		
Total	27	2195.43			
SEm±	3.79	SEd	5.36	CD	11.14

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	2638.47	439.75	9.64*	2.57
Error	21	958.16	45.63		
Total	27	3596.64			
SEm±	3.38	SEd	4.78	CD	9.93

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	4281.75	713.62	6.22*	2.57
Error	21	2408.04	114.67		
Total	27	6689.79			
SEm±	5.35	SEd	7.57	CD	15.75

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	5273.23	879.87	18.05*	2.57
Error	21	1023.43	48.73		
Total	27	6302.66			
SEm±	3.49	SEd	4.94	CD	10.27

* Significant at 5% level of significance

ANOVA 11. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

1(a) Mortality (%)

i) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	213.92	30.56	0.49	2.42
Error	24	1490.53	62.11		
Total	31	1704.45			
SEm±	3.94	SEd	5.57	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	499.45	71.35	1.94	2.42
Error	24	884.48	36.85		
Total	31	1383.93			
SEm±	3.04	SEd	4.29	CD	NS

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1154.17	164.88	2.53*	2.42
Error	24	1565.77	65.24		
Total	31	2719.94			
SEm±	4.04	SEd	5.71	CD	11.79

* Significant at 5% level of significance

ANOVA 12. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	157.57	26.26	0.62	2.57
Error	21	882.38	42.02		
Total	27	1039.95			
SEm±	3.24	SEd	4.58	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	640.65	106.77	2.37	2.57
Error	21	946.87	45.09		
Total	27	1587.52			
SEm±	3.36	**SEd**	4.75	**CD**	NS

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1478.33	246.39	3.51*	2.57
Error	21	1472.85	70.14		
Total	27	2951.18			
SEm±	4.19	**SEd**	5.92	**CD at 5%**	12.31

* Significant at 5% level of significance

ANOVA 13. Virulence of *Beauveria bassiana* isolates (1x 10^6 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

1(a) Mortality (%)

i) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	489.44	69.92	1.79	2.42
Error	24	937.53	39.06		
Total	31	1426.97			
SEm±	3.13	**SEd**	4.42	**CD**	NS

ii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	908.39	129.77	3.76*	2.42
Error	24	829.33	34.56		
Total	31	1737.72			
SEm±	2.94	**SEd**	4.16	**CD**	8.58

iii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1150.38	164.34	4.40*	2.42
Error	24	896.43	37.35		
Total	31	2046.81			
SEm±	3.06	**SEd**	4.32	**CD**	8.92

iv) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1563.42	223.35	6.30*	2.42
Error	24	850.91	35.45		
Total	31	2414.32			
SEm±	2.98	**SEd**	4.21	**CD**	8.69

* Significant at 5% level of significance

ANOVA 14. Virulence of *Beauveria bassiana* isolates (1x 10^6 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	409.68	68.28	1.53	2.57
Error	21	937.53	44.64		
Total	27	1347.20			
SEm±	3.34	**SEd**	4.72	**CD**	NS

ii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	727.35	121.23	2.45	2.57
Error	21	1039.27	49.49		
Total	27	1766.62			
SEm±	3.52	**SEd**	4.97	**CD**	10.34

iii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1609.51	268.25	6.89*	2.57
Error	21	817.79	38.94		
Total	27	2427.30			
SEm±	3.12	**SEd**	4.41	**CD**	9.18

iv) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	471.62	78.60	1.96	2.57
Error	21	842.62	40.12		
Total	27	1314.23			
SEm±	3.71	**SEd**	4.48	**CD**	NS

ANOVA 15. Virulence of *Beauveria bassiana* isolates (1x 10^7 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	213.92	30.56	1.02	2.42
Error	24	717.66	29.90		
Total	31	931.58			
SEm±	2.73	SEd	3.87	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	348.76	49.82	0.67	2.42
Error	24	1784.13	74.34		
Total	31	2132.89			
SEm±	4.31	SEd	6.10	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1049.26	149.89	4.87*	2.42
Error	24	738.19	30.76		
Total	31	1787.44			
SEm±	2.77	SEd	3.92	CD	8.09

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1652.98	236.14	4.21*	2.42
Error	24	1346.25	26.09		
Total	31	2999.23			
SEm±	3.74	SEd	5.30	CD	10.93

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1633.55	233.36	3.91*	2.42
Error	24	1432.54	59.69		
Total	31	3066.08			
SEm±	3.86	SEd	5.46	CD	11.27

* Significant at 5% level of significance

ANOVA 16. Virulence of *Beauveria bassiana* isolates (1x 10^7 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	189.08	31.51	0.92	2.46
Error	21	716.93	34.14		
Total	27	906.01			
SEm±	2.92	SEd	4.13	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	392.16	65.36	1.04	2.57
Error	21	1316.59	62.69		
Total	27	1708.75			
SEm±	3.96	SEd	5.60	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	506.52	84.42	2.29	2.57
Error	21	773.47	36.83		
Total	27	1279.99			
SEm±	3.03	SEd	4.29	CD	NS

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1079.56	179.93	1.72	2.57
Error	21	2195.32	104.54		
Total	27	3274.88			
SEm±	5.11	SEd	7.23	CD	NS

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	344.27	57.38	0.60	2.57
Error	21	1998.45	95.16		
Total	27	2342.72			
SEm±	4.88	SEd	6.90	CD	NS

ANOVA 17. Virulence of *Beauveria bassiana* isolates (1x 10^8 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	489.44	69.92	1.32	2.42
Error	24	1268.42	52.85		
Total	31	1757.87			
SEm±	3.63	SEd	5.14	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	969.11	138.44	5.14*	2.42
Error	24	646.15	26.92		
Total	31	1615.26			
SEm±	2.59	SEd	3.67	CD	7.57

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1622.44	231.78	8.50*	2.42
Error	24	654.80	27.28		
Total	31	2277.24			
SEm±	2.61	SEd	3.69	CD	7.62

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2473.79	353.40	12.79*	2.42
Error	24	662.97	27.62		
Total	31	3136.76			
SEm±	2.63	SEd	3.72	CD	7.67

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3139.52	448.50	19.76*	2.42
Error	24	544.85	22.70		
Total	31	3684.37			
SEm±	2.38	SEd	3.37	CD	6.95

* Significant at 5% level of significance

ANOVA 18. Virulence of *Beauveria bassiana* isolates (1x 10^8 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	267.87	44.64	0.74	2.57
Error	21	1268.42	60.40		
Total	27	1536.29			
SEm±	3.89	SEd	5.50	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	276.30	46.05	1.50	2.57
Error	21	646.15	30.77		
Total	27	922.45			
SEm±	2.77	SEd	8.53	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	389.23	64.87	1.67	2.57
Error	21	814.14	38.77		
Total	27	1203.37			
SEm±	3.11	SEd	4.40	CD	NS

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1105.41	184.24	4.95*	2.57
Error	21	781.33	37.21		
Total	27	1886.74			
SEm±	3.05	SEd	4.31	CD	8.97

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	841.47	140.24	4.28*	2.57
Error	21	688.45	32.78		
Total	27	1529.92			
SEm±	2.86	SEd	4.05	CD	8.42

* Significant at 5% level of significance

ANOVA 19. Virulence of *Beauveria bassiana* isolates (1x 10^9 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	845.65	120.81	4.84*	2.42
Error	24	599.66	24.99		
Total	31	1445.31			
SEm±	2.50	SEd	3.53	CD	7.29

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1906.98	272.43	7.77*	2.42
Error	24	841.23	35.05		
Total	31	2748.21			
SEm±	2.96	SEd	4.19	CD	8.64

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3504.85	500.69	13.72*	2.42
Error	24	876.15	36.51		
Total	31	4381.00			
SEm±	3.02	SEd	4.27	CD	8.82

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	4903.75	700.54	20.84*	2.42
Error	24	806.63	33.61		
Total	31	5710.38			
SEm±	2.90	SEd	4.10	CD	8.46

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	4696.91	670.99	22.34*	2.42
Error	24	720.98	30.04		
Total	31	5417.89			
SEm±	2.74	SEd	3.88	CD	8.00

* Significant at 5% level of significance

ANOVA 20. Virulence of *Beauveria bassiana* isolates (1x 10^9 spores ml^{-1}) to *Tuta absoluta* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of Variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	252.61	42.10	1.47	2.57
Error	21	599.66	28.56		
Total	27	852.27			
SEm±	2.67	SEd	3.78	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	279.20	46.53	1.14	2.57
Error	21	856.28	40.92		
Total	27	1138.48			
SEm±	3.20	SEd	4.52	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	936.01	156.00	4.30*	2.57
Error	21	762.41	36.31		
Total	27	1698.43			
SEm±	3.01	SEd	4.26	CD	8.86

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	2239.97	373.33	10.62*	2.57
Error	21	738.01	35.14		
Total	27	2977.98			
SEm±	2.96	SEd	4.19	CD	8.72

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	985.40	164.23	3.97*	2.57
Error	21	868.01	41.33		
Total	27	1853.41			
SEm±	3.21	SEd	4.55	CD	9.45

* Significant at 5% level of significance

ANOVA 21. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

1(a) Mortality (%)

i) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	82.31	11.83	0.17	2.42
Error	24	1656.15	69.01		
Total	31	1738.95			
SEm±	4.15	SEd	5.87	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	139.77	19.97	0.88	2.42
Error	24	544.51	22.69		
Total	31	684.28			
SEm±	2.38	SEd	3.37	CD	NS

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	57.84	8.26	0.50	2.42
Error	24	396.59	16.52		
Total	31	454.43			
SEm±	2.03	SEd	2.87	CD	NS

ANOVA 22. Virulence of *Beauveria bassiana* isolates (1x 10^5 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	78.78	13.13	0.83	2.57
Error	21	330.89	15.76		
Total	27	409.68			
SEm±	1.98	SEd	2.81	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	166.41	27.73	0.65	2.57
Error	21	898.23	42.77		
Total	27	1064.64			
SEm±	3.27	SEd	4.62	CD	NS

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	107.91	17.98	0.25	2.57
Error	21	1510.73	71.94		
Total	27	1618.63			
SEm±	4.24	SEd	6.00	CD	NS

ANOVA 23. Virulence of *Beauveria bassiana* isolates (1x 10^6 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

1(a) Mortality (%)

ii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	110.41	15.77	0.14	2.42
Error	24	2644.87	110.20		
Total	31	2755.28			
SEm±	5.25	SEd	7.42	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	574.99	82.14	2.28	2.42
Error	24	865.86	36.08		
Total	31	1440.84			
SEm±	3.00	SEd	4.25	CD	8.77

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	478.86	68.41	2.16	2.42
Error	24	760.32	31.68		
Total	31	1239.18			
SEm±	2.81	SEd	3.98	CD	NS

ANOVA 24. Virulence of *Beauveria bassiana* isolates (1x 10^6 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	94.54	15.76	0.22	2.57
Error	21	1512.32	72.02		
Total	27	1606.86			
SEm±	4.24	SEd	6.00	CD	NS

ii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	957.24	159.54	2.26	2.57
Error	21	1483.74	70.65		
Total	27	2440.97			
SEm±	4.20	SEd	5.94	CD	NS

iii) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	792.21	132.03	1.23	2.57
Error	21	2246.75	106.99		
Total	27	3038.96			
SEm±	5.17	SEd	7.31	CD	NS

ANOVA 25. Virulence of *Beauveria bassiana* isolates (1x 10^7 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

1(a) Mortality (%)

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	355.40	50.77	1.41	2.42
Error	24	861.45	35.89		
Total	31	1216.85			
SEm±	3.00	SEd	4.24	CD	NS

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	978.36	139.77	1.93	2.42
Error	24	1731.04	72.13		
Total	31	2709.40			
SEm±	4.25	**SEd**	6.01	**CD**	NS

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	930.77	132.97	6.42*	2.42
Error	24	497.23	20.72		
Total	31	1428.00			
SEm±	2.28	**SEd**	3.22	**CD**	6.42

* Significant at 5% level of significance

ANOVA 26. Virulence of *Beauveria bassiana* isolates (1x 10^7 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	94.54	15.76	0.50	2.46
Error	21	661.78	31.51		
Total	27	756.33			
SEm±	2.81	**SEd**	3.94	**CD**	NS

ii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	346.34	57.72	0.99	2.57
Error	21	1229.90	58.57		
Total	27	1576.24			
SEm±	3.83	**SEd**	5.41	**CD**	NS

iii) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1370.70	228.45	2.66*	2.57
Error	21	1806.00	86.00		
Total	27	3176.69			
SEm±	4.64	**SEd**	6.65	**CD**	13.64

iv) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1067.02	177.84	3.45*	2.46
Error	21	1082.25	51.54		
Total	27	2149.27			
SEm±	3.59	SEd	5.08	CD	10.56

* Significant at 5% level of significance

ANOVA 27. Virulence of *Beauveria bassiana* isolates (1x 10^8 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	82.81	11.83	0.86	2.42
Error	24	331.23	13.80		
Total	31	414.04			
SEm±	1.86	SEd	2.63	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	414.04	59.15	1.07	2.42
Error	24	1324.92	55.20		
Total	31	1738.95			
SEm±	3.72	SEd	5.25	CD	10.84

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	492.77	70.40	1.71	2.42
Error	24	988.60	41.19		
Total	31	1481.37			
SEm±	3.21	SEd	4.54	CD	9.37

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1318.18	188.31	5.22*	2.42
Error	24	865.20	36.05		
Total	31	2183.38			
SEm±	3.00	SEd	4.25	CD	8.76

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1275.51	182.22	5.03*	2.42
Error	24	869.36	36.22		
Total	31	2144.87			
SEm±	3.01	**SEd**	4.26	**CD**	8.78

* Significant at 5% level of significance

ANOVA 28. Virulence of *Beauveria bassiana* isolates (1x 10^8 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	78.78	13.13	0.83	2.57
Error	21	330.89	15.76		
Total	27	409.68			
SEm±	1.98	**SEd**	2.81	**CD**	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	267.87	44.64	0.74	2.57
Error	21	1268.42	60.40		
Total	27	1536.29			
SEm±	3.89	**SEd**	5.50	**CD**	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	865.39	144.23	1.66	2.57
Error	21	1828.95	87.09		
Total	27	2694.34			
SEm±	4.67	**SEd**	6.60	**CD**	NS

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1145.95	190.99	2.07	2.57
Error	21	1939.02	92.33		
Total	27	3084.98			
SEm±	4.80	**SEd**	6.79	**CD**	NS

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	502.50	83.75	1.47	2.57
Error	21	1194.42	56.88		
Total	27	1696.93			
SEm±	3.77	SEd	5.33	CD	NS

ANOVA 29. Virulence of *Beauveria bassiana* isolates (1x 10^9 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

1(a) Mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	434.74	62.11	1.29	2.42
Error	24	1159.30	48.30		
Total	31	1594.04			
SEm±	3.48	SEd	4.91	CD	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	871.62	124.52	1.88	2.42
Error	24	1587.06	66.13		
Total	31	2458.68			
SEm±	4.07	SEd	5.75	CD	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	1332.32	190.33	4.14*	2.42
Error	24	1103.40	45.98		
Total	31	2435.73			
SEm±	3.39	SEd	4.79	CD	9.90

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	2672.67	381.81	8.71*	2.42
Error	24	1052.12	43.84		
Total	31	3724.79			
SEm±	3.31	SEd	4.68	CD	9.66

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	3251.38	464.48	16.57*	2.42
Error	24	672.76	28.03		
Total	31	3924.13			
SEm±	2.65	**SEd**	3.74	**CD**	7.73

* Significant at 5% level of significance

ANOVA 30. Virulence of *Beauveria bassiana* isolates (1x 10^9 spores ml^{-1}) to *Bemisia tabaci* under *in-vitro* conditions

2(a) Corrected mortality (%)

i) 24 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	315.14	52.52	0.95	2.57
Error	21	1158.12	55.15		
Total	27	1473.26			
SEm±	3.71	**SEd**	5.25	**CD**	NS

ii) 48 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	407.34	67.89	0.90	2.57
Error	21	1586.26	75.54		
Total	27	1993.60			
SEm±	4.35	**SEd**	6.15	**CD**	NS

iii) 72 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1387.00	231.17	2.96*	2.57
Error	21	1637.36	77.97		
Total	27	3024.36			
SEm±	4.42	**SEd**	6.24	**CD**	12.98

iv) 96 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1665.49	277.58	3.40*	2.57
Error	21	1714.18	81.63		
Total	27	3379.67			
SEm±	4.52	**SEd**	6.39	**CD**	13.29

* Significant at 5% level of significance

v) 120 hours after treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	1573.79	262.30	6.36*	2.46
Error	21	866.20	41.25		
Total	27	2439.99			
SEm±	3.21	SEd	4.54	CD	9.44

ANOVA 31. Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Helicoverpa armigera* (2nd instar larvae)

i) 24 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	911.55	151.92	4.26*	2.19	0.30	0.42	0.84
Spore concentration	4	3615.37	903.84	25.33*	2.46	0.21	0.30	0.60
Isolate x Spore concentration	24	565.06	23.54	0.66	1.62	1.49	2.11	NS
Error	105	3746.99	35.69	-	-	-	-	-
Total	139	8838.97		-	-	-	-	-

ii) 48 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	2842.35	473.73	22.68	2.19	0.23	0.32	0.64
Spore concentration	4	8110.81	2027.70	97.07	2.46	0.16	0.23	0.46
Isolate x Spore concentration	24	583.59	24.32	1.16	1.62	1.14	1.62	NS
Error	105	2193.33	20.89	-	-	-	-	-
Total	139	13730.09		-	-	-	-	-

iii) 72 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	5619.51	936.59	29.21*	2.19	0.28	0.40	0.79
Spore concentration	4	12477.93	3119.48	97.29*	2.46	0.20	0. 29	0.57
Isolate x Spore concentration	24	1574.76	65.62	2.05	1.62	1.42	2.11	3.97
Error	105	3366.68	32.06	-	-	-	-	-
Total	139	23038.88		-	-	-	-	-

* Significant at 5% level of significance

iv) 96 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	7459.60	1243.27	25.46*	2.19	0.35	0.49	0.98
Spore concentration	4	15372.39	3843.10	78.70*	2.46	0.25	0.35	0.70
Isolate x Spore concentration	24	2437.38	101.56	2.08*	1.62	1.75	2.11	4.90
Error	105	5127.68	48.84	-	-	-	-	-
Total	139	30397.06		-	-	-	-	-

v) 120 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	12121.65	2020.28	36.80*	2.19	0.37	0.52	1.04
Spore concentration	4	17659.47	4414.87	80.42*	2.46	0.26	0.37	0.74
Isolate x Spore concentration	24	1594.78	66.45	1.21	1.62	1.85	2.62	NS
Error	105	5764.38	54.90	-	-	-	-	-
Total	139	37140.29		-	-	-	-	-

ANOVA 32. Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Tuta absoluta* (2nd instar larvae)

i) 24 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	360.20	60.03	2.05	2.19	0.27	0.38	NS
Spore concentration	4	3366.65	841.66	28.69*	2.46	0.19	0.27	0.54
Isolate x Spore concentration	24	366.63	15.28	0.52	1.62	1.35	1.91	NS
Error	105	3080.50	29.34	-	-	-	-	-
Total	139	7173.98		-	-	-	-	-

ii) 48 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	948.86	158.14	3.69*	2.19	0.33	0.46	0.92
Spore concentration	4	7370.61	1842.65	43.02*	2.46	0.23	0.33	0.66
Isolate x Spore concentration	24	503.16	20.97	0.49	1.62	1.64	2.31	NS
Error	105	4497.13	42.83	-	-	-	-	-
Total	139	13319.76		-	-	-	-	-

* Significant at 5% level of significance

iii) 72 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	1723.62	287.27	5.58*	2.19	0.36	0.42	1.01
Spore concentration	4	9699.48	2424.87	47.12*	2.46	0.26	0.30	0.72
Isolate x Spore concentration	24	549.08	22.88	0.44	1.62	2.54	2.11	NS
Error	105	5403.35	51.46	-	-	-	-	-
Total	139	17375.53		-	-	-	-	-

iv) 96 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	3215.87	535.98	16.76*	2.19	0.28	0.40	0.79
Spore concentration	4	11987.30	2996.82	93.70*	2.46	0.20	0.29	0.57
Isolate x Spore concentration	24	1327.92	55.33	1.73	1.62	1.41	2.00	NS
Error	105	3358.25	31.98	-	-	-	-	-
Total	139	19889.33		-	-	-	-	-

v) 120 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	2455.46	409.24	25.55*	2.19	0.20	0.28	0.56
Spore concentration	4	11459.76	2864.94	178.87*	2.46	0.14	0.20	0.40
Isolate x Spore concentration	24	668.09	27.84	1.74	1.62	1.00	1.41	NS
Error	105	1681.81	16.02	-	-	-	-	-
Total	139	16265.12		-	-	-	-	-

ANOVA 33. Impact of *Beauveria bassiana* isolates and spore concentration on mortality of *Bemisia tabaci* (3rd instar nymph)

i) 24 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	132.36	22.06	1.40	2.19	0.20	0.28	NS
Spore concentration	4	715.36	178.84	11.35*	2.46	0.14	0.20	0.40
Isolate x Spore concentration	24	277.32	11.55	0.73	1.62	0.99	1.40	NS
Error	105	1654.46	15.76	-	-	-	-	-
Total	139	2779.50		-	-	-	-	-

* Significant at 5% level of significance

ii) 48 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	479.85	79.98	3.64*	2.19	0.23	0.33	0.66
Spore concentration	4	3162.67	790.67	35.95*	2.46	0.17	0.24	0.47
Isolate x Spore concentration	24	490.31	20.43	0.93	1.62	1.17	1.66	NS
Error	105	2309.27	21.99	-	-	-	-	-
Total	139	6442.11		-	-	-	-	-

iii) 72 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	1801.32	300.22	4.80*	2.19	0.40	0.56	1.11
Spore concentration	4	3478.99	869.75	13.92*	2.46	0.28	0.40	0.79
Isolate x Spore concentration	24	643.45	26.81	0.43	1.62	1.98	2.80	NS
Error	105	6562.58	62.50	-	-	-	-	-
Total	139	12486.34		-	-	-	-	-

iv) 96 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	2764.28	460.71	7.84*	2.19	0.38	0.54	1.07
Spore concentration	4	7348.67	1837.17	31.27*	2.46	0.27	0.39	0.77
Isolate x Spore concentration	24	666.57	27.77	0.47	1.62	1.92	2.71	NS
Error	105	6169.45	58.76	-	-	-	-	-
Total	139	16948.97		-	-	-	-	-

v) 120 hrs after treatment

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Isolates	6	1437.20	239.53	20.54*	2.19	0.17	0.24	0.48
Spore concentration	4	7757.89	1939.47	166.35*	2.46	0.12	0.17	0.34
Isolate x Spore concentration	24	911.64	37.99	3.26*	1.62	0.85	1.21	2.39
Error	105	1224.20	11.66	-	-	-	-	-
Total	139	11330.93		-	-	-	-	-

2. Identification and standardization of economic substrate for mass production of the virulent isolate of *B. bassiana*

ANOVA 34: Spore count at different days after inoculation

ANOVA 34(a) : 10DAI

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	7.37	0.92	9.20*	2.51
Error	18	4.80	0.10		
Total	26	9.17			
SEm±	0.11	SEd	0.15	CD	0.22

DAI = Days after inoculation; * Significant at 5% level of significance

ANOVA 31(b): 20DAI

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	7.17	0.90	14.14*	2.51
Error	18	1.14	0.06		
Total	26	8.31			
SEm±	0.08	SEd	0.12	CD	0.17

ANOVA 31(c): 30DAI

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @ 5%
Treatments	8	10.38	1.30	17.23*	2.51
Error	18	1.36	0.08		
Total	26	11.74			
SEm±	0.09	SEd	0.13	CD	0.19

ANOVA 31(d): Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	7.50	0.94	3.46*	2.51
Error	18	4.88	0.27		
Total	26	12.38			
SEm±	0.17	SEd	0.25	CD	0.36

* Significant at 5% level of significance

ANOVA 35. Rate of increase in growth of *B. bassiana* (%)

(a) 10 to 20 DAI

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @ 5%
Treatments	8	2846.15	355.77	0.90	2.51
Error	18	7145.13	396.95		
Total	26	9991.27			
SEm±	6.64	SEd	9.39	CD	NS

(b) 20 to 30 DAI

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	746.20	93.28	0.24	2.51
Error	18	7119.71	395.54		
Total	26	7865.91			
SEm±	6.63	SEd	9.68	CD at 5%	NS

ANOVA 36: Economics of *Beauveria bassiana* production on different substrates

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	300126.27	37515.78	20.20*	2.51
Error	18	33430.97	1857.28		
Total	26	333557.24			
SEm±	14.37	SEd	20.32	CD at 5%	29.79

3 . To study the compatibility of virulent isolate of *B.bassiana* with various additives

3 (a). Impact of additives on growth and development of *Beauveria bassiana* on 10th day after inoculation

ANOVA 37: Radial growth (mm) at 0.1% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	12671.23	1055.94	181.78*	2.15
Error	26	151.03	5.81		
Total	38	12822.26			
SEm±	0.80	SEd	1.14	CD	1.67

ANOVA 38: Radial growth (mm) at 0.5% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	15730.21	1310.85	185.43*	2.15
Error	26	183.80	7.07		
Total	38	15914.01			
SEm±	0.89	SEd	1.25	CD	1.84

ANOVA 39: Radial growth (mm) at 1.0% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	19523.19	1626.93	220.64*	2.15
Error	26	191.71	7.37		
Total	38	19714.91			
SEm±	0.91	SEd	1.28	CD	1.88

* Significant at 5% level of significance

ANOVA 40: Mean radial growth (mm)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	15607.11	1300.59	62.09*	2.15
Error	26	544.61	20.95		
Total	38	16151.72			
SEm±	1.53	SEd	2.16	CD	3.16

ANOVA 41: Growth inhibition (%) at 0.1% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	11	4854.81	441.35	70.80*	2.22
Error	24	149.61	6.23		
Total	35	5004.43			
SEm±	0.83	SEd	1.18	CD	1.73

ANOVA 42: Growth inhibition (%) at 0.5% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	11	6933.33	630.30	68.17*	2.22
Error	24	221.90	9.25		
Total	35	7155.23			
SEm±	1.01	SEd	1.43	CD	2.10

ANOVA 43: Growth inhibition (%) at 1.0% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	11	9296.39	845.13	103.48*	2.22
Error	24	196.00	8.17		
Total	35	9492.39			
SEm±	0.95	SEd	1.35	CD	1.98

ANOVA 44: Mean growth inhibition (%)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	11	6631.37	602.85	39.12*	2.22
Error	24	369.86	15.41		
Total	35	7001.23			
SEm±	1.31	SEd	1.85	CD	2.71

ANOVA 45: Mean spore count ($1x10^9$ spores ml^{-1}) at 0.1% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	4.96	0.41	4.85*	2.15
Error	26	2.22	0.09		
Total	38	7.18			
SEm±	0.10	SEd	0.14	CD	0.20

* Significant at 5% level of significance

ANOVA 46: Mean spore count ($1x10^9$ spores ml^{-1}) at 0.5% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	5.81	0.48	7.22*	2.15
Error	26	1.74	0.07		
Total	38	7.55			
SEm±	0.09	SEd	0.12	CD	0.18

ANOVA 47: Mean spore count ($1x10^9$ spores ml^{-1}) at 1.0% concentration

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	6.57	0.55	7.96*	2.15
Error	26	1.79	0.07		
Total	38	8.35			
SEm±	0.09	SEd	0.12	CD	0.18

ANOVA 48: Mean spore count ($1x10^9$ spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	4.93	0.41	5.07*	2.15
Error	26	2.11	0.08		
Total	38	7.04			
SEm±	0.09	SEd	0.13	CD	0.20

3 (b) . Interaction effect of additives and concentrations

ANOVA 49: Radial growth (mm)

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Factor-A	12	46821.33	3901.77	577.99*	1.87	0.87	1.22	2.44
Factor-B	2	530.47	265.23	39.29*	3.11	0.41	0.58	1.17
Factor-A x B	24	1103.33	45.97	6.81*	1.65	1.50	2.12	4.23
Error	78	526.54	6.75	-	-	-	-	-
Total	116	48981.70		-	-	-	-	-

ANOVA 50: Growth inhibition (%)

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Factor-A	11	20216.59	1837.87	233.16*	1.92	0.93	1.32	2.64
Factor-B	2	286.39	143.20	18.17*	3.12	0.47	0.66	1.32
Factor-A x B	22	867.90	39.45	5.00*	1.69	1.62	2.29	4.57
Error	72	567.52	7.88	-	-	-	-	-
Total	107	21938.42		-	-	-	-	-

* Significant at 5% level of significance

ANOVA 51: Mean spore count (1x 10^9 spores ml^{-1})

Source of variation	DF	SS	MSS	F (cal)	F (tab) @5%	SEm±	SEd	CD
Factor-A	12	15.81	1.31	17.92*	0.86	0.09	0.12	0.25
Factor-B	2	4.62	2.31	31.44*	0.41	0.04	0.06	0.12
Factor- A x B	24	1.60	0.06	0.90*	1.50	0.15	0.22	NS
Error	78	5.73	0.07	-	-	-	-	-
Total	116	27.78		-	-	-	-	-

4. Shelf life studies of *B. bassiana* formulations and to evaluate the effective formulations against insect-pest complex on tomato under protected conditions

4(a) Shelf life studies of *B. bassiana* formulations under *in-vitro* conditions

ANOVA 52:Spore count in wettable powder formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	14.85	1.24	73.62*	2.02
Error	26	0.04	0.02		
Total	38	15.28			
SEm±	0.06	SEd	0.09	CD	0.19

ANOVA 53: Spore count in talc formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	6.69	0.96	29.50*	2.44
Error	16	0.52	0.03		
Total	23	7.21			
SEm±	0.09	SEd	0.13	CD	0.27

ANOVA 54: Spore count in aqueous formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	10	11.51	1.15	66.10*	2.14
Error	22	0.38	0.02		
Total	32	11.90			
SEm±	0.07	SEd	0.09	CD	0.19

ANOVA 55: Spore count in wettable powder formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	9	8.35	0.93	16.18*	2.25
Error	18	1.03	0.06		
Total	27	9.38			
SEm±	0.12	SEd	0.17	CD	0.36

* Significant at 5% level of significance

ANOVA 56: Spore count in talc formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	6.10	1.02	14.09*	2.60
Error	14	1.01	0.07		
Total	20	7.11			
SEm±	0.13	SEd	0.19	CD	0.41

ANOVA 57: Spore count in aqueous formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	9.68	1.38	32.56*	2.44
Error	16	0.68	0.04		
Total	23	10.36			
SEm±	0.10	SEd	0.15	CD	0.31

ANOVA 58: Spore reduction in wettable powder formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	12	24314.21	2026.18	86.05*	2.02
Error	26	612.19	23.55		
Total	38	24926.40			
SEm±	2.43	SEd	3.43	CD	7.05

ANOVA 59: Spore reduction in talc formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	8885.49	1480.92	24.40*	2.60
Error	14	849.53	60.68		
Total	20	9735.03			
SEm±	3.89	SEd	5.51	CD	11.81

ANOVA 60: Spore reduction in aqueous formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	9	14026.43	1558.49	50.58*	2.22
Error	20	616.19	30.81		
Total	29	14642.62			
SEm±	2.78	SEd	3.92	CD	8.19

ANOVA 61: Spore reduction in wettable powder formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	20379.71	2547.46	205.16*	2.32
Error	18	223.50	12.42		
Total	26	20603.21			
SEm±	1.76	SEd	2.49	CD	5.23

* Significant at 5% level of significance

ANOVA 62: Spore reduction in talc formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	4	7275.99	1819.00	36.57*	5.04
Error	10	49.74	49.74		
Total	14				
SEm±	3.53	SEd	4.99	CD	11.11

ANOVA 63: Spore reduction in aqueous formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	12449.37	2074.89	48.10*	2.60
Error	14	603.94	43.14		
Total	20	43053.31			
SEm±	3.28	SEd	4.64	CD	9.96

ANOVA 64: Spore viability in wettable powder formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	10	8.44	0.84	18.59*	2.93
Error	22	1.00	0.05		
Total	32	9.44			
SEm±	0.11	SEd	0.15	CD	0.31

ANOVA 65: Spore viability in talc formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	5	2.36	0.47	20.82*	2.81
Error	12	0.27	0.02		
Total	17	2.63			
SEm±	0.08	SEd	0.11	CD	0.23

ANOVA 66: Spore viability in aqueous formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	9.33	1.17	46.08*	2.32
Error	18	0.46	0.03		
Total	26	9.48			
SEm±	0.08	SEd	0.11	CD	0.24

ANOVA 67: Spore viability in wettable powder formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	9	9.27	1.03	22.64*	3.09
Error	20	0.91	0.05		
Total	29	10.18			
SEm±	0.11	SEd	0.15	CD	0.31

* Significant at 5% level of significance

ANOVA 68: Spore viability in talc formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	5	5.04	1.01	13.38*	2.81
Error	12	0.90	0.08		
Total	17	5.95			
SEm±	0.14	SEd	0.19	CD	0.42

ANOVA 69: Spore viability in aqueous formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	6	6.98	1.16	46.55*	2.60
Error	14	0.35	0.02		
Total	20	7.33			
SEm±	0.08	SEd	0.11	CD	0.24

ANOVA 70: Spore viability reduction in wettable powder formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	9	11307.35	1256.37	19.46*	2.22
Error	20	1291.47	64.57		
Total	29	12598.81			
SEm±	4.02	SEd	5.68	CD	11.85

ANOVA 71: Spore viability reduction in talc formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	4	3998.20	999.55	22.74*	3.11
Error	10	439.49	43.95		
Total	14	4437.69			
SEm±	3.31	SEd	4.69	CD	10.44

ANOVA 72:Spore viability reduction in aqueous formulation (1x 10^8 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	7	12048.56	1721.22	15.46*	2.44
Error	16	1781.62	111.35		
Total	23	13830.18			
SEm±	5.28	SEd	7.46	CD	15.82

ANOVA 73: Spore viability reduction in wettable powder formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	8	12109.70	1513.71	150.20*	2.32
Error	18	181.41	10.08		
Total	26	12291.11			
SEm±	1.59	SEd	2.24	CD	4.72

* Significant at 5% level of significance

ANOVA 74: Spore viability reduction in talc formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	4	4157.87	1043.97	13.72*	3.11
Error	10	761.05	76.11		
Total	14	4936.92			
SEm±	4.36	SEd	6.17	CD	13.74

ANOVA 75:Spore viability reduction in aqueous formulation (1x 10^9 spores ml^{-1})

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	5	4895.27	979.05	15.71*	2.81
Error	12	748.05	62.34		
Total	17	5643.33			
SEm±	3.95	SEd	5.58	CD	12.16

ANOVA 76: Effect of storage period on the virulence of *B.bassiana* formulations against *H.armigera* under *in-vitro* conditions (1x 10^8 spores ml^{-1})

i) 0 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	12811.96	4270.65	33.39*	3.29
Error	12	1534.64	127.89		
Total	15	14346.60			
SEm±	5.65	SEd	8.00	CD	16.58

ii) 90 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	6189.14	2063.05	64.95*	3.29
Error	12	381.16	31.76		
Total	15	6570.29			
SEm±	2.82	SEd	3.99	CD	8.27

iii) 180 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	3086.23	1028.74	61.78*	3.29
Error	12	199.81	16.65		
Total	15	3286.03			
SEm±	2.04	SEd	2.89	CD	5.98

iv) 270 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	2912.91	970.97	20.61*	3.29
Error	12	565.37	47.11		
Total	15	3478.28			
SEm±	3.43	SEd	4.85	CD	10.07

* Significant at 5% level of significance

v) 360 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	372.25	124.08	9.00*	3.29
Error	12	165.45	13.79		
Total	15	537.70			
SEm±	1.86	SEd	2.63	CD	5.45

ANOVA 77: Effect of storage period on the virulence of *B.bassiana* formulation against *H.armigera* under *in-vitro* conditions 1x 10^9 spores ml^{-1})

i) 0 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	15202.71	5067.57	71.72*	3.29
Error	12	847.92	70.66		
Total	15	16050.63			
SEm±	4.20	SEd	5.94	CD	12.33

ii) 90 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	6679.88	2226.63	62.84*	3.29
Error	12	425.22	35.43		
Total	15	7105.10			
SEm±	2.98	SEd	4.21	CD	8.37

iii) 180 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	3798.36	1266.12	49.90*	3.29
Error	12	304.48	25.37		
Total	15	4102.84			
SEm±	2.52	SEd	3.56	CD	7.39

iv) 270 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	3193.69	1064.56	17.19*	3.29
Error	12	743.07	61.92		
Total	15	3936.76			
SEm±	3.93	SEd	5.56	CD	11.54

v) 360 days after storage

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Treatments	3	372.25	124.08	9.00*	3.29
Error	12	165.45	13.79		
Total	15	537.70			
SEm±	1.86	SEd	2.63	CD	5.45

* Significant at 5% level of significance

4(b) To evaluate the effective formulations against insect-pest complex on tomato under protected conditions

i) Bioefficacy of *B.bassiana* formulations against *H. armigera* (2016-17)

ANOVA 78: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.05	0.01	1.82	3.49
Treatments	4	0.03	0.01	0.99	3.26
Error	12	0.11	0.01		
Total	19	0.20			
SEm±	0.05	SEd	0.07	CD	NS

ANOVA 79: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.007	0.002	2.54	3.49
Treatments	4	0.220	0.055	59.96*	3.26
Error	12	0.015	0.001		
Total	19	0.23			
SEm±	0.015	SEd	0.021	CD	0.047

ANOVA 80: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.0011	0.0004	1.05	3.49
Treatments	4	0.4938	0.1234	351.21*	3.26
Error	12	0.0042	0.0004		
Total	19	0.4991			
SEm±	0.009	SEd	0.013	CD	0.029

ANOVA 81 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.0028	0.0009	1.53	3.49
Treatments	4	0.5117	0.1279	211.24*	3.26
Error	12	0.0073	0.0006		
Total	19	0.5217			
SEm±	0.012	SEd	0.017	CD	0.038

ANOVA 82 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	1.2815	0.4272	68.47*	3.49
Treatments	4	0.2412	0.0603	9.66*	3.26
Error	12	0.0749	0.0062		
Total	19	1.5979			2
SEm±	0.039	SEd	0.056	CD	0.12

* Significant at 5% level of significance

ii) Bioefficacy of *B.bassiana* formulations against *H. armigera* (2017-18)

ANOVA 83: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.036	0.012	3.29	3.49
Treatments	4	0.025	0.006	1.73	3.26
Error	12	0.044	0.004		
Total	19	0.105			
SEm±	0.030	SEd	0.043	CD	NS

ANOVA 84: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.004	0.001	2.07	3.49
Treatments	4	0.416	0.104	164.94*	3.26
Error	12	0.008	0.001		
Total	19	0.428			
SEm±	0.013	SEd	0.018	CD	0.39

ANOVA 85: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.0009	0.0003	0.50	3.49
Treatments	4	0.6255	0.1561	256.90*	3.26
Error	12	0.0073	0.0006		
Total	19	0.6338			
SEm±	0.012	SEd	0.01	CD	0.03

ANOVA 86 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.006	0.006	2.28	3.49
Treatments	4	0.602	0.152	164.80*	3.26
Error	12	0.011	0.001		
Total	19	0.624			
SEm±	0.015	SEd	0.021	CD	0.047

ANOVA 87 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.004	0.001	2.07	3.49
Treatments	4	0.416	0.104	164.94*	3.26
Error	12	0.008	0.001		
Total	19	0.428			
SEm±	0.013	SEd	0.018	CD	0.039

* Significant at 5% level of significance

iii) Bioefficacy of *B.bassiana* formulations against *H. armigera* (Pooled)

ANOVA 88: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.03	0.029		
Replications within year	6	0.09	0.015		
Treatment	4	0.04	0.011	2.22	6.39
Year x Treatment	4	0.02	0.005	0.75	2.78
Pooled error	24	0.16	0.007		
Total	39	0.34			
SEm±	0.041	SEd	0.058		0.119

ANOVA 89: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.315	0.315		
Replications within year	6	0.011	0.002		
Treatment	4	0.618	0.154	33.43*	6.39
Year x Treatment	4	0.018	0.005	5.97	2.78
Pooled error	24	0.019	0.001		
Total	39	0.981			
SEm±	0.011	SEd	0.020	CD	0.041

ANOVA 90: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.2040	0.2044		
Replications within year	6	0.0020	0.0003		
Treatment	4	1.1136	0.2788	196.08*	6.39
Year x Treatment	4	0.0157	0.0001	2.96	2.78
Pooled error	24	0.0115	0.0005		
Total	39	1.337			
SEm±	0.0111	SEd	0.0148	CD	0.0321

ANOVA 91 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.1736	0.1736		
Replications within year	6	0.009	0.0015		
Treatment	4	1.1110	0.2777	151.91*	6.39
Year x Treatment	4	0.0073	0.0018	2.40	2.78
Pooled error	24	0.0183	0.0008		
Total	39	1.3192			
SEm±	0.014	SEd	0.0195	CD	0.0403

* Significant at 5% level of significance

ANOVA 92 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.022	0.022		
Replications within year	6	1.285	0.2185		
Treatment	4	0.645	0.1645	50.89*	6.39
Year x Treatment	4	0.013	0.003	0.92	2.78
Pooled error	24	0.082	0.003		
Total	39	2.048			
SEm±	0.02	SEd	0.041	CD at 5%	0.086

iv) Bioefficacy of *B.bassiana* formulations against *T.absoluta* (2016-17)

ANOVA 93: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.005	0.005	0.18	3.49
Treatments	4	0.029	0.007	0.75	3.26
Error	12	0.117	0.010		
Total	19	0.152			
SEm±	0.048	SEd	0.070	CD	0.152

ANOVA 94: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.010	0.003	1.17	3.49
Treatments	4	0.638	0.160	54.37*	3.26
Error	12	0.035	0.000		
Total	19	0.685			
SEm±	0.027	SEd	0.038	CD	0.084

ANOVA 95: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.010	0.003	0.63	3.49
Treatments	4	0.878	0.220	42.81*	3.26
Error	12	0.062	0.005		
Total	19	0.950			
SEm±	0.036	SEd	0.051	CD	0.110

ANOVA 96 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.008	0.003	0.15	3.49
Treatments	4	1.721	0.430	26.06*	3.26
Error	12	0.198	0.017		
Total	19	1.927			
SEm±	0.064	SEd	0.091	CD	0.198

* Significant at 5% level of significance

ANOVA 97 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.127	0.042	2.708	3.49
Treatments	4	0.638	0.160	10.185	3.26
Error	12	0.188	0.016		
Total	19	0.953			
SEm±	0.063	**SEd**	0.088	**CD**	0.193

v) Bioefficacy of *B. bassiana* formulations against *T.absoluta* (2017-18)

ANOVA 98: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.052	0.017	2.006	3.49
Treatments	4	0.080	0.020	2.302	3.26
Error	12	0.105	0.008		
Total	19	0.237			
SEm±	0.047	**SEd**	0.066	**CD**	NS

ANOVA 99: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.003	0.001	2.24	3.49
Treatments	4	0.237	0.058	124.36*	3.26
Error	12	0.006	0.001		
Total	19	0.246			
SEm±	0.011	**SEd**	0.015	**CD**	0.034

ANOVA 100: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.004	0.001	2.25	3.49
Treatments	4	0.621	0.155	258.27*	3.26
Error	12	0.007	0.001		
Total	19				
SEm±	0.012	**SEd**	0.017	**CD**	0.038

ANOVA 101 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.005	0.002	1.47	3.49
Treatments	4	0.837	0.208	196.05*	3.26
Error	12	0.013	0.001		
Total	19				
SEm±	0.016	**SEd**	0.023	**CD**	0.050

* Significant at 5% level of significance

ANOVA 102 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.055	0.018	2.24	3.49
Treatments	4	0.376	0.084	124.36*	3.26
Error	12	0.069			
Total	19				
SEm±	0.034	**SEd**	0.015	**CD**	0.117

vi) Bioefficacy of *B.bassiana* formulations against *T.absoluta* (Pooled)

ANOVA 103: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.027	0.027		
Replications within year	6	0.058	0.010		
Treatment	4	0.095	0.024	6.32	6.39
Year x Treatment	4	0.015	0.004	0.41	2.78
Pooled error	24	0.222	0.009		
Total	39	0.416			
SEm±	0.048	**SEd**	0.068	**CD**	NS

ANOVA 104: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.0002	0.0002		
Replications within year	6	0.0135	0.0023		
Treatment	4	0.8232	0.2050	15.57*	6.39
Year x Treatment	4	0.0528	0.0132	7.74*	2.78
Pooled error	24	0.0410	0.0017		
Total	39	0.9307			
SEm±	0.0210	**SEd**	0.0288	**CD**	0.060

ANOVA 105: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.009	0.009		
Replications within year	6	0.014	0.002		
Treatment	4	1.482	0.370	83.78*	6.39
Year x Treatment	4	0.018	0.004	1.54	2.78
Pooled error	24	0.069	0.003		
Total	39	1.59			
SEm±	0.027	**SEd**	0.038	**CD**	0.078

* Significant at 5% level of significance

ANOVA 106 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.002	0.002		
Replications within year	6	0.012	0.002		
Treatment	4	2.473	0.618	29.05*	6.39
Year x Treatment	4	0.085	0.021	2.42	2.78
Pooled error	24	0.211	0.009		
Total	39	2.783			
SEm±	0.047	SEd	0.066	CD	0.137

ANOVA 107 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.01	0.01		
Replications within year	6	0.13	0.02		
Treatment	4	0.83	0.21	16.62*	6.39
Year x Treatment	4	0.05	0.01	1.54	2.78
Pooled error	24	0.19	0.01		
Total	39	1.21			
SEm±	0.05	SEd	0.06	CD	0.15

vii) Bioefficacy of *B.bassiana* formulations against *B.tabaci* (2016-17)

ANOVA 108: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	1.31	0.43	14.41*	3.49
Treatments	4	0.10	0.02	0.89	3.26
Error	12	0.35	0.03		
Total	19	1.77			
SEm±	0.08	SEd	0.12	CD	NS

ANOVA 109: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.037	0.012	2.16	3.49
Treatments	4	0.418	0.105	18.58*	3.26
Error	12	0.068	0.006		
Total	19	0.523	3		
SEm±	0.038	SEd	0.053	CD	0.128

* Significant at 5% level of significance

ANOVA 110: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.017	0.006	0.71	3.49
Treatments	4	0.419	0.105	13.19*	3.26
Error	12	0.095	0.008		
Total	19	0.53			
SEm±	0.042	**SEd**	0.532	**CD**	0.141

ANOVA 111 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.022	0.007	3.59*	3.49
Treatments	4	0.642	0.161	78.20*	3.26
Error	12	0.025	0.002		
Total	19	0.688			
SEm±	0.022	**SEd**	0.032	**CD**	0.070

ANOVA 112 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.355	0.118	17.75*	3.49
Treatments	4	0.318	0.080	11.97*	3.26
Error	12	0.080	0.007		
Total	19	0.754			
SEm±	0.041	**SEd**	0.034	**CD**	0.126

viii) Bioefficacy of *B.bassiana* formulations against *B.tabaci* (2017-18)

ANOVA 113: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.49	0.16	0.82	3.49
Treatments	4	0.39	0.09	0.488	3.26
Error	12	2.41	0.20		
Total	19	3.30			
SEm±	0.22	**SEd**	0.31	**CD**	NS

ANOVA 114: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.088	0.030	8.80	3.49
Treatments	4	0.472	0.118	35.13*	3.26
Error	12	0.040	0.003		
Total	19	0.601			
SEm±	0.028	**SEd**	0.041	**CD**	0.088

* Significant at 5% level of significance

ANOVA 115: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.040	0.013	3.02	3.49
Treatments	4	0.618	0.154	34.91*	3.26
Error	12	0.053	0.004		
Total	19	0.711			
SEm±	0.033	SEd	0.047	CD	0.102

ANOVA 116 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.027	0.009	2.51	3.49
Treatments	4	0.669	0.167	46.49*	3.26
Error	12	0.043	0.004		
Total	19	0.740			
SEm±	0.030	SEd	0.042	CD	0.092

ANOVA 117 : Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	0.174	0.058	6.13*	3.49
Treatments	4	0.364	0.091	9.61*	3.26
Error	12	0.113	0.009		
Total	19				
SEm±	0.049	SEd	0.069	CD	0.150

ix) Bioefficacy of *B.bassiana* formulations against *B.tabaci* (Pooled)

ANOVA 118: Pre-treatment

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.66	0.66		
Replications within year	6	1.81	0.30		
Treatment	4	0.25	0.06	1.03	6.39
Year x Treatment	4	0.25	0.06	0.53	2.78
Pooled error	24	2.77	0.12		
Total	39	5.74			
SEm±	0.17	SEd	0.24	CD	NS

ANOVA 119: 3DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.010	0.010		
Replications within year	6	0.125	0.021		
Treatment	4	0.885	0.221	153.91*	6.39
Year x Treatment	4	0.006	0.001	0.32	2.78
Pooled error	24	0.108	0.004		

Total	39	1.135			
SEm±	0.034	**SEd**	0.047	**CD**	0.098

* Significant at 5% level of significance

ANOVA 120: 7DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.047	0.047		
Replications within year	6	0.057	0.010		
Treatment	4	1.027	0.257	95.52*	6.39
Year x Treatment	4	0.011	0.003	0.43	2.78
Pooled error	24	0.148	0.006		
Total	39	1.290			
SEm±	0.038	**SEd**	0.056	**CD**	0.115

ANOVA 121 : 10 DAS

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.014	0.014		
Replications within year	6	0.049	0.008		
Treatment	4	1.307	0.327	314.66*	6.39
Year x Treatment	4	0.004	0.001	0.37	2.78
Pooled error	24	0.068	0.003		
Total	39	1.44			
SEm±	0.027	**SEd**	0.038	**CD**	0.078

ANOVA 122: Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	0.049	0.049		
Replications within year	6	0.529	0.088		
Treatment	4	0.675	0.169	87.33*	6.39
Year x Treatment	4	0.008	0.002	0.24	2.78
Pooled error	24	0.193	0.002		
Total	39	1.454			
SEm±	0.045	**SEd**	0.063	**CD**	0.131

X) Bioefficacy of *Beauveria bassiana* formulations on tomato fruit damage by insect pest complex and fruit yield (2016-17 and 2017-18)

ANOVA 123: Tomato fruit damage by *H.armigera* (2016-17)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	237.80	79.26	3.21	3.49
Treatments	4	494.71	123.67	5.01*	3.26
Error	12	296.19	24.68		
Total	19	1028.70			

SEm±	2.48	**SEd**	3.51	**CD**	7.65

ANOVA 124: Tomato fruit damage by *H.armigera* (2017-18)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	172.47	57.49	4.48*	3.49
Treatments	4	223.19	55.79	4.35*	3.26
Error	12	153.81	12.81		
Total	19	549.49			
SEm±	1.79	**SEd**	2.53	**CD**	5.51

ANOVA 125: Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	5.36	5.36		
Replications within year	6	410.28	68.38		
Treatment	4	661.89	165.47	11.82*	6.39
Year x Treatment	4	56.02	14.00	0.75	2.78
Pooled error	24	450.01	18.75		
Total	39	1583.56			
SEm±	2.16	**SEd**	3.06	**CD**	6.31

ANOVA 126: Tomato fruit damage by *T.absoluta* (2016-17)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	79.27	26.42	1.92	3.49
Treatments	4	3710.93	927.73	67.42*	3.26
Error	12	165.11	13.75		
Total	19	3955.31			
SEm±	1.85	**SEd**	2.62	**CD**	5.71

ANOVA 127: Tomato fruit damage by *T.absoluta* (2017-18)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	16.78	5.59	0.48	3.49
Treatments	4	3411.32	852.83	73.27*	3.26
Error	12	139.66	11.63		
Total	19	3567.77			
SEm±	1.70	**SEd**	2.41	**CD**	5.25

* Significant at 5% level of significance

ANOVA 128: Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	32.59	32.59		
Replications within year	6	96.05	16.01		
Treatment	4	7116.29	1779.07	1190.47*	6.39
Year x Treatment	4	5.98	1.49	0.12	2.78
Pooled error	24	304.78	12.70		
Total	39	7555.69			
SEm±	1.78	**SEd**	2.52	**CD**	5.20

ANOVA 129: Healthy fruit yield (2016-17)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	8.10	2.70	0.19	3.49
Treatments	4	1289.19	322.29	22.93*	3.26
Error	12	168.64	14.05		
Total	19	1465.94			
SEm±	1.87	**SEd**	2.65	**CD**	5.77

ANOVA 130: Healthy fruit yield (2017-18)

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Replications	3	6.82	2.27	0.25	3.49
Treatments	4	1477.85	369.46	40.76*	3.26
Error	12	108.75	9.06		
Total	19	1593.43			
SEm±	1.50	**SEd**	2.12	**CD**	4.63

ANOVA 131: Mean

Source of variation	D.F.	SS	MSS	F (cal)	F (tab) @5%
Year	1	48.80	48.80		
Replications within year	6	14.93	2.49		
Treatment	4	2750.22	687.56	163.49*	6.39
Year x Treatment	4	16.82	4.21	0.36	2.78
Pooled error	24	277.40	11.56		
Total	39	3108.17			
SEm±	1.70	**SEd**	2.40	**CD**	4.96

* Significant at 5% level of significance

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P Swathi
Department of Entomology,
JNKVV, Jabalpur,
Madhya Pradesh, India

PN Ganga Visalakshy
Division of Entomology and
Nematology, ICAR-IIHR,
Bengaluru, Karnataka, India

SB Das
Department of Entomology,
JNKVV, Jabalpur,
Madhya Pradesh, India

Correspondence
P Swathi
Department of Entomology,
JNKVV, Jabalpur,
Madhya Pradesh, India

Potentiality of *Beauveria bassiana* strains against *Helicoverpa armigera* through laboratory bioassay

P Swathi, PN Ganga Visalakshy and SB Das

Abstract
The susceptibility of second instar *Helicoverpa armigera* (L-2) to seven strains of *Beauveria bassiana*, was tested under laboratory conditions at ICAR-IIHR, Bengaluru during 2016-17 using the larval immersion method. The highest virulence (100%) was recorded in strain-4 with LC_{50} value (2.02 x 10^5spores ml^{-1}) followed by strain-1(92.50%) with LC_{50} value (2.48 x 10^5 spores ml^{-1}) at 120 hours after treatment. Corrected mortalities ranging from 59.50 to 100% at different hours after treatment were recorded in all the strains with more profound effects in strain- 4 and strain-1.

Keywords: *Beauveria bassiana*, *Helicoverpa armigera*, biocontrol, dose–mortality relationship and LC_{50}.

1. Introduction
Helicoverpa armigera Hubner (Noctuidae: Lepidoptera) is a major pest that attacks wide range of different vegetable crops and causes serious economic damage and yield loss. It is considered as an important insect-pest in different geographical regions due to its polyphagous nature [6].
Recently increased resistance and reduced susceptibility of *H. armigera* to several insecticides have been recorded worldwide [9]. Hence, interest in bio-pesticides is growing as they are showing promise for the protection of agricultural crops [7]. One potential alternative for *H. armigera* management is the use of entomopathogenic fungi which are known for their efficacy combined with environmental safety. The role of fungal pathogens as natural enemies for vegetable insect pests has been explored and many strains have been identified. The entomopathogenic fungus *Beauveria bassiana* of deuteromycetes have emerged as a good tool for the biological control of many pests. It is an obligate killer and ubiquitous insect pathogenic fungus with a very wide host range.
The main objective of this study was to critically evaluate the potential strain of *B. bassiana* for control of *H. armigera* larvae. Therefore, the efficacy of several strains of *B. bassiana* were evaluated under laboratory conditions.

2. Materials and methods
The *in-vitro* studies were carried at Bio-control laboratory (Insect Pathology laboratory) at Division of Entomology and Nematology, ICAR-IIHR, Bengaluru during 2016-17.The experiments were carried out with seven fungal strains with Completely Randomized Design (CRD).

2.1 Rearing of *Helicoverpa armigera*
Helicoverpa armigera (Hubner) larvae were collected from tomato fields and brought into the laboratory for the second generation rearing. It was reared on artificial diet as per standard procedures given by Krishnareddy and Hanur (2015) [8] at a temperature of 27±1 °C and 60±10 % relative humidity to get pure line culture. Freshly emerged second instar larvae from pure line culture were used for experiments.

2.2 Source of *Beauveria bassiana* strains
Seven strains of *Beauveria bassiana* were obtained from different places (Table1). Fungi were cultured on Potato Dextrose Agar (PDA).Fungal cultures were kept in an incubator at 25±1 °C and 65±5% RH till further use.

Table 1: Source of *Beauveria bassiana* strains

Strains	Source
Strain - 1	National Bureau of Agriculturally Important Microorganisms (NBAIIM), Mau, Uttar Pradesh
Strain - 2	IIHR strain-1,Bengaluru, Karnataka
Strain - 3	IIHR strain-2,Bengaluru, Karnataka
Strain - 4	Isolated from *Bombyx mori* larva from Nellore, Andhra Pradesh
Strain - 5	National Bureau of Agriculturally Important Microorganisms(NBAIIM), Mau, Uttar Pradesh
Strain - 6	IIHR strain-3,Bengaluru, Karnataka
Strain - 7	IIHR strain-4,Bengaluru, Karnataka

2.3 Efficacy of *B. bassiana* strains against *H. armigera* second instar larvae

The immersion method was used in this study as described by Goettel and Inglis [3]. *Helicoverpa armigera* second instar larvae were dipped into five different spore concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores ml^{-1}) in seven different strains of *B. bassiana*. Larvae were immersed individually for 10 seconds into different fungal suspension and larvae dipped in water served as control. Treated larvae were allowed to freely crawl in a Petri dish to remove excess moisture before placing them individually onto a filter paper, which was moistened with distilled water on the first day and subsequently after every 2 days. Tomato leaves were added as food source for the larvae which were regularly replaced by fresh ones. The Petri dishes were kept in BOD at 27±1 °C, 65±5% RH. Insect mortality and sporulation was assessed daily over 5 days and dead larvae were transferred to a Petri dish containing a piece of moistened cotton to promote outgrowth and sporulation of the fungi. Ten individuals were tested in each treatment and the experiments were repeated four times.

2.4 Dose–mortality effect of *B. bassiana* against *H. armigera* second instar larvae

Corrected percent mortality was calculated using Abbott's formula [1] and prior to analysis, percent mortality was transformed and LC_{50} values were determined using Probit analysis software [12].

3. Results

All tested fungal strains of *B. bassiana* were highly virulent against second instar *H. armigera* at different concentrations. However, at higher concentration of 1 x 10^9spores ml^{-1} all the strains showed higher degree of mortality at varied time interval. Significantly highest virulence (100%) was recorded in strain-4 with LC_{50} value (2.02 x 10^5 spores ml^{-1}) followed by strain-1(92.50%) with LC_{50} value (2.48 x 10^5 spores ml^{-1}) at 120 hours after treatment (Table-2e , 4).

Similar trend for efficacy of strain-4 was observed after 24hours (Table-2a), 48 hours (Table-2b), 72 hours (Table-2c) and 96 hours (Table-2d) after treatment. In addition to higher efficacy, strain-4 also caused significantly faster death and sporulation of *H. armigera* compared to that treated with other strains of *B. bassiana* (Table-3, Figure-1and Figure-2).

Table 2a: Efficacy of different strains of *Beauveria bassiana* on *H. armigera* (2^{nd} instar) at 24hr after treatment

Dosage (spores/ml)	Strain/s						
	S-1	S-2	S-3	S-4	S-5	S-6	S-7
	Mortality of *H. armigera* larvae (%)						
$1x10^9$	12.50 (20.91)	7.50 (13.48)	7.50 (13.48)	17.50 (24.57)	7.50 (15.20)	7.50 (13.48)	10.00 (17.20)
$1x10^8$	10.00 (15.49)	5.00 (11.48)	7.50 (13.48)	15.00 (22.92)	7.50 (15.20)	5.00 (11.48)	2.50 (7.77)
$1x10^7$	5.00 (11.48)	5.00 (11.48)	2.50 (7.77)	7.50 (15.20)	2.50 (7.77)	0.00 (4.05)	2.50 (7.77)
$1x10^6$	2.50 (7.77)	2.50 (7.77)	0.00 (4.05)	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
$1x10^5$	2.50 (7.77)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
SEm±	3.95	3.72	3.62	2.75	2.63	2.91	2.90
CD@5%	NS	NS	NS	8.07	7.70	NS	8.50

Note: Values in parentheses are arcsin transformed values (x+0.5) and values outside parentheses are per-cent mortality values

Table 2b: Efficacy of different strains of *Beauveria bassiana* on *H. armigera* (2^{nd} instar) at 48 hr after treatment

Dosage (spores/ml)	Strain/s						
	Mortality of *H. armigera* larvae (%)						
	S-1	S-2	S-3	S-4	S-5	S-6	S-7
$1x10^9$	22.50 (28.07)	22.50 (28.22)	22.50 (25.99)	42.50 (40.41)	22.50 (28.22)	12.50 (19.20)	15.00 (22.92)
$1x10^8$	20.00 (26.57)	20.00 (26.57)	17.50 (22.34)	35.00 (35.81)	17.50 (24.57)	10.00 (17.20)	10.00 (18.91)
$1x10^7$	12.50 (18.85)	15.00 (22.92)	10.00 (17.20)	20.00 (23.82)	15.00 (22.92)	7.50 (13.39)	7.50 (13.48)
$1x10^6$	12.50 (16.63)	7.50 (13.48)	7.50 (15.20)	12.50 (19.20)	7.50 (15.20)	2.50 (7.77)	2.50 (7.77)
$1x10^5$	5.00	0.00	0.00	7.50	0.00	0.00	0.00

	(11.48)	(4.05)	(4.05)	(13.48)	(4.05)	(4.05)	(4.05)
Control	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)
SEm±	5.04	3.13	5.03	4.34	2.70	4.02	2.93
CD@5%	14.77	9.17	14.76	12.72	7.91	NS	8.59

Table 2c: Efficacy of different strains of *Beauveria bassiana* on *H. armigera* (2nd instar) at 72 hr after treatment

Dosage (spores/ml)	**Strain/s**						
	Mortality of *H. armigera* larvae (%)						
	S-1	**S-2**	**S-3**	**S-4**	**S-5**	**S-6**	**S-7**
$1x10^9$	52.50 (46.80)	45.00 (42.41)	42.50 (40.91)	70.00 (57.89)	37.50 (37.96)	25.00 (29.87)	25.00 (30.22)
$1x10^8$	40.00 (39.20)	32.50 (35.02)	30.00 (33.37)	55.00 (48.18)	30.00 (33.37)	20.00 (26.57)	17.50 (24.92)
$1x10^7$	27.50 (31.87)	27.50 (31.72)	27.50 (31.72)	40.00 (39.46)	25.00 (30.07)	17.50 (24.45)	15.00 (22.92)
$1x10^6$	7.50 (15.20)	22.50 (28.57)	22.50 (28.22)	30.00 (33.37)	22.50 (28.22)	10.00 (17.20)	10.00 (17.20)
$1x10^5$	5.00 (11.48)	2.50 (7.77)	12.50 (19.20)	17.50 (20.85)	5.00 (11.48)	2.50 (7.77)	2.50 (7.77)
Control	2.50 (7.77)	2.50 (7.77)	2.50 (7.77)	2.50 (7.77)	2.50 (7.77)	2.50 (7.77)	2.50 (7.77)
SEm±	4.04	2.72	3.62	4.07	3.39	3.78	3.25
CD@5%	11.85	7.99	10.62	11.93	9.93	11.08	9.53

Note: Values in parentheses are arcsin transformed values (x+0.5) and values outside parentheses are per-cent mortality values

Table 2d: Efficacy of different strains of *Beauveria bassiana* on *H. armigera* (2nd instar) at 96 hr after treatment

Dosage (spores/ml)	**Strain/s**						
	Mortality of *H. armigera* larvae (%)						
	S-1	**S-2**	**S-3**	**S-4**	**S-5**	**S-6**	**S-7**
$1x10^9$	85.00 (73.71)	57.50 (49.69)	57.50 (49.69)	90.00 (77.29)	70.00 (57.26)	42.50 (40.91)	47.50 (43.85)
$1x10^8$	65.00 (55.16)	47.50 (43.86)	50.00 (45.30)	72.50 (58.94)	50.00 (45.29)	37.50 (37.96)	37.50 (40.92)
$1x10^7$	57.50 (49.98)	45.00 (42.35)	40.00 (39.46)	57.50 (49.69)	42.50 (40.91)	32.50 (34.87)	30.00 (33.37)
$1x10^6$	47.50 (43.79)	30.00 (33.16)	32.50 (35.02)	47.50 (43.79)	45.00 (42.41)	30.00 (33.16)	20.00 (26.57)
$1x10^5$	20.00 (25.51)	22.50 (28.22)	20.00 (26.57)	27.50 (31.37)	20.00 (26.57)	5.00 (11.48)	7.50 (13.48)
Control	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)	5.00 (11.48)
SEm±	6.68	4.01	3.33	4.69	3.05	3.66	4.18
CD@5%	19.58	11.77	9.76	13.74	8.95	10.74	12.27

Table 2e: Efficacy of different strains of *Beauveria bassiana* on *H. armigera* (2nd instar) at 120hr after treatment

Dosage (spores/ml)	**Strain/s**						
	Mortality of *H. armigera* larvae (%)						
	S-1	**S-2**	**S-3**	**S-4**	**S-5**	**S-6**	**S-7**
$1x10^9$	92.50 (78.96)	62.50 (52.64)	77.50 (62.51)	100.00 (90.00)	80.00 (64.18)	65.00 (54.15)	62.50 (52.64)
$1x10^8$	82.50 (69.06)	57.50 (49.92)	72.50 (58.94)	90.00 (77.29)	65.00 (54.08)	50.00 (45.29)	57.50 (49.69)
$1x10^7$	72.50 (58.94)	47.50 (43.79)	60.00 (51.13)	72.50 (58.94)	57.50 (49.69)	42.50 (42.35)	40.00 (39.46)
$1x10^6$	65.00 (54.31)	37.50 (37.96)	42.50 (40.91)	65.00 (54.31)	47.50 (43.85)	42.50 (40.91)	35.00 (36.31)
$1x10^5$	47.50 (43.65)	22.50 (28.22)	27.50 (31.37)	50.00 (45.36)	32.50 (33.37)	12.50 (19.20)	20.00 (26.22)
Control	7.50 (15.20)	7.50 (15.20)	7.50 (15.20)	7.50 (15.20)	7.50 (15.20)	7.50 (15.20)	7.50 (15.20)
SEm±	5.51	3.83	3.38	4.46	2.84	3.63	3.39
CD@5%	16.17	11.23	9.93	13.08	8.33	10.66	9.94

Note: Values in parentheses are arcsin transformed values (x+0.5) and values outside parentheses are per-cent mortality values

Table 3: Percent corrected mortality of different strains of *B. bassiana* strains at higher concentration of $1x10^9$ spores ml^{-1}

Hours after treatment	Strains						
	S-1	S-2	S-3	S-4	S-5	S-6	S-7
24	12.50	10.00	7.50	17.50	7.50	7.50	7.50
48	22.50	15.00	22.50	42.50	17.50	12.50	28.20
72	51.30	23.10	39.50	69.23	32.50	23.10	44.70
96	84.20	44.70	55.30	89.47	68.40	39.50	50.00
120	91.90	59.50	75.50	100.00	78.40	62.20	59.50

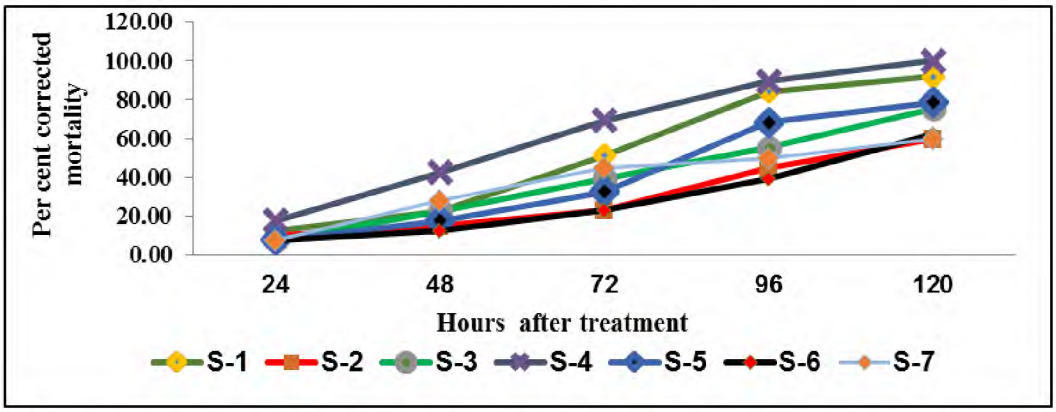

Fig 1: Percent corrected mortality of different strains of *B. bassiana* strains at different hours after treatment

Table 4: Spore concentration mortality response of *H. armigera* to different fungal strains of *B. bassiana* at 120 hrs after treatment

Strains	Heterogeneity ($\chi 2$)	Regression equation	LC_{50} (spores ml^{-1})	Fiducial limits	
				Lower	Upper
S-1	0.50	y = 0.370x + 2.995	$2.48 x10^5$	$4.55x10^4$	$1.36x10^6$
S-2	1.20	y = 0.331x + 2.342	$1.01 x10^8$	$2.19 x 10^7$	$4.65 x 10^8$
S-3	0.96	y = 0.380x + 2.398	$6.59 x10^6$	$2.17 x 10^6$	$2.00 x 10^7$
S-4	5.03	y = 0.805x + 0.452	$2.02 x 10^5$	$2.58 x 10^4$	$1.57 x 10^6$
S-5	0.43	y = 0.327x + 2.791	$5.58 x10^6$	$1.52 x 10^6$	$2.05 x 10^7$
S-6	5.34	y = 0.418x + 1.636	$1.09 x 10^8$	$2.76 x 10^7$	$4.30 x 10^8$
S-7	1.80	y = 0.358x + 2.127	$1.01 x10^8$	$2.37x 10^7$	$4.33 x 10^8$

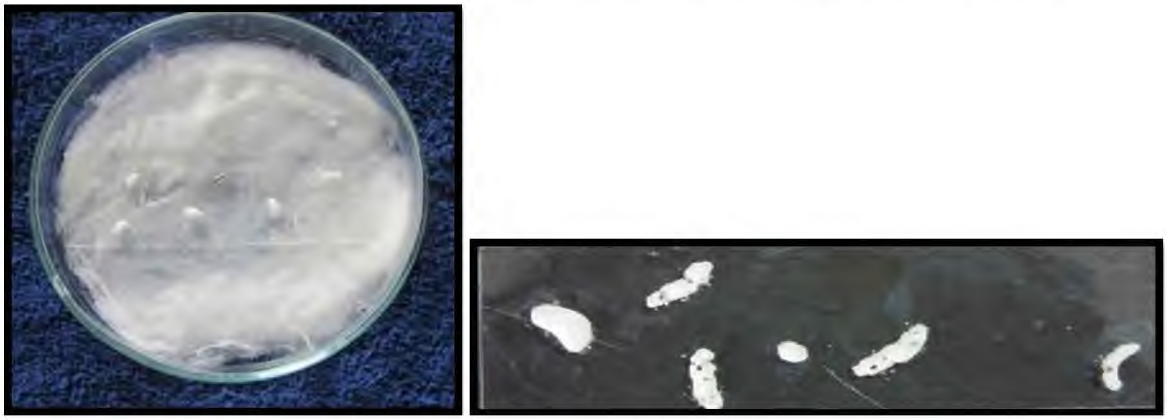

Fig 2: *Beauveria bassiana* infected larvae of *H. armigera*

5. Discussion

Entomopathogens have great potential to control *H. armigera*. All seven tested strains of *B. bassiana* showed high efficacy against second instar *H. armigera* larvae in the screening test and was in accordance with the findings of Hassani [5]. However, strain- 4 of *B. bassiana* was more virulent than the other strains. These results were similar to the previous laboratory findings demonstrating high virulence of *B. bassiana* against *H. armigera* larvae [11]. The efficiency of *B. bassiana* on *H. armigera* increased with the time and reached to 100 percent mortality after 120 hours after treatment and these observations were in accordance with study of Quesada-Moraga *et al.* that the efficiency of the entomopathogenic fungi began clearly after 48 hours after inoculation and the hyphae penetrated the integument, the trachea and the epithelial cells and after 72 hours, the fat tissue was damaged and lethality reached to 100 per cent after 96 hours [10]. In addition to high virulence, sporulation of entomopathogenic fungi on cadavers is a key factor for proliferation and spread of the disease within a pest population and is important consideration in the selection of entomopathogens for biological control [4, 7].

In the present study, high mortality levels were obtained for *H. armigera* 2nd instar larva treated with $1x10^9$spores ml^{-1}. The study revealed that there is an increase in mortality with increase in dosage in all strains against *H. armigera*. Dhembare and Siddique tested the pathogenicity of *B. bassiana* against *H. armigera* and found that per cent larval

mortality was increased with increase in spore concentration [2].

6. Conclusion

The study revealed that strain-4 is the most potent showing 100 percent mortality at 120 hours after treatment at $1x10^9$spores ml^{-1}.

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RESEARCH Open Access

In vitro evaluation for compatibility of additives with *Beauveria bassiana* (Balsamo) Vuillemin

P. Swathi[1*], P. N. Ganga Visalakshy[2] and S. B. Das[1]

Abstract

An in vitro evaluation was conducted for compatibility of 12 commonly used additives at three different concentrations of (0.1, 0.5, and 1.00%) with *Beauveria bassiana* through poisoned food technique. The results were expressed as radial growth and growth inhibition of *B. bassiana* on an additive treated medium. All the additives showed an inhibition in mycelial growth of *B. bassiana*, either partially or completely depending on their concentrations. On overall basis, carboxylmethyl cellulose (CMC) showed the highest radial growth with a least growth inhibition of (77.16 mm and 8.03%, respectively), followed by Kaolite (69.07 mm and 17.65%, respectively) and silica gel (65.20 mm and 22.25%, respectively). These findings concluded that CMC could be used in formulations of *B. bassiana* with the highest spore load of 4.67×10^8 spore's ml^{-1}.

Keywords: Compatibility, *Beauveria bassiana*, Additives, Radial growth and growth inhibition

Background

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, has attracted significant interest as a biological control agent since it infects a wide range of insect pests in diverse agro-ecosystems (Ambethgar et al. 2009). *B. bassiana* is registered biopesticide that act on a broad host range of approximately 700 insect species used for management of several crop insect pests. Entomopathogenic fungi are usually applied in the form of spores, which need a stabilizing agent to facilitate application, stability, and enhancement of activity (Meikle et al. 2008). Bioactivity of *B. bassiana* has been established against several pests at the laboratory level, while efforts are underway to simulate these results in practical scenarios and under field conditions (Mishra et al. 2013). However, successful implementation of entomopathogenic activity shown at laboratory level to field scale necessitates the development of a suitable formulation (Amutha et al. 2010). The present investigation was carried out to study the compatibility of additives with *B. bassiana* under in vitro condition.

* Correspondence: padavalaswathi107@gmail.com
[1]Department of Entomology, JNKVV, Jabalpur, India
Full list of author information is available at the end of the article

Materials and methods

The present study was conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. The experiment was carried out using a completely block design of 12 additives at three different concentrations (0.1, 0.5 and 1.00%) (Table 1) in three replicates and a control treatment.

Fungal isolate

B. bassiana was isolated from mulberry silk worm larvae, *Bombyx mori*. The fungus was grown on Potato dextrose agar slants and selected based on its virulence against tomato insect pest complex through laboratory bioassay studies with a standard concentration of 1×10^8 spore's ml^{-1}.

Experimental procedure

Effect of the additives was evaluated on the basis of radial growth and germination of *B. bassiana*. The additives including wetting agents and emulsifiers (T_1 to T_5); humectants'(T_6); desiccants (T_7 and T_8); crude/refined oils (T_9 to T_{11}); and detergent carrier (T_{12}) along with a control set were evaluated by poisoned food technique in Potato Dextrose Agar (PDA) medium (Moorhouse et al. 1992). Sterilized 20 ml PDA with the additives of

Table 1 Additives for compatibility studies of *B. bassiana*

Tr. Code	Category	Treatments	Importance	References
T_1	Wetting agents and Emulsifiers	Tween-20	Help to rehydrate spores stored dry and to disperse clumps	Burges (2012)
T_2		Tween-40		
T_3		Tween-60		
T_4		Tween-80		
T_5		Triton-X		
T_6	Humectants'	Glycerol	Delays the evaporation of the liquid and favors spore germination	Kubicek and Druzhinina (2007)
T_7	Desiccants	Kaolite	Regulate water availability to microorganisms and help in absorption of harmful metabolic by-products	Onions (1971)
T_8		Silica gel		
T_9	Oils	Sunflower oil	Improve spore survival and reduce sensitivity to UV radiations	Mishra et al. (2013)
T_{10}		Neem oil		
T_{11}		Pongamia oil		
T_{12}	Detergent carrier	Carboxylmethyl cellulose (CMC)	Enhances the ability of *B. bassiana* to reduce cellulolytic enzymes	Petlamul et al. (2017)
T_{13}	Control			

the concentrations (0.1, 0.5, and 1.0%) were incorporated into 25-mm diameter sterile petri dishes, and they were allowed to solidify under laminar flow cabinet. An agar disc along with mycelium mat of *B. bassiana* was cored with the help of cork borer and transferred onto the center of the PDA plate. Growth medium (PDA) without additive, but inoculated with mycelial disc, served as untreated check (control).The plates were sealed with parafilm and incubated at room temperature to allow maximum growth. The diameter of growing culture, i.e., the radial growth in excess of the plugs in each Petri dish, was measured on 10th day after inoculation (DAI). The data were expressed as percentage growth inhibition of *B. bassiana* by additive treated PDA (Hokkanen and Kotiluoto 1992).

$$X = \frac{Y\text{-}Z}{Y} \times 100$$

where *X*, *Y*, and *Z* stand for the percentage of growth inhibition, radial growth of fungus in untreated check, and radial growth of fungus in poisoned medium, respectively.

Results and discussion

All the additives showed significant differences relating to control in terms of all the observed parameters. Data on growth performance of *B. bassiana* 10DAI in different additives are presented in Table 2 and depicted in Fig. 1.

Radial growth and growth inhibition

Among the wetting agents and emulsifiers (T_1 to T_5) tested, Tween-80 @ 0.5% followed by Tween-80 @ 0.1% showed a maximum radial growth of 51.49 and 50.07 mm with percentage growth inhibition of 38.58 and 40.30%, respectively.

Among the different concentrations of humectants'(T_6), Glycerol @ 0.5%, followed by Glycerol @ 0.1%, presented maximum radial growth of 52.23 and 52.93 mm and growth inhibition percentage of 37.72 and 36.87%, respectively.

Among the desiccants (T_7 and T_8) tested, Kaolite @ 0.5%, followed by Kaolite @ 0.1%, showed a maximum radial growth of 73.83 and 66.48 mm and a growth inhibition percentage of 11.97 and 20.69%, respectively.

Among the tested oils (T_9 to T_{11}), sunflower oil @ 0.5%, followed by 0.1%, showed maximum radial growth of 55.45 and 45.78 mm and growth inhibition percentage of 33.98 and 45.42%, respectively.

The data further revealed that among different concentrations of the detergent carrier, i.e., CMC (T_{12}) @ highest concentration of 1.0%, showed a maximum radial growth of 81.29 mm and a growth inhibition percentage of 3.13%, respectively.

Thus, among the additives of various categories tested, CMC was relatively less toxic to *B. bassiana* at all the tested concentrations.

Spore load

Data on sporulation of *B. bassiana* in relation to additives treated media are presented in Table 2 and depicted in Fig. 1.

On the overall basis among the various additives tested at various concentrations, the highest mean spore load was recorded in control (T_{13}) (5.22×10^8 spores ml^{-1}) that was at par with CMC (T_{12}) (4.67×10^8 spores ml^{-1}). This was followed by Tween-80 (T_4) (3.44×10^8 spores ml^{-1}) which was at par with Kaolite (T_{10}) (2.89×10^8 spores ml^{-1}) and Glycerol (T_5) (2.78×10^8 spores ml^{-1}). Meanwhile, the least spore load was recorded in Tween-

Table 2 Compatibility of *Beauveria bassiana* with various additives on 10th day after inoculation

Trt. codes	Additives (Factor A)	Performance of *B. bassiana* in different additives at 3 different concentrations											
		Concentrations % (Factor B)											
		Radial growth (mm)				Growth inhibition (%)[a]				Mean spore count (1×10^8 spores ml^{-1})[b]			
		0.1	0.5	1.0	Mean	0.1	0.5	1.0	Mean	0.1	0.5	1.0	Mean
T_1	Tween-20	43.67	44.96	42.51	43.71	47.91 (43.77)	46.38 (42.91)	49.33 (44.60)	47.87 (43.76)	1.67 (1.46)	2.00 (1.56)	1.00 (1.17)	1.56 (1.43)
T_2	Tween-40	34.00	32.92	30.49	32.47	59.45 (50.43)	60.74 (51.20)	63.60 (52.87)	61.26 (51.50)	1.00 (1.17)	1.33 (1.34)	0.67 (1.05)	1.00 (1.22)
T_3	Tween-60	39.91	40.33	39.23	39.82	52.44 (46.39)	51.95 (46.09)	53.22 (46.82)	52.54 (46.43)	2.33 (1.68)	1.67 (1.46)	1.00 (1.22)	1.67 (1.46)
T_4	Tween-80	50.07	51.49	49.03	50.20	40.30 (39.35)	38.58 (38.16)	41.58 (40.14)	40.16 (39.29)	4.00 (2.11)	4.33 (2.20)	1.67 (1.46)	3.44 (1.96)
T_5	Glycerol	52.23	52.93	50.32	51.83	37.72 (37.84)	36.87 (37.32)	40.08 (39.25)	38.22 (38.15)	3.00 (1.86)	3.33 (1.95)	2.00 (1.58)	2.78 (1.80)
T_6	Triton-X	27.92	23.69	17.42	23.01	66.72 (54.76)	71.73 (57.86)	79.27 (62.90)	72.58 (58.50)	1.00 (1.17)	1.00 (1.17)	0.33 (0.88)	0.89 (1.16)
T_7	Neem oil	31.17	24.67	15.86	23.90	62.87 (52.44)	70.56 (57.14)	81.18 (64.32)	71.54 (57.92)	3.00 (1.86)	3.00 (1.86)	0.67 (1.05)	2.22 (1.61)
T_8	Pongamia oil	26.03	23.97	12.94	20.98	69.02 (56.16)	71.39 (57.71)	84.54 (66.89)	74.98 (60.20)	2.67 (1.77)	2.33 (1.68)	0.33 (0.88)	1.78 (1.46)
T_9	Sunflower oil	64.15	55.45	45.78	55.13	23.48 (28.84)	33.98 (35.60)	45.42 (42.35)	34.29 (35.63)	2.00 (1.56)	2.00 (1.56)	1.00 (1.17)	1.78 (1.50)
T_{10}	Kaolite	66.90	73.83	66.48	69.07	20.28 (26.70)	11.97 (19.74)	20.69 (26.82)	17.65 (24.63)	2.67 (1.74)	3.67 (2.04)	2.33 (1.68)	2.89 (1.83)
T_{11}	Silica gel	65.20	68.33	62.07	65.20	22.25 (27.97)	18.52 (25.37)	25.98 (30.56)	22.25 (28.06)	2.67 (1.77)	3.33 (1.93)	1.33 (1.34)	2.44 (1.70)
T_{12}	CMC	73.25	76.93	81.29	77.16	12.69 (20.72)	8.28 (16.29)	3.13 (9.34)	8.03 (15.86)	4.67 (2.26)	5.67 (2.48)	3.67 (2.04)	4.67 (2.27)
T_{13}	Control	83.95	83.95	83.95	83.95	–	–	–	–	5.33 (2.41)	5.33 (2.41)	4.67 (2.27)	5.22 (2.39)
	Mean	50.64	50.27	45.95		42.93 (40.44)	43.41 (40.45)	49.00 (43.90)		5.33 (2.41)	5.33 (2.41)	5.33 (2.41)	
	SEm±	0.80	0.89	0.91	1.53	0.83	1.01	0.95	1.31	0.10	0.09	0.09	0.09
	CD at 5%	1.67	1.84	1.88	3.16	1.73	2.10	1.98	2.71	0.20	0.18	0.18	0.20

Analyzed data for interactions

	SEm±	CD ($P = 0.05$)	SEm±	CD ($P = 0.05$)	SEm±	CD ($P = 0.05$)
Factor A	0.87	2.44	0.93	2.64	0.09	0.25
Factor B	0.41	1.18	0.47	1.32	0.04	0.12
Factor A + B	1.50	4.23	1.62	4.58	0.15	NS

[a]Arcsine transformed values
[b]Square root transformed values ($x + 0.5$)

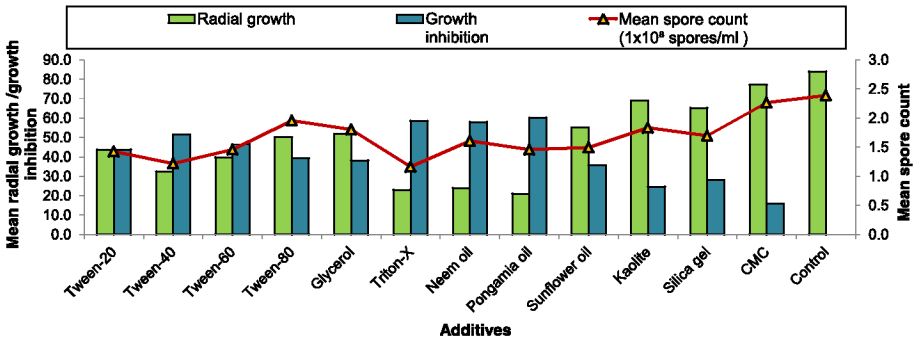

Fig. 1 Mean growth rate (mm), growth inhibition (%), and spore load of *B. bassiana* on various additives on the 10th day after inoculation

40(T_2) and Triton-X (T_6) with 1.00×10^8 spores ml^{-1} and 0.89×10^8 spores ml^{-1}, respectively.

Impact of additives and their tested concentrations on *B. bassiana*

Perusal of data in Table 2 revealed the following:

a) Radial growth and growth inhibition percentages

Factor A: additives

Different tested additives showed significant impact of the radial growth and growth inhibition percentages on *B. bassiana*.

Factor B: concentrations

Evaluation of additives at varied concentrations with *B. bassiana* revealed that they had significant impact on the radial growth and growth inhibition percentages of *B. bassiana*.

Interactions: additives × concentrations

The interaction effect of additives and concentrations had significant effect on the radial growth and growth inhibition percentages of *B. bassiana*.

b) Spore load

Factor A: additives

Different tested additives showed significant impact on the mean spore load of *B. bassiana*.

Factor B: concentrations

Evaluation of the additives at varied concentrations of *B. bassiana* revealed that they had significant impact on the mean spore load of *B. bassiana*.

Interactions: additives × concentrations

Different tested additives and concentrations had non-significant interaction effect on the spore load of *B. bassiana*.

In the present study, CMC at all the tested concentrations caused the highest mean radial growth (77.16 mm) with least inhibition percentage (8.03%) after control (83.95 mm); it was found to be relatively less toxic to *B. bassiana*. Results of the growth inhibition of *B. bassiana* in the additives were in accordance with those of Tanuja et al. (2010) who stated that the negative impact of surfactants to microorganism was probably due to increased cell permeability and amino acid leakage through inner membrane. Formulation of myco-insecticide must be compatible with the agent and must enhance its performance and, ideally, must maintain an adequate shelf-life of the agent in order to be successful (Derakhshan et al. 2008).

The highest mean spore load was recorded in T_{13} (5.22×10^8 spores ml^{-1}) which was at par with CMC (T_{12}) (4.67×10^8 spores ml^{-1}). The present results were in accordance with the findings of Petlamul et al. (2017) who revealed that *B. bassiana* had the ability to release cellulolytic enzymes on CMC for cellulose degradation to carbon source led to their growth.

The assessment of spores compatibility with surfactants is the primary requirement in successful development of surfactant based formulation, viz. emulsion. Particularly, fungi with hydrophobic conidia render the use of surfactants indispensable for laboratory bioassays and field trials (Jin et al. 2008).

Further studies need to be carried out for as a combination in order to achieve additive and develop or increase the efficacy of the fungus.

Conclusion

The findings conclude that CMC could be used in formulations of *B. bassiana* that helps to enhance its shelf life.

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Authors' contributions

All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Department of Entomology, JNKVV, Jabalpur, India. [2]Division of Entomology and Nematology, ICAR-IIHR, Bengaluru, India.

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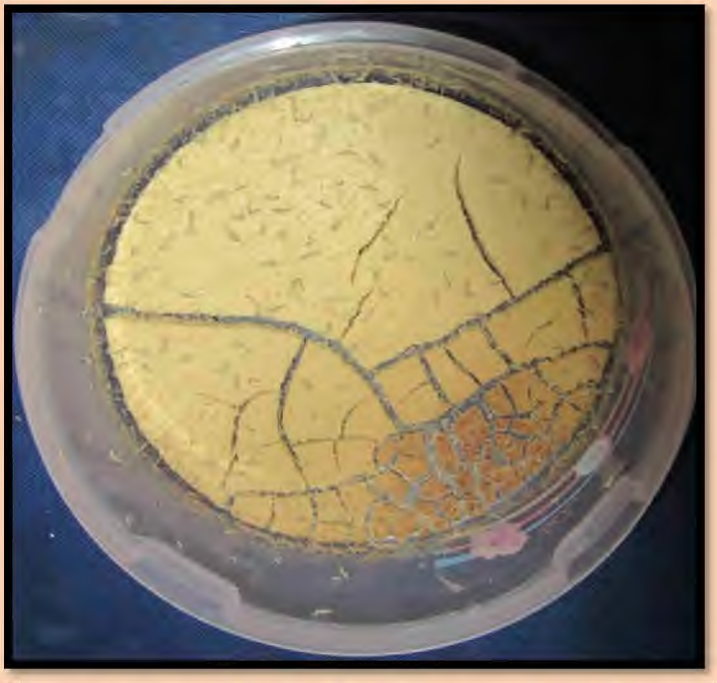


Plate 2: Rearing of *Helicoverpa armigera* larvae on artificial diet

Plate 3: *H. armigera* 2nd instar larvae (encircled) feeding on artificial diet

Plate 4 : Mass rearing of *T. absoluta* and *B. tabaci* on tomato plants under caged condition in polyhouse

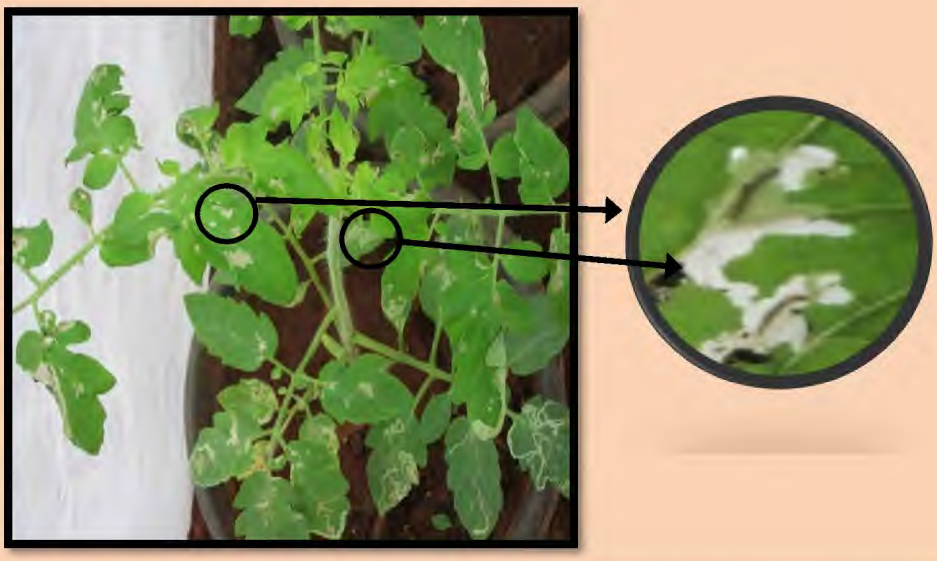
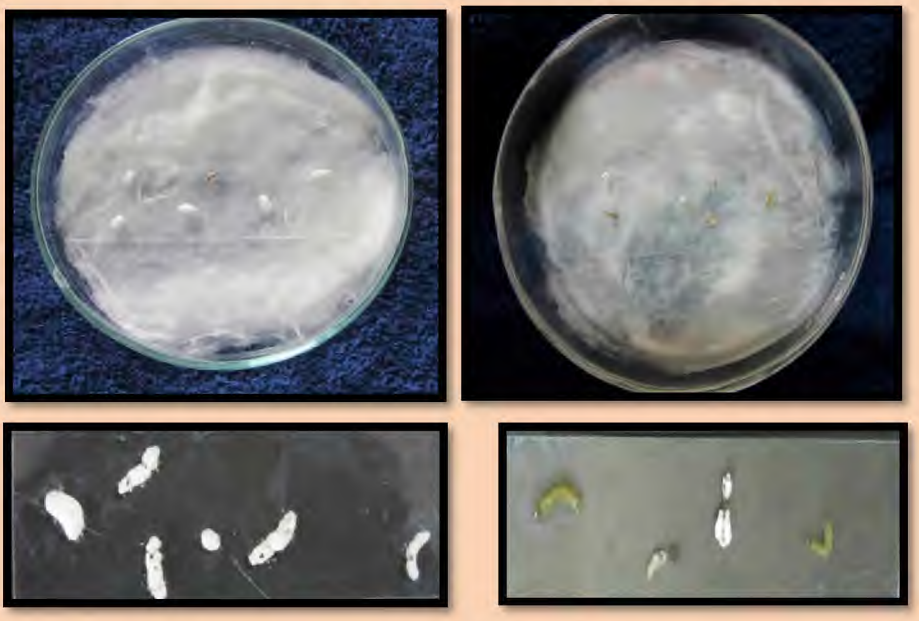


Plate 5: *T. absoluta* infested tomato plant with larvae (encircled)

6 (a) 2nd instar larvae of *H. armigera* on soaked chickpea seeds

6 (b) 2nd instar larvae of *T.absoluta* and 3rd instar nymph of *B. tabaci* on tomato leaves

Plate 6 Bioassay studies with different isolates of *B.bassiana* at varied spore concentration against tomato insect-pests



7 (a) *H.armigera* larvae

7 (b) *T.absoluta* larvae

7 (c) *B. tabaci* 3rd instar nymphal stage and adult

Plate 7 *B. bassiana* infected cadavers of tomato insect pests

Plate 9 : Methodology and establishment of *B. bassiana* on different solid substrates

a. Potato Dextrose Broth (PDB)

b. Czapek Dox Broth (CDB)

c. Sabouraud Dextrose Broth (SDB)

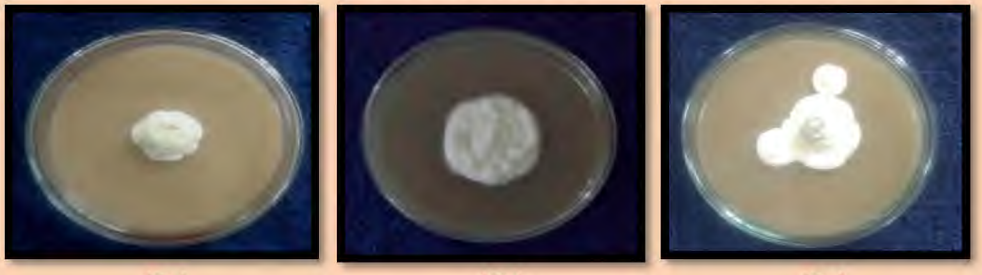
Plate 10 : Establishment of *B. bassiana* on different liquid media at 30 DAI

0.1 % concentration (C_1) 0.5 % concentration (C_2) 1.00 % concentration(C_3)

Tween- 20 (A_1)

(C_1) (C_2) (C_3)

Tween- 40 (A_2)



(C_1) (C_2) (C_3)

Tween- 60 (A_3)

(C_1) (C_2) (C_3)

Tween- 80 (A_4)

(C_1) (C_2) (C_3)

CMC (A_5)

Plate 11 a : Additives A_1 to A_5

Plate 11: Effect of different concentrations of additives on mycelial growth of *B.bassiana* (10 days after inoculation)

(C_1) (C_2) (C_3)

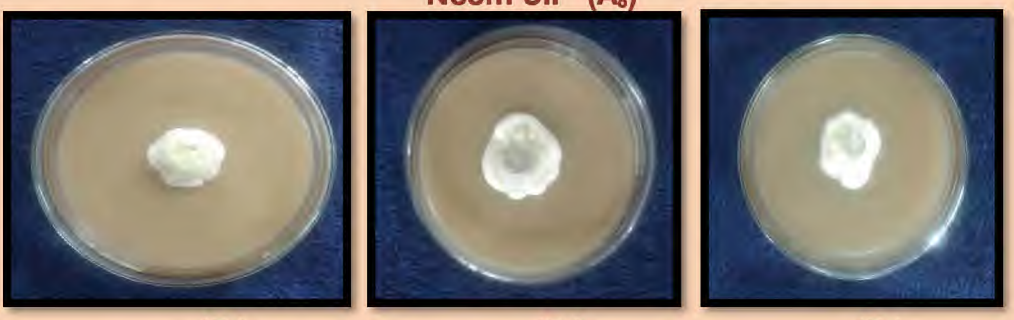
Sunflower oil (A_6)

(C_1) (C_2) (C_3)

Kaolite (A_7)

(C_1) (C_2) (C_3)

Neem oil (A_8)



(C_1) (C_2) (C_3)

Pongamia oil (A_9)

(C_1) (C_2) (C_3)

Triton - X (A_{10})

Plate 11 b :Additives: A_6 to A_{10}

(C_1) (C_2) (C_3)

Silica gel (A_{11})

(C_1) (C_2) (C_3)

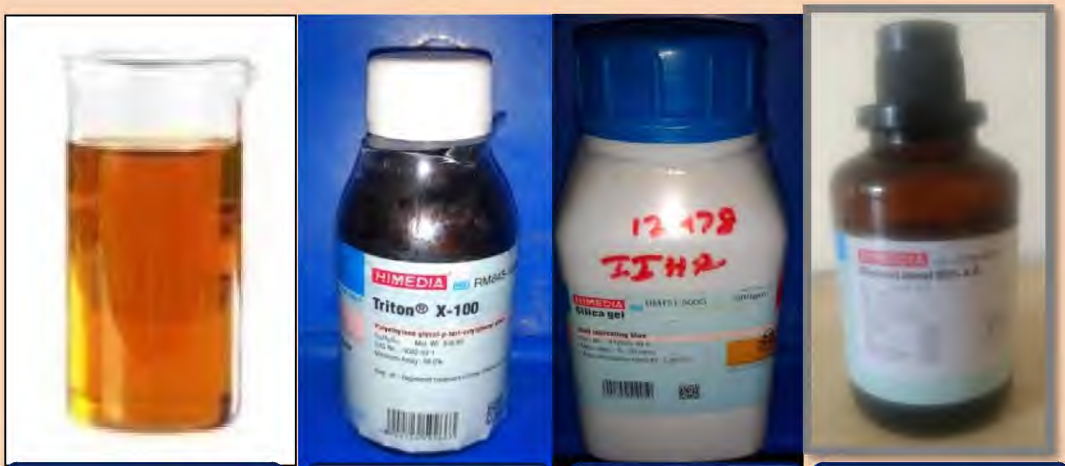
Glycerol (A_{12})

Control

Plate 11 c : Additives A_{11} to A_{12} and Control

Tween-20(A_1) | Tween-40 (A_2) | Tween-60 (A_3) | Tween-80(A_4)

CMC (A_5) | Sunflower oil (A_6) | Kaolin (A_7) | Neem oil (A_8)



Pongamia oil (A_9) | Triton-X (A_{10}) | Silica gel (A_{11}) | Glycerol (A_{12})

Plate 10: Additives used for compatibility studies of *B.bassiana*

a. Marketable healthy fruits

b. Damaged fruits

b (i). *H.armigera*

b (ii). *T.absoluta*

Plate 12 : Tomato fruits

Plate 13: *B. bassiana* infected *T.absoluta* larva

CURRICULUM VITAE

The author of this thesis, Ms. P.Swathi D/o Mr. Padavala Ramu and Smt. Padavala Sridevi was born on 16 February 1990 in Hyderabad (Telangana). After post-graduation, for further studies, she got admission in Ph.D.(Ag.) in Entomology at the College of Agriculture, JNKVV, Jabalpur (M.P) where she successfully completed all the course requirement for doctoral degree with OGPA 8.23 out of 10 point scale in the year 2018.

For the partial fulfilment of the doctoral degree she was allotted a topic entitled **"Developing effective formulation of *Beauveria bassiana* (Balsamo) Vuillemin for management of insect pest complex on tomato"**, which is successfully conducted by her and being submitted in the form of this thesis.

She was awarded with DST-INSPIRE fellowship from Department of Science and Technology, for carrying out her Ph.D research work .

She completed her schooling from K.V.Bolarum, Secundrabad in R.R. District, securing first division (82.00%) in Central Board of Secondary Examinations and her higher schooling was from Intermediate Board of Examinations, Hyderabad (Telangana) with distinction , securing 92.29%.

She joined Agricultural College, Bapatla, Acharya N G Ranga Agricultural University, in the year 2008 and successfully completed her B.Sc. (Ag.) degree in the year 2012-13 with an OGPA of 8.76 out of 10.00 scales.

Subsequently, for the post-graduation she joined the Entomology Section at College of Agriculture, Indore , Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, for Master Degree in the year 2013-2014 She completed her M.Sc. (Ag.) course with an OGPA of 8.64 out of 10.00 scale. For partial fulfillment of Masters Degree she was allotted a topic entitled **"Performance of sorghum germplasm against insect pests and succession of insect pests in established varieties"** which was successfully completed and submitted by her in the form of thesis.