'Effect of UV-protectants on the Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)'

By

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B. Sc. (Ag.)

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CERTIFICATE

Ms. Billakurthi Sabitha has satisfactorily prosecuted the course of research and that the thesis entitled **"Effect of UV-protectants on the Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)"** submitted is the result of original research work and of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by her for a degree of any University.

Date :
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(**Dr.P.V.Krishnayya**)
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CERTIFICATE

This is to certify that the thesis entitled **"Effect of UV-protectants on the Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE** in the major field of **ENTOMOLOGY** of the Acharya N.G.Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms. B. Sabitha** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I, Ms. Billakurthi Sabitha here by declare that the thesis entitled **"Effect of UV-protectants on the Pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)"** submitted to the Acharya N.G. Ranga Agricultural University for the degree of Master of Science in Agriculture in the major field of **Entomology** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier.

Date : **(B. SABITHA)**

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	=	Per cent
/	=	Per
@	=	At the rate
+	=	Plus
=	=	Is equal to
µg	=	Microgram
µl	=	Micro litre
^{0}C	=	Degree centigrade
b	=	Slope
B. t	=	*Bacillus thuringiensis* Berlinerr
CD (P=0.05)	=	Critical difference at 5 per cent probability
Cfu	=	Conidial forming units
cm	=	Centimeter
DAT	=	Days after treatment
et al.	=	And others
Fig.	=	Figure
g	=	Gram
i.e.,	=	That is
Lbs/inch^2	=	Pounds per square inch
LC_{50}	=	Median lethal concentration
LT_{50}	=	Median lethal time
Ltd.	=	Limited
M/s	=	Manufactures
ml	=	Milli litre
N.S	=	Non significant
NPV	=	Nuclear polyhedrosis virus
RH	=	Relative humidity
SDA	=	saborauds dextrose agar
SED ±	=	Standard error difference
Sig.	=	Significant
ULV	=	Ultra low volume
UV	=	Ultra violet
Viz.	=	Namely
WDP	=	Water dispersible powder
χ^2	=	Chi-square

ABSTRACT

Name	:	**B. Sabitha**
Title of the thesis	:	**'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)'**
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The present investigation entitled 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' was undertaken in the Department of Entomology, Agricultural College, Bapatla during 2004-05. The median lethal concentration (LC_{50}) values for *Beauveria bassiana* as pure culture were $20.4x10^4$ and $9.4x10^4$ spores/ml after nine and eleven days after treatment (DAT) while, the LC_{50} values for *B. bassiana* as Bio-power, a commercial formulation were $94.6x10^4$ and $56.2x10^4$ spores/ml at six and seven DAT, respectively. The LC_{50} values for *Metarhizium anisopliae* as pure culture were $265.2x10^4$ and $98.9x10^4$ spores/ml at nine and eleven DAT, respectively.

The median lethal times (LT_{50}) for *B. bassiana* as pure culture @ $25x10^4$ to $10x10^4$ spores/ml and as Bio-power @ $125x10^4$ to $50x10^4$ spores/ml, and *M. anisopliae* as pure culture @ $300x10^4$ to $100x10^4$ spores/ml ranged between 180.0 to 302.4 hours, 129.6 to 172.8 hours and 196.8 to 256.8 hours, respectively.

B. bassiana and *M. anisopliae* at their respective LC_{50}s with UV-protectants resulted significantly higher larval mortalities, which ranged between 20.61 to 71.20 per cent and 31.51 to 75.51 per cent as against 18.14 to 34.54 per cent and 21.55 to 33.17 per cent in *B. bassiana* and *M. anisopliae,* respectively without any UV-protectant for 30 minutes to three hours of exposure to UV-light.

The LT_{50} values for *B. bassiana* and *M. anisopliae* at their respective LC_{50}s with and without the UV-protectants when exposed for 30 minutes and one hour of UV- light ranged between 91.2 to 194.4 hours and 55.2 to 182.4 hours, respectively.

Whereas, the LT_{50} values of *B. bassiana* at its LC_{50} with charcoal 1% and indian ink 1% were 235.2 and 242.4 hours and LT_{50}values for *M. anisopliae* at its LC_{50} with charcoal 1%, indian ink 1%, congo red 1% and robin blue 1% ranged between 199.2 to 230.4 hours after three hours exposure to UV-light, respectively.

The fungal growths of *B. bassiana* and *M. anisopliae* at their respective LC_{50}s with UV-protectants were significantly protected (1.45 to 7.77 cm and 1.47 to 7.45 cm, diameter), when compared to their growths in (*B. bassiana* 1.40 to 5.52 cm, diameter and *M. anisopliae* 1.45 to 5.07 cm, diameter)

without any UV-protectant after 30 minutes to three hours of exposure to UV-light.

Spore productions of *B. bassiana* and *M. anisopliae* at their respective LC_{50}s with UV-protectants was significantly higher (8.75 to 617.5x10^4 spores/ml and 8.75 to 632.5x10^4 spores/ml) compared to that (*B. bassiana* 8.75 to 422.5x10^4 spores/ml and *M. anisopliae* 7.50 to 315.0x10^4 spores/ml) without any UV-protectant after 30 minutes to three hours exposure to UV-light, respectively.

CHAPTER – I

INTRODUCTION

Green revolution ushered in an era of high yielding crop varieties requiring improved agricultural practices including the use of high doses of fertilizers and pesticides. In India, the annual pesticide consumptions has increased 20-25 folds during the past four decades, from a meager value of 4000 tonnes in 1960 – 61 to 86,469 tonnes in 2001 – 02. (The Hindu, Survey of Indian Agriculture, 2003). However, there was a resurgence of pests and recent years have witnessed the development of resistance to pesticides in many pest species.

Concerns about the negative effects of chemical insecticides have led to emphasis on alternative strategies for pest control that are eco-friendly, bio-safe, economically viable and socially acceptable. There is a world wide resurgence of interest in the use of entomopathogenic fungi as biological control agents (Khachatourians, 1986). Moreover, fungi unlike bacteria and viruses, donot have to be ingested to cause infection, but can penetrate the insect cuticle directly using a combination of enzymes (Ferron, 1978).

Of all the entomopathogenic fungi that have been evaluated as pest control products, the most successful by so far has been the fungi belonging to fungi imperfecti. They are *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin, that cause fatal white and green muscardine diseases in insects by the production of a cyclodepsipeptide called, beauvericin and insecticidal metabolites, destruxins.

Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera have been recorded as hosts of *B. bassiana* (Li, 1988). Like wise *M. anisopliae* is known to attack over 200 species of insects covering seven orders including Coleopterans as the most common hosts (Yendol and Robert, 1971).

The most important limiting factor in the use of fungi as microbial agents in the field is that they require high degree of atmospheric humidity for spore germination. Further, Ultra Violet light in the natural sunlight is one of the major limiting factors against the field persistence of microbial insecticides (Edgington *et al.,* 2000). This has led to the present study, aimed at the evaluation of UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% on the pathogenicity of *B. bassiana* and *M. anisopliae* with the following objectives.

1. To fix the median lethal concentrations (LC_{50}) and median lethal times (LT_{50}) of *B. bassiana* and *M. anisopliae* against the third instar larvae of *Spodoptera litura* (Fab.), and

2. To evaluate the addition of UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% on the pathogenicity of selected entomopathogenic fungi.

CHAPTER– II

REVIEW OF LITERATURE

Entomopathogenic fungi (Mycoinsecticides) are gaining increased attention as environmentally friendly insect control agents. *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin appear to have broadest potential as viable insect control agents (Feng *et al.,* 1994). All microbial insecticides are inactivated by exposure to sunlight (Ignoffo *et al.*, 1977; Gaugler and Boush, 1979) and photoinactivation has emerged as the major environmental factor limiting their effectiveness (Cohen *et al.,* 1990). The objective of the present study is to identify whether the photo-protectant substances *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5%, and yeast extract 2% would increase the persistence of *B. bassiana* and *M. anisopliae* spores exposed for the specified durations to Ultra Violet light *viz.,* 30 minutes, one hour, three hours and five hours, and to record the intensity of the infection caused by the entomopathogenic fungi against *Spodoptera litura* (Fab.). Hence, the literature available on the influence of UV-protectants on the pathogenicity of *B. bassiana* and *M. anisopliae* has been reviewed in this chapter.

The literature published was reviewed in three main sections *viz.,* effect of *B. bassiana* against *S. litura*, effect of *M. anisopliae* against *S. litura* and effect of UV-protectants on microbial insecticides.

2.1 EFFECT OF *B. bassiana* AGAINST LEPIDOPTERAN PESTS

2.1.1 Larval Mortality:

Deva Prasad *et al.* (1989) bioassayed five isolates of three entomogeneous fungi, *B. bassiana*, *Paecilomyces fumosorosus* (Wize) Brown and Smith, and *P. farinosus* (Holm ex Gray) for their infectivity to the second instar larvae of *S. litura*. He reported that *B. bassiana* (from Bapatla) was the most virulent fungus recording the lowest LC_{50} of $19.9x10^5$ conidia/ml. The respective LC_{50}s of isolates of *B. bassiana* from Bangalore and Delhi were 5.5 $x10^8$ and $7.5x10^8$ conidia/ml.

Sivasankaran *et al.* (1990) studied the infectivity of *B. bassiana* and determined that the second and third instar larvae of the shoot borer, *Chilo infuscatellus* Snellen were more susceptible (51.47 to 65.2 per cent) even at the lowest LC_{50} of 10^5 spores/ml. At 10^7 spores/ml, the mortality observed was 68.53 to 75.93 per cent.

Gopalakrishnan and Narayanan (1990) experimented on host-pathogen relationships with *B. bassiana* against *Helicoverpa armigera* (Hub.) at four different concentrations *i.e.,* $1x0^7$,$1x10^8$, $1x10^9$ and $1x10^{10}$ spores/ml. They revealed that all the five instars were highly susceptible to $1x10^7$ and $1x 10^8$ spores/ml concentrations recording 60 to100 per cent mortality with an incubation period of 2 to15 days.

Deva Prasad *et al.* (1990) studied the infectivity of *B. bassiana*, *P. farinosus* and *P. fumosorosus* to the second instar larvae of *H. armigera.* Of them, *B. bassiana* was found to be the most potent with the lowest LC_{50} of

$2.17x10^5$ conidia/ml. The bioassay on the second, third and fourth instar larvae with Bapatla isolate of *B. bassiana* showed LC_{50} values of $1.61x10^5$, $6.45x10^5$ and $1.17x10^6$ conidia/ml, respectively.

Fluents and Carballo (1995) evaluated the pathogenicity of different isolates (447, Achi 5, 165, A4 and Achi 2) of *B. bassiana* for the control of *Plutella xylostella* (L.). The isolate 447 has the least LC_{50} of $2.2x10^5$ conidia/ml and LC_{95} of $5.1x10^7$ conidia/ml indicating the isolate 447 as the most effective one.

Kamalajayanthi and Padmavathamma (1996) conducted experiments on the infectivity of *B. bassiana* against first, second, third, fourth and fifth instar larvae of *S. litura*. The mortalities recorded at $1x10^9$ spores/ml concentration for first, second, third, fourth and fifth instar larvae were 96.67, 80.67, 80.00, 70.67 and 61.33 per cent, respectively.

Manjula and Padmavathamma (1999) conducted experiments on the infectivity of *B. bassiana* against different instars of *H. armigera.* The mean mortalities for the third instar larvae were 80.00, 74.67, 53.11, 42.11 and 40.00 per cent for the concentrations 10^9, 10^7, 10^5, 10^3 and 10^1 spores/ml, respectively.

Laboratory and net house assays of *B. bassiana* isolate CS-1 were conducted by Yoon *et al.* (1999) for the control of *P. xylostella.* Treatment of conidial suspension at various concentrations indicated that corrected mortality at $1x\ 0^8$ conidia/ml was 86.23 per cent.

Bioassay of *B. bassiana* against the second instar larvae of cotton leaf roller, *Sylepta derogata* Fab. as reported by Ramesh *et al.* (1999) showed a cumulative per cent mortality of 52.65, 58.18 and 49.49 for the concentrations $3.1x10^6$, $2.8x10^6$ and $2.9x10^6$ conidia/ml, respectively after three days of exposure.

Glorina *et al.* (2000) tested the pathogenicity of the entomopathogenic microbe, *B. bassiana* on different larval stages of *S. litura* and *Pericalia ricini* F. The LC_{50} value for *P. ricini* ranged from 8.5 to19.5 log conidia/ml and for *S. litura* it was log 11.6 to12.9 conidia/ml.

Toxicity of wettable formulation of *B. bassiana* (Daman $2x10^9$ cfu/g) was evaluated by Sood *et al.* (2001) against the third instar larvae of *S. litura*. The larval mortality varied from 6.7 to 86.7 per cent under different treatments with mean larval mortality of 56.3 ($2x10^7$ cfu/ml), 39.2 ($1x10^7$ cfu/ml) and 26.3 per cent ($5x10^6$ cfu/ml).

Mondal *et al.* (2001) evaluated the median lethal concentration of *B. bassiana* against ten day old larvae of *H. armigera*. The LC_{50} values after six days of treatment were $2.47x10^7$ and $1.06x10^7$ conidia/ml by crawling on treated leaf and direct topical spraying methods, respectively.

Haseeb *et al.* (2001) bioassayed BB 10 isolate of *B. bassiana* against the second, third, fourth and fifth instar larvae of Bihar hairy caterpillar, *Spilosoma obliqua* Walk. The LC_{50} values for the second, third, fourth and fifth instar larvae were $0.24x10^5$, $0.45x10^5$, $387.47x10^5$ and $3226.26x10^5$ spores/ml, respectively.

Dayakar and Kanaujia (2001) tested the pathogenicity of different isolates of entomopathogenic fungi, *B. bassiana* (MUCL – 38502, MUCL – 1025, MUCL – 1555 and MTCC – 2028) against the second instar larvae of *S. litura*. The LC_{50} values indicated that among the different isolates of *B. bassiana*, MUCL – 38502 was the most virulent with the lowest LC_{50} value $5.23x10^5$ conidia/ml.

Laboratory assays of two isolates of *B. bassiana* (CPD 3 and CPD 10) were conducted by Ekesi *et al.* (2002) for the control of legume pod borer, *Maruca vitrata* F. and the pod sucking bug, *Clavigralla tomentosicollis* Stal. at a concentration of $1x10^8$ conidia/ml. The larval and nymphal mortality ranged from 94 to 100 per cent in *M. vitrata* and 91 to 100 per cent in *C. tomentosicollis*.

Dayakar and Kanaujia (2003b) reported the infectivity of *B.bassiana* against different larval instars of castor spiny caterpillar, *Ergolis merione* L. The LC_{50} value for the second instar larvae was $3.6x10^5$ conidia/ml. The LC_{50} value increased by 3.02, 5.63 and 12.56 folds for third, fourth and fifth instars, respectively.

Laboratory evaluation of pathogenicity of *B. bassiana* to *S. litura* by Dayakar and Kanaujia (2003a) resulted LC_{50} values between 14.85 to 71.69x 10^5 conidia/ml against the third and fourth instar larvae of *S. litura*.

2.1.2 Lethal Time

The results of the bioassay studies by Deva Prasad *et al.* (1989 and 1990) using Bapatla isolate of *B. bassiana* against larvae of *S. litura* and

H. armigera showed that the susceptibility to infection decreased with the age of the larvae. The LT_{50} at 10^7conidia/ml was also increased with the age of the larvae showing 112.87, 120.03 and 130.86, and 101.16, 111.13 and 128.35 hours for the second, third and fourth instar larvae of *S. litura* and *H. armigera*, respectively.

Sivasankaran *et al.* (1990) reported that the time taken to kill the second, third, fourth and fifth instar larvae of *C. infuscatellus* using *B. bassiana* ranged from 3.67 to 5.73; 4.60 to 6.20; 4.90 to 6.50 and 5.40 to 6.83 days, respectively at concentrations ranging from 10^3 to 10^7 spores/ml. This revealed that the incubation period increased with increase in the age of the larvae.

Sandhu (1993) investigated different routes of infection of *B. bassiana* against *H. armigera* and reported that the pathogen was capable of infecting the larvae through all the routes tested with varying mortality rates. Hundred per cent mortality was obtained by dorsal and ventral infection.

Yoon *et al.* (1999) assayed the potential of *B. bassiana* CS-1 for the control of *P. xylostella*. The corrected mortality at $1x10^8$ conidia/ml was 86.23 per cent with LT_{50} of 1.63 days under laboratory conditions. While it was 66.5 per cent with LT_{50} of 3.61 days in the net house assay.

Haseeb *et al.* (2001) determined the pathogenicity of *B. bassiana* to different larval instars of *S. obliqua*. The LT_{50} at concentrations from 10^4 to 10^{10} conidia/ml was increased with the age of the larvae showing 76.68, 79.30, 180.11 and 188.09 hours for the second, third and fourth instar larvae, respectively.

Dayakar and Kanaujia (2001) studied the infectivity of four isolates of *B. bassiana* (MUCL – 38502, 1025, 1555 and MTCC- 2028) to the second instar larvae of *S. litura* through laboratory bioassays. Of the four strains, MUCL – 38502 was the most aggressive with an LT_{50} of 4.45 days.

Dayakar and Kanaujia (2003b) evaluated the virulence of *B. bassiana* to different instars of *E. merione*. The LT_{50} at conidial concentration of $5x10^7$ conidia/ml showed 4.07, 4.45, 5.08 and 5.68 days for the second, third, fourth and fifth instar larvae, respectively.

The results of the bioassay studies by Dayakar and Kanaujia (2003a) using *B. bassiana* against the third and fourth instar larvae of *S. litura* showed an LT_{50} ranging between 5.13 to 6.45 days.

2.2 EFFECT OF *M. anisopliae* AGAINST LEPIDOPTERAN PESTS

2.2.1 Larval Mortality:

Laboratory evaluation of pathogenicity of *M. anisopliae* to *H. armigera* by Gopalakrishnan and Nayaranan (1988) showed that the fungus was highly virulent inflicting 100 per cent mortality to all the instars except in the case of fifth instar, where the mortality was 80 per cent with a spore suspension of 1.8 $x10^9$ spores/ml.

Gopalakrishnan and Narayanan (1989) studied the infectivity of *M. anisopliae* to larval instars, pre pupae and pupae of *H. armigera*. The results showed 80 to 100 per cent mortality in all stages with the conidial suspension concentration of $1.8x10^9$ conidia/ml.

Dayakar and Kanaujia (2001) tested the pathogenicity of different isolates of entomopathogenic fungi, *M. anisopliae* (MUCL – 8237, 4181 and Lucknow) against the second instar larvae of *S. litura*. The LC_{50} values indicated that among the different isolates of *M. anisopliae*, MUCL – 8237 was the most infective with LC_{50} value of $12.53x10^5$ conidia/ml.

Laboratory assays with two isolates of *M. anisopliae* (CPD 5 and 12) were conducted by Ekesi *et al.* (2002) for the control of *M. vitrata* and *C. tomentosicollis* at a concentration of $1x10^8$ conidia/ml. The larval and nymphal mortality ranged from 94 to 100 per cent in *M. vitrata* and 91 to 100 per cent in *C. tomentosicollis*.

Dayakar and Kanaujia (2003b) conducted experiments on the infectivity of *M. anisopliae* against different larval instars of *E. merione*. The LC_{50} for the second instar larvae was $9.8x10^5$ conidia/ml. The LC_{50} value increased by 1.94, 4.15 and 8.54 folds for third, fourth and fifth instars, respectively.

Laboratory evaluation of the pathogenicity of *M. anisopliae* to *S. litura* by Dayakar and Kanaujia (2003a) resulted an LC_{50} ranged from 14.85 to 71.69 $x10^5$ conidia/ml against the third and fourth instar larvae of *S. litura*.

Studies conducted by Ajay Kumar Pandey and Kanaujia (2003) on the pathogenicity of two isolates of *M. anisopliae* cultured on SDA medium supplemented with and without larval extract were carried out against *S. litura* indicated that the virulence of both the isolates increased when cultures were grown on SDA medium supplemented with larval extract. The LC_{50} values of pantnagar isolate and MUCL – 8237 grown on SDA medium supplemented

with larval extract were 1.08 and $1.42x10^6$ conidia/ml, 1.73 and $6.42x10^6$ conidia/ml and 2.65 and $7.58x10^6$ conidia/ml against the second, third and fourth instar larvae, respectively.

Kulat *et al.* (2003) assessed the virulence of *M. anisopliae* on *H. armigera*. The fungus at $2.28x10^{10}$ conidia/ml caused highest cumulative mean larval mortality of 97.5 per cent followed by 92.5, 85.0, 80.0 and 67.5 per cent in the fungal suspensions containing $2.28x\ 10^9$, $2.28x10^8$, $2.28x10^7$ and $2.28x10^6$ conidia/ml, respectively at 192 hours after treatment.

Pathogenicity studies on *M. anisopliae* conducted by Wadyalkar *et al.* (2003) with 10^8 spores/ml concentration of fungal suspension revealed larval mortality of 100.00, 90.00, 76.67 and 56.67 per cent against the first, second, third and fourth instar larvae of *H. armigera*.

2.2.2 Lethal Time

Dayakar and Kanaujia (2001) studied the infectivity of three isolates of *M. anisopliae* (MUCL – 8237, 4181 and Lucknow) to the second instar larvae of *S. litura* through laboratory bioassays. Of the three strains, MUCL – 8237 was the most aggressive with an LT_{50} of 4.94 days.

Dayakar and Kanaujia (2003b) evaluated the virulence of *M. anisopliae* to different instars of *E. merione*. The LT_{50} at $5x10^7$ conidia/ml spore suspension were 4.32, 4.75, 5.49 and 6.09 days for the second, third, fourth and fifth instar larvae, respectively. The LT_{50} values increased by 1.10, 1.27 and 1.41 folds for third, fourth and fifth instars, respectively.

The results of the bioassay studies by Dayakar and Kanaujia (2003a) using *M. anisopliae* against the third and fourth instar larvae of *S. litura* showed LT_{50} values ranging between 5.13 to 6.45 days.

Studies conducted by Ajay Kumar Pandey and Kanaujia (2003) on the pathogenicity of the two isolates of *M. anisopliae* (Pantnagar and MUCL–8237) cultured on SDA medium supplemented with and without larval extract against *S. litura* showed LT_{50} values of 154.9, 157.1 and 161.1 hours, respectively in case of pantnagar isolate and 158.4, 168.3 and 169 hours in case of MUCL–8237 to the second, third and fourth instar larvae, respectively.

The bioassay studies of *M. anisopliae* against the second instar larvae of *H. armigera* by Kulat *et al.* (2003) resulted LT_{50} values of 79.43 hours for 2.28 $x10^{10}$, 85.11 hours for $2.28x10^{9}$, 97.72 hours for $2.28x10^{8}$, 104.71 hours for $2.28x10^{7}$ and 123.02 hours for $2.28x10^{6}$ conidia/ml of fungal suspensions.

2.3 EFFECT OF UV-PROTECTANTS ON MICROBIALS

2.3.1 Effect of Charcoal as UV-protectant

Pishokha and Timchenko (1988) studied the effect of various additives including charcoal on Gomelin (*Bacillus thuringiensis* Ber.) against inactivation by light. Charcoal had the best protective effect, which preserved the insecticidal activity of the preparation up to 12 days longer than that of the control without additive.

Im *et al.* (1990) reported that viral insecticide formulations when combined with UV-protectants like white carbon 1%, persisted for five and ten

days when sprayed on the upper surface of the leaves and on the lower surface of the leaves, respectively.

Ramakrishnan and Chaudhary (1991) reported that addition of UV-light protectants including charcoal (0.5 and 1%) provided protection for nuclear polyhedrosis virus (NPV) of *S. litura* against the far and near UV-light. In case of far UV-protection, it was concentration dependent and charcoal 1% resulted in 100 per cent mortality of larvae.

Koa and Huang (1992) reported that addition of 1% activated carbon to a viral suspension of NPV provided significant UV-protection.

Gailce Leo Justin *et al.* (1999) stated that original activity of *B. t.* was enhanced as evidenced by the mortality of diamond back moth larvae, which was maximum with UV-protectants *viz.,* yeast extract 2% (34%) followed by indian ink 1% (24%) and carbon 1% (22%) as against 8 per cent in *B. t.* alone.

Srivastava and Prasad (2000) compared WDP formulation of *B. t. k.* named Pusa *B. t.* containing charcoal 10% as UV-protectant with a commercial formulation of *B. t.,* HIL *B. t. k.* of M/s Hindustan Insecticide Limited, India against *S. litura* and recorded 83 per cent larval mortality in Pusa *B. t.* compared to HIL *B. t. k.* with 73 per cent.

2.3.2 Effect of Indian ink as UV-protectant

Pishokha and Timchenko (1988) studied the effect of various additives including black ink on bacterial preparation, Gomelin (*B. t.*) against inactivation by light. It had best protective effect, which preserved the

insecticidal activity of the preparation up to 12 days longer than that of the control without additive.

Ramakrishnan and Chaudhary (1991) reported that addition of UV-light protectants including black ink (0.5 and 1%) provided protection for NPV of *S. litura* against the far and near UV-light.

Padmavathamma and Veeresh (1995) reported that indian ink 2%, when added as sunlight protectant to NPV of *P. xylostella* caused the greatest reduction in populations of *P. xylostella*.

Gailce Leo Justin *et al.* (1999) stated that original activity of *B. t.* was enhanced as evidenced by the mortality of diamond back moth larvae, which was maximum with UV-protectants *viz.,* yeast extract 2% (34%) followed by indian ink 1% (24%) and carbon 1% (22%) as against 8 per cent in *B. t.* alone.

2.3.3 Effect of Congo red as UV-protectant

Shaprio (1989) proved that the addition of congo red provided significant UV-protection to NPV of the gypsy moth, *Lymantria dispar* (L.). At 1.0% concentration of the dye, 100 per cent of the original viral activity remained after 60 minutes of exposure to UV-radiation.

Dunkle and Shasha (1989) encapsulated crystals and spores of *B. t.* with congo red and folic acid as UV-screens. Congo red 1%was the most effective treatment followed by folic acid and retained at least 50 per cent of their original toxicity after 12 days.

Michael *et al.* (1990) studied the effect of *B. t. sub species kurstaki* Ber. encapsulated by corn starch granules with the feeding stimulant coax and UV- screen congo red 1% against European corn borer, *Ostrinia nubilalis* (Hub.). Congo red showed UV absorbing properties and prolonged activity of *B. t.* for up to 12 days in laboratory tests when incorporated into starch granules.

Michael *et al.* (1991) conducted experiments on different formulations of *B. t.* containing feeding stimulant and UV-light screens. The formulation with congo red 13% as UV-light screen produced 35 per cent mortality as compared to control (31%) after exposing for a period of 21 days.

Baskaran *et al.* (1998) evaluated the effect of congo red in different concentrations (0.1 to 1%) suspended in NPV of groundnut red hairy caterpillar, *Amsacta albistriga* Wlk. (Aa NPV). Its effect was concentration dependent and wet viruses were comparatively resistant to UV-rays than the dry viruses. It resulted in 83.3 and 100.0 per cent of original viral activity remained, respectively after 60 minutes of exposure to UV-radiation.

Prabakaran *et al.* (2000) assessed the effect of UV-screens (congo red 0.1%, methyl green 0.1% and acriflavin 0.1%) added to alginate encapsulated, slow release and floating formulations of *B. t.* variety *israelensis* (*B. t. i.*) toxin. The results obtained indicated that all UV-screens used gave significant level of protection to *B. t. i.* toxin from inactivation by sunlight even after exposure to four hours of direct sunlight daily till 45th day. Compared to acriflavin or methyl green, congo red provided excellent UV-protection.

Hoffmann *et al.* (2000) tested the effect of three chemical protectants *viz.,* P-amino benzoic acid (PABA), congo red and flourescent brightner 28 for their ability to decrease the rate of degradation of ultra low volume malathion. Congo red 1% offered less degradation of 10.8 per cent by six days after treatment.

Prasad *et al.* (2003) concluded that the overall performance of a WDP formulation of *B. t.,* containing 1% (w/w) congo red 1% as UV-protectant was superior to ranipal and untreated check.

2.3.4 Effect of Robin blue as UV-protectant

Rabindra and Jayaraj (1988) reported that when NPV was applied to *Cicer arietinum* L. plants @ 10^5 polyhedral bodies/ml and exposed to field conditions, its persistence was increased by the addition of robin blue 1%.

Rabindra *et al.* (1989) reported that addition of robin blue (0.5%) to ULV sprays of NPV effectively controlled the noctuid pests, reduced pod damage and significantly increased yields of chickpea.

Laboratory evaluation by Reddy *et al.* (2001) revealed that persistence of a virus can be raised by the addition of robin blue 1% which acts as UV-protectant for NPV. Larval mortality in *H. armigera* after five days of persistence was 74.7 per cent (NPV + robin blue) and 59.1 per cent in NPV alone.

2.3.5 Effect of Yeast extract as UV-protectant

Gailce Leo Justin *et al.* (1999) stated that original activity of *B. t.* was enhanced as evidenced by the mortality of diamond back moth larvae which was maximum with UV-protectants *viz.,* yeast extract 2% (34%) followed by indian ink 1% (24%) and carbon 1% (22%) as against 8 per cent in *B. t.* alone.

CHAPTER – III

MATERIALS AND METHODS

The present investigation on the 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' was carried out under laboratory conditions in the Department of Entomology, Agricultural College, Bapatla during 2004-05. The pathogenicity of *B. bassiana* as pure culture and a commercial formulation, and *M. anisopliae* as pure culture was evaluated against the third instar larvae of *Spodoptera litura* (Fab.). Further, both the entomopathogens were tested for their bioefficacy, growth and spore production with and without Ultra Violet (UV)-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5%, and yeast extract 2% after exposing them to UV-light (30 watts) for 30 minutes, one hour, three hours and five hours. The different materials used in conducting the experiment and methods employed during the course of investigations are detailed in this chapter.

3.1 TEST INSECT

The tobacco caterpillar, *S. litura* was reared on the leaves of castor, *Ricinus communis* (L.) in the laboratory under hygenic conditions at room temperature (27 ± 2^{O}C) with egg masses collected from the field, which formed the initial culture.

3.1.1 Rearing of the Test Insect

The test insect was reared in cages, after disinfecting them with four per cent formaldehyde and sun drying. The glasswares were cleaned with glass cleaning solution (concentrated/diluted potassium dichromate + concentrated sulphuric acid) and subsequently swabbed with four per cent formaldehyde and sterilized in UV-light chamber for two to three hours before use.

The egg masses were kept for hatching between two young tender castor leaves in pneumatic plastic troughs (25 cm height and 15 cm diameter) and the petioles of the leaves were dipped in water in a small conical flask to keep leaves fresh throughout the day. The open ends of the troughs were closed with white muslin cloth. Every day, the hatched larvae were changed to new containers with fresh leaves and the used containers were cleaned and sterilized regularly. As the larvae grew larger, the number of larvae per glass jar (15 x 15 cm) was limited to 20 to 25 to give sufficient space. The grown up larvae were fed twice a day with fresh clean castor leaves (Plate 1).

When the larvae reached pre-pupal stage, they were transferred to glass jars containing a layer (15 to 20 cm) of fine sterilized moist sand. After three to four days of pupation, pupae were taken out and kept in a glass jar lined inside with butter paper. The emerged adults were transferred to fresh glass jar with butter paper placed along the inner walls and they were fed with four per cent honey solution through a cotton swab afresh daily. The egg batches laid on the butter paper were collected day wise and maintained separately in between two castor leaves with petioles dipped in water till the

eggs hatched. Different overlapping generations of the test insect were maintained. Intermittently field collected egg batches were added to the culture to maintain the vigour.

3.2 INSECT PATHOGENS

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* as pure cultures and Bio-power, a commercial formulation of *B. bassiana* (M/s T Stanes Ltd., Coimbatore.) were used in different bioassays against *S. litura.*

3.2.1 Culturing of Insect Pathogens

The test cultures of *B. bassiana* and *M. anisopliae,* which were popularly known as white muscardine and green muscardine fungi, respectively were obtained from Biological control laboratory, ANGRAU, Rajendra Nagar, Hyderabad and were subcultured on Saboraud's Dextrose Agar (SDA) medium (Delarosa *et al.* 1997).

3.2.1.1 Preparation of saborauds dextrose agar medium

The different ingredients of the medium used were dextrose (40g), neopeptone (10g), agar agar (20g), yeast extract (2g) and distilled water (1000 ml). The medium was prepared by adding 20 g of agar powder to 500 ml of distilled water while boiling. Other ingredients were dissolved in the remaining 500 ml of distilled water and added to the gelating agar solution with constant stirring. The medium was transferred to 5 to 6 conical flasks (250 ml). The conical flasks were sealed with cotton plugs and sterilized for 20 minutes at 15 Lbs/inch^2 in an autoclave. The solidified medium was stored at 4^{0}C in a refrigerator.

3.2.1.2 Inoculation and harvesting of spores

The solidified SDA medium was melted in a hot waterbath and poured into the sterilized petriplates in aseptic conditions using UV-laminar air flow chamber. The pure cultures of *B. bassiana and M. anisopliae* were inoculated into sterilized petriplates after the molten media was cooled to 44^0C just before the solidification of the media. Sporulation was observed in five to eight days after the inoculation of the fungus. The pure cultures were maintained continuously by further sub culturing. The spores were harvested aseptically using 10 ml of sterile water and the number of spores were counted by using improved Neaubar haemocytometer and mono-occular compound microscope at 40x (Plates 2 and 3). This served as stock solution and different working concentrations were prepared from the stock solution by serial dilution. The left over stock suspension of pure culture was stored in a sterile test tube plugged with cotton at 4^0C in refrigerator for further use (Plates 4 and 5).

3.3 UV-PROTECTANTS TESTED

The particulars of UV- protectants tested (Plate 6) are as follows.

S. No.	UV- protectant	Concentration	Source
1.	Charcoal	1%	M/s. S.d.fine Chemicals Ltd., Mumbai – 400 255
2.	Indian ink	1%	M/s. S.d.fine Chemicals Ltd., Mumbai – 400 255
3.	Congo red	1%	M/s. S.d.fine Chemicals Ltd., Mumbai – 400 255
4.	Robin blue	0.5%	M/s. Reckitt Benckiser (India) Ltd., Kolkata – 700 071.
5.	Yeast extract	2%	M/s. S.d.fine Chemicals Ltd., Mumbai – 400 255

3.4 BIOEFFICACY OF THE TEST PATHOGENS

Bioefficacy of the test pathogens, *B. bassiana* as pure culture and Bio-power, as formulation, and *M. anisopliae* as pure culture against the third instar larvae of *S. litura* (6 days) was evaluated for median lethal concentrations (LC_{50}) and lethal times (LT_{50}).

The third instar larvae of *S. litura* were dipped for few seconds (Desh Mukh and Mathai,1991) in different concentrations of fungal spore suspensions of *B. bassiana* and *M. anisopliae* prepared by serial dilution. After air drying, the treated larvae were fed with fresh and clean castor leaves in the laboratory (27 $\pm 2^0$C and 60 to 70% RH). Twenty larvae per treatment with four replications were maintained for each experiment. The data on larval mortalities were recorded daily till pupation (Plate 7).

3.5 EFFECT OF UV-PROTECTANTS ON THE PATHOGENICITY AND GROWTH OF ENTOMOPATHOGENIC FUNGI

The selected UV-protectants at the specified concentrations were added to the LC_{50} concentrations of the two fungal spore suspensions and were exposed to UV-light for specific durations *viz.,* 30 minutes, one hour, three hours and five hours.

A fresh batch of third instar, *S. litura* larvae were treated with the combinations of the fungal spore suspensions and UV-protectants that were exposed for the specified durations to UV-light and the data on larval mortality was recorded to calculate the LT_{50} values.

The LC_{50} concentrations of the fungal spore suspensions with the UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% were exposed to UV-light for the specified durations and were inoculated using 20 µl of test solution at the centre of the petri plate containing the SDA medium. The test pathogens were assessed for their growth inhibition (diameter in cm) and spore production (Number of spores/ml) at ten days after incubation.

3.6 STATISTICAL ANALYSIS

The LC_{50} and LT_{50} values were derived from logarithmic calculations (Finney, 1984) using multiple linear programme on computer. The remaining experiments were conducted in completely randomized block design with four replications. The data obtained were analysed by using analysis of variance technique (Gomez and Gomez, 1984). The data pertaining to percent larval mortality was subjected to arc sine percentage transformation before going for analysis of variance.

CHAPTER – IV

RESULTS

The results obtained in the present investigation on the 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' conducted under laboratory conditions in the Department of Entomology, Agricultural College, Bapatla during 2004-05 are presented in this chapter.

The results obtained are basically from three experiments *viz.,* pathogenicity of *B. bassiana* on *S. litura,* pathogenicity of *M. anisopliae* on *S. litura* and effect of charcoal, indian ink, congo red, robin blue and yeast extract as UV-protectants on the efficacy of *B. bassiana* and *M. anisopliae* as pure cultures at their respective LC_{50} values exposed for the specified durations to UV-light *viz.,* 30 minutes, one hour and three hours.

4.1 PATHOGENICITY OF *B. bassiana* AND *M. anisopliae* ON *S. litura*

The median lethal concentrations (LC_{50}) and median lethal times (LT_{50}) of *B. bassiana* (as pure culture and as Bio-power, a commercial formulation) and *M. anisopliae* (as pure culture) were determined with reference to 50 per cent mortality of the third instar *S. litura* larvae after dipping them in fungal spore suspension for few seconds.

The LC_{50} values of *B. bassiana* as pure culture at nine and eleven days after treatment were 20.4×10^4 and 9.4×10^4 spores/ml, respectively. However,

the LC_{50} values of Bio-power at six and seven days after treatment were $94.6x10^4$ and $56.2x10^4$ spores/ml, respectively.

The LC_{50} values of *M. anisopliae* as pure culture at nine and eleven days after treatment were $265.2x10^4$ and $98.9x10^4$ spores/ml, respectively (Table 1).

The values of χ^2, regression equations and fiducial limits corresponding to different LC_{50} values are also presented in the table 1 and the regression lines drawn for the concentrations are presented in the fig. 1 to 6.

The LT_{50} values of *B. bassiana* as pure culture against *S. litura* larvae were180.0, 220.8, 261.6 and 302.4 hours at $25x10^4$, $20x10^4$, $15x10^4$ and $10x10^4$ spores/ml concentrations, respectively. At $5x10^4$ spores/ml concentration *B. bassiana* as pure culture did not result in 50 per cent larval mortality (Table 2).

The LT_{50} values of Bio-power, a commercial formulation of *B. bassiana* at $125x10^4$, $100x10^4$, $75x10^4$ and $50x10^4$ spores/ml were 129.6, 136.8, 163.2 and 172.8 hours, respectively. However, at $25x10^4$spores/ml Bio-power did not result in 50 per cent larval mortality (Table 3).

The LT_{50} values of *M. anisopliae* as pure culture against *S. litura* larvae were 196.8, 199.2, 237.6, 240.0 and 256.8 hours at $300x10^4$, $250x10^4$, $200x10^4$, $150x10^4$ and $100x10^4$ spores/ml, respectively (Table 4).

The values of χ^2, regression equations and fiducial limits corresponding to the LT_{50} values of *B. bassiana* as pure culture and Bio-power and

M. anisopliae as pure culture are presented in table 2, table 3 and table 4, respectively.

4.2 EFFECT OF UV-PROTECTANTS ON THE PATHOGENICITY of *B. bassiana* AND *M. anisopliae*

A laboratory investigation was conducted to study the effect of UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% on the LC_{50} concentrations of *B. bassiana* and *M. anisopliae* fungal spore pure culture suspensions after exposing to UV-light for the specified durations *viz.,* 30 minutes, one hour, three hours and five hours against third instar larvae of *S. litura.*

A fresh batch of third instar, *S. litura* larvae were treated with the combinations of the spore suspensions and UV-protectants that were exposed to UV-light to record the mortality over untreated check.

4.2.1 Larval Mortality and Median Lethal Times (LT_{50}) of *B. bassiana*

The treatments of *B. bassiana* $9.4x10^4$ spores/ml (LC_{50}) with and without UV-protectants exposed for the specified durations to UV-light were evaluated in terms of larval mortality of *S. litura* at different days after treatment (DAT).

4.2.1.1 UV exposure for 30 minutes

With 30 minutes of exposure to UV-light (Table 5) *B. bassiana* with charcoal 1%, as UV-protectant resulted in significantly the highest larval mortality of *S. litura* (21.26 per cent) at two DAT followed by *B. bassiana* with

indian ink 1% (8.92 per cent) and congo red 1% (6.46 per cent). There was no larval mortality in *B. bassiana* either with robin blue 0.5% or yeast extract 2% and *B. bassiana* without any UV-protectant, but these treatments were on par with *B. bassiana* with indian ink 1% and congo red 1%.

At four and six DAT, the combinations of *B. bassiana* with charcoal 1% (45.72 and 71.20 per cent, respectively) and indian ink 1% (45.00 and 66.41 per cent, respectively), as UV-protectants were on par with each other and significantly superior to all the other treatments, and resulted in the highest larval mortality. Next to them were, *B. bassiana* with congo red 1% (37.01 and 60.91per cent, respectively) and robin blue 0.5% (23.83 and 56.82 per cent, respectively). In the remaining treatments, the combinations with the UV-protectant yeast extract 2% (6.46 and 36.24 per cent) was on par with *B. bassiana* without any UV-protectant (3.23 and 34.54 per cent) in resulting the lowest per cent larval mortality at four and six DAT, respectively.

4.2.1.2 UV exposure for one hour

With one hour of exposure to UV-light (Table 6) *B. bassiana* with charcoal 1%, as UV-protectant resulted in the highest larval mortality at four, five and six DAT (25.39, 36.98 and 47.87 per cent, respectively). It was followed by *B. bassiana* with indian ink 1% (19.23, 31.33 and 44.28 per cent, respectively). The order of efficacy for the remaining UV-protectants in terms of larval mortality was *B. bassiana* + congo red 1% (12.44, 21.55 and 35.44 per cent), *B. bassiana* + robin blue 0.5% (6.46, 19.23 and 33.05 per cent) and *B. bassiana* + yeast extract 2% (3.23, 20.61 and 31.56 per cent) were on par with *B. bassiana* without any UV-protectant (3.23, 11.06 and 28.71 per cent)

indicating significantly lower per cent larval mortality after four, five and six DAT, respectively.

4.2.1.3 UV exposure for three hours

At five, seven and nine days of treatment, *B. bassiana* with UV-protectants exposed for three hours to UV-light indicated (Table 7) that charcoal 1%, (12.15, 28.71 and 39.17 per cent, respectively) and indian ink 1% (4.60, 23.50 and 37.71 per cent, respectively) as UV-protectants were on par with each other resulting in the highest larval mortality. The order of efficacy in the remaining UV-protectants in terms of larval mortality was *B. bassiana* + congo red 1% (3.23, 6.46 and 23.58 per cent), *B. bassiana* + robin blue 0.5% (0.00, 3.23 and 23.58 per cent) and *B. bassiana* + yeast extract 2% (0.00, 0.00 and 20.61 per cent) at five, seven and nine DAT, respectively but these were on par with each other. However, the later was on par with *B. bassiana* without any UV-protectant (0.00, 0.00 and 18.14 per cent) resulting significantly the lowest per cent larval mortality at five, seven and nine DAT, respectively.

The LT_{50} values of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) with the UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% for 30 minutes exposure to UV-light against the third instar *S. litura* larvae were 91.2, 93.6, 105.6, 112.8 and 156.0 hours, respectively as against 160.8 hours in *B. bassiana* without any UV-protectant (Table 8). The LT_{50} values of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) with the UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 1% and yeast extract 2% for one hour exposure to UV-light were 134.4,

144.0, 160.8, 158.4 and 187.2 hours, respectively as against 194.4 hours in the treatment of *B. bassiana* without any UV-protectant (Table 9).

The LT_{50} values of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) with charcoal 1% and indian ink 1% as UV-protectants for three hours exposure to UV-light were 235.2 and 242.4 hours, respectively. In case of *B. bassiana* with congo red 1%, robin blue 0.5% and yeast extract 2% as UV-protectants and without any UV-protectant for three hours exposure to UV-light did not record 50 per cent larval mortality hence, LT_{50} values were not obtained (Table 10).

Similarly no LT_{50} values were obtained either in case of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) either with or without the UV-protectants for five hours exposure to UV-light as 50 per cent larval mortalities were not resulted.

The corresponding values of χ^2, regression equations and fiducial limits of the LT_{50} values of *B. bassiana* at the LC_{50} with and without the UV-protectants exposed for 30 minutes, one hour and three hours to UV-light are also presented in the table 8, 9 and 10.

4.2.2 Larval Mortality and Median Lethal Times (LT_{50}) of *M. anisopliae*

The different treatments with *M. anisopliae* $98.9x10^4$ spores/ml (LC_{50}) with and without the UV-protectants exposed for the specified durations to UV-light were evaluated in terms of larval moratlity of *S.litura* at different DAT.

4.2.2.1 UV exposure for 30 minutes

With 30 minutes exposure to UV-light (Table 11), *M. anisopliae* with charcoal 1% (42.12, 54.78 and 75.51 per cent), indian ink 1% (38.47, 51.57

and 73.05 per cent) and congo red 1% (37.68, 49.34 and 66.49 per cent) as UV-protectants were on par with each other resulting in the highest per cent larval mortality at two, three and four DAT, respectively. However, the later two were also on par with *M. anisopliae* + robin blue 0.5%, which recorded 35.44, 39.86 and 63.52 per cent larval mortality at two, three and four DAT, respectively. Whereas, *M. anisopliae* + yeast extract 2% (13.10 per cent) was on par with *M. anisopliae* without any UV-protectant at two DAT (3.23 per cent). However, the larval mortality in *M. anisopliae* + yeast extract 2% (28.55 and 44.28 per cent) proved effective over *M. anisopliae* without any UV-protectant (18.14 and 33.17 per cent) at three and four DAT, respectively.

4.2.2.2 UV exposure for one hour

With one hour exposure to UV-light (Table 12), *M. anisopliae* with charcoal 1%, as UV-protectant resulted in significantly the highest per cent larval mortality at four, five and six DAT (34.74, 46.44 and 57.74 per cent, respectively). The next best UV-protectants were indina ink 1% (23.50, 39.15 and 53.81 per cent) and congo red 1% (17.05, 32.31 and 45.00 per cent) with the larval mortality significantly superior to that of the remaining UV-protectants at four, five and six DAT, respectively. Robin blue 0.5% (6.46, 24.44 and 35.48 per cent) and yeast extract 2% (3.23, 24.30 and 34.67 per cent) at four, five and six DAT and also *M. anisopliae* without any UV-protectant at four DAT (3.23 per cent) were on par with eachother. However, robin blue 0.5% and yeast extract 2% were significantly superior to *M. anisopliae* without any UV-protectant at five and six DAT (14.29 and 26.47 per cent, respectively) by giving significantly superior larval mortality.

4.2.2.3 UV exposure for three hours

With three hours exposure to UV-light (Table 13), *M. anisopliae* with charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%, as UV-protectants provided 16.76, 11.06, 8.92, 7.83 and 7.83 per cent larval mortality compared to *M. anisopliae* without any UV-protectant (3.23 per cent) at three DAT. However, all the five treatments with UV-protectants including *M. anisopliae* without any UV-protectant were on par with eachother at three DAT. At five and seven DAT charcoal 1% (28.99 and 38.47 per cent, respectively), indian ink 1% (28.94 and 37.71 per cent, respectively), congo red 1% (24.44 and 36.90 per cent, respectively), as UV-protectants for *M. anisopliae* were on par with each other in terms of larval mortality. However, the larval mortality in *M. anisopliae* + yeast extract 2% (22.64 and 31.51 per cent, respectively) was on par with *M. anisopliae* + congo red 1% and robin blue 0.5% at five DAT. Whereas, the larval mortality in *M. anisopliae* + yeast extract 2% was on par with robin blue 0.5% at seven DAT.

The LT_{50} values of *M. anisopliae* at $98.9x10^4$ spores/ml (LC_{50}) with charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%, as UV-protectants for 30 minutes exposure to UV-light against the third instar *S. litura* were 55.2, 55.2, 60.0, 60.0 and 98.4 hours, respectively as against 117.6 hours in *M. anisopliae* without any UV-protectant (Table 14).

The LT_{50} values of *M. anisopliae* at $98.9x10^4$ spores/ml (LC_{50}) with charcoal 1%, indian ink 1%, congo red 1%, robin blue 1% and yeast extract 2%, as UV-protectants for one hour exposure to UV-light were 115.2,

127.2,141.6, 160.8 and 156.0 hours as against 182.4 hours in *M. anisopliae* without any UV-protectant (Table 15).

The LT_{50} values of *M. anisopliae* at $98.9x10^4$ spores/ml (LC_{50}) with charcoal 1%, indian ink 1%, congo red 1% and robin blue 0.5% for three hours exposure to UV-light were 199.2, 196.8, 213.6 and 230.4 hours, respectively. But LT_{50} values were not obtained in case of *M. anisopliae* without any UV-protectant and with yeast extract 2% after three hours exposure to UV-light as 50 per cent larval mortalities were not resulted (Table 16).

Like wise, no LT_{50} values were obtained either in case of *M. anisopliae* at $98.9x10^4$ spores/ml (LC_{50}) either with or without the UV-protectants after five hours exposure to UV-light as 50 per cent larval mortalities were not resulted.

The corresponding values of χ^2, regression equations and fiducial limits of the LT_{50} values of *M. anisopliae* at the LC_{50} with and without the UV-protectants exposed to UV-light for 30 minutes, one hour and three hours are also presented in the table 14, 15 and 16.

4.3 GROWTH RESPONSE OF *B. bassiana*

4.3.1 Diameter of Fungal Growth

The data on the response of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) with UV-protectants exposed for the specified durations to UV-light indicated that charcoal 1%, indian ink 1% and congo red 1% significantly reduced the lethal effect of UV-light on the fungal growth (diameter) .

The diameter of fungal growth of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) (Table 17) with charcoal 1% and indian ink 1% was significantly the highest with 30 minutes exposure (7.77and 7.45 cm, respectively), one hour exposure (5.90and 5.82 cm, respectively) and three hours exposure (3.15and 2.75 cm, respectively) to UV-light and were on par. Next were *B. bassiana* + congo red 1%, *B. bassiana* + robin blue 0.5%, *B. bassiana* + yeast extract 2% and *B. bassiana* without any UV-protectant indicating significantly lesser cm diameter with 30 minutes, one hour and three hours of exposure to UV-light. The diameters of fungal growth was 6.77, 4.92 and 2.00 cm in case of *B. bassiana* + congo red 1% **;** 6.42,4.55 and 1.82 cm in case of *B. bassiana* + robin blue 0.5% **;** 6.10, 4.55 and 1.45 cm in case of *B. bassiana* + yeast extract 2% and 5.52, 4.15 and 1.40 cm in case of *B. bassiana* without any UV-protectant with 30 minutes, one hour and three hours of UV exposure, respectively.

Further, exposure of *B. bassiana* either with or without UV-protectants to UV-light for the specified durations *i.e.,* 30 minutes, one hour and three hours clearly reduced the fungal diameter indicating its lethal effect .

4.3.2 Spore production ($x10^4$ spores/ml)

With reference to the spore production of *B. bassiana* at $9.4x10^4$ spores/ml (LC_{50}) exposed for the specific durations to UV-light (Table 18), the UV-protectant charcoal 1% gave the best protection resulting 617.5, 222.5 and $20.0x10^4$ spores/ ml with 30 minutes, one hour and three hours of UV exposure, respectively. With 30 minutes of exposure, indian ink 1% and congo red 1%, as UV-protectants with *B. bassiana* were on par with each

other (557.5 and 532.5x10^4 spores/ml, respectively) and significantly superior to the remaining treatments. Next to them were, *B. bassiana* with robin blue 0.5%, yeast extract 2% as UV-protectants and *B. bassiana* without any UV-protectant (447.5, 430.0 and 422.5x10^4 spores/ml, respectively) and were on par with each other. Whereas, with one hour of UV exposure, *B. bassiana* + indian ink 1% had the second highest spore production of 170.0x10^4 spores/ml and was significantly superior to the remaining treatments. All the remaining treatments; *B. bassiana* + congo red 1%, *B. bassiana* + robin blue 0.5%, *B. bassiana* + yeast extract 2% and *B. bassiana* without any UV-protectant (125.0 to 110.5x10^4 spores/ml) were on par with each other. However, with three hours of UV exposure, *B. bassiana* +indian ink 1% (15.0x10^4 sproes/ml) was on par with *B. bassiana* with congo red 1%, robin blue 0.5%, yeast extract 2% and *B. bassiana* without any UV-protectant (11.25 to 8.75x10^4 spores/ml).

The results indicated that 30 minutes to three hours of UV-light exposure has significant effect on the spore production of *B. bassiana* at 9.4x10^4 spores/ml (LC_{50}) either without any UV-protectant (422.5 to 8.75x10^4 spores/ml) or with charcoal 1% (617.5 to 20x10^4 spores/ml), indian ink 1% (557.5 to 15.0 x 10^4 spores/ml), congo red 1% (532.5 to 11.25x10^4 spores/ml), robin blue 0.5% (447.5 to 8.75x10^4 spores/ml) and yeast extract 2% (430.0 to 8.75x10^4 sproes/ml) as UV-protectants, respectively.

4.4 GROWTH RESPONSE OF *M. anisopliae*

4.4.1 Diameter of Fungal Growth

The data on the response of *M. anisopliae* $98.9x10^4$ spores/ml (LC_{50}) with and without the UV-protectants exposed for the specified durations to UV-light (Table19) indicated that charcoal 1% and indian ink 1% significantly reduced the lethal effect of UV-light on the diameter of fungal growth (7.45 and 7.45 cm, respectively) were on par and significantly superior to the other treatments. Next to them were congo red 1%, robin blue 0.5% and yeast extract 2% (6.77, 6.50, 6.22 cm, respectively), which were on par with each other with 30 minutes of exposure to UV-light as against 5.07 cm diameter in *M. anisopliae* without any UV-protectants.

Whereas, with one hour exposure, *M. anisopliae* $98.9x10^4$ spores/ml (LC_{50}) with charcoal 1%,as UV-protectant gave the best protection against UV-light resulting in a fungal growth of 6.20 cm diameter. Next came indian ink 1% (5.70 cm) and congo red 1% (5.57 cm) were the next best followed by robin blue 0.5% (5.40 cm) and yeast extract 2% (5.32 cm) that were significantly superior to *M. anisopliae* without any UV-protectant (4.02 cm). Among these, *M. anisopliae* with indian ink 1% and congo red 1% were on par with each other. whereas, *M. anisopliae* with robin blue 0.5% and yeast extract 2% were on par with each other.

With three hours exposure to UV-light also, *M. anisopliae* $98.9x10^4$ spores/ml (LC_{50}) with charcoal 1% was the best UV-protectant resulting in the highest diameter of fungal growth (2.92 cm) followed by indian ink 1% (2.47 cm). whereas, congo red 1% and robin blue 0.5% (2.00 and 1.87 cm,

respectively) were on par with each other and stood third in position. But yeast extract 2% (1.47 cm) did not provide significant protection from UV-light and stood on par with *M. anisopliae* without any UV-protectant (1.45 cm).

The duration of exposure to UV-light for 30 minutes, one hour and three hours has significant effect on the diameter of fungal growth of *M. anisopliae* even in the presence of the UV-protectants. The diameter of fungal growth was reduced from 7.45 to 2.92 cm in case of *M. anisopliae* + charcoal 1%; 7.45 to 2.47 cm in case of *M. anisopliae* + indian ink 1%; 6.77 to 2.00 cm in case of *M. anisopliae* + congo red 1%; 6.50 to 1.87 cm in case of *M. anisopliae* + robin blue 0.5% and 6.22 to 1.47 cm in case of *M. anisopliae* + yeast extract 2% compared to 5.07 to 1.45 cm in case of *M. anisopliae* without any UV-protectant.

4.4.2 Spore production (x10^4 spores/ml)

With regard to the spore production by *M. anisopliae* exposed for the specified durations to UV-light (Table 20) charcoal 1% gave the best protection resulting in 632.5x10^4 spores/ ml) with 30 minutes of exposure. Whereas, indian ink 1% (530.0x10^4 spores/ml) and congo red 1% (500.0x10^4 spores/ml) were the second best and were on par with each other. Whereas, robin blue 0.5% and yeast extract 2% (405.0 and 355.0x10^4 spores/ ml, respectively) were third in sequence and were on par with each other, while yeast extract 2% was also on par with *M. anisopliae* without any UV-protectant (315.0x10^4 spores/ml).

With one hour of exposure to UV-light also charcoal 1% gave the best protection resulting 185.0x10^4 spores/ml and was significantly superior to all

the other treatments. It was followed by indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% (160.0, 150.0, 146.0 and 125.0x10^4 spores/ ml, respectively) with more than 125.0x10^4 spores/ml, which were significantly superior to *M. anisopliae* without any UV-protectant (85.0x10^4 spores/ml).

Similarly, with three hours exposure to UV-light, charcoal 1% (23.75x10^4 spores/ ml) was again the best UV-protectant, while all other UV-protectants tested, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% were on par with each other and were also not significantly different with the *M. anisopliae* without any UV-protectant (12.50 to 7.50x10^4 spores/ ml).

Table 1 : Median Lethal Concentrations (LC_{50}) of *B. bassiana* and *M. anisopliae* against the third instar larvae of *S. litura.*

Sl. No	Entomopathogenic Fungi	DAT	$\chi 2$ value	Regression Equation (Y= a + bx)	LC_{50} $\times 10^4$ spores/ml	Fiducial Limits (95%)
1	*B. bassiana* (pure culture)	9	5.388	Y= -1.0952+1.9942 x	20.4	17.6 ± 25.1
		11	0.188	Y= 2.6666+2.3200 x	9.4	8.6 ± 15.7
2	*B. bassiana* (Bio- power)	6	0.551	Y= -1.1428+0.4182 x	94.6	83.0 ± 111.7
		7	4.661	Y= 3.3333+0.5400 x	56.2	49.1 ± 63.4
3	*M. anisopliae* (pure culture)	9	6.102	Y= 3.2857+0.1462 x	265.2	219.5 ± 386.1
		11	1.162	Y= 12.2142+0.2097 x	98.9	63.3 ± 122.9

DAT : Days After Treatment

Table 2 : Median Lethal Times (LT_{50}) for different concentrations of *B. bassiana* as pure culture against the third instar larvae of *S. litura.*

Sl. No.	*B. bassiana* Concentrations spores/ml	$\chi 2$ value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	5×10^4	0.011	Y= - 1.6509+1.3927 x	*	*
2	10×10^4	1.353	Y= - 3.1781+2.1745 x	302.4	271.2 ± 384.0
3	15×10^4	0.481	Y= - 4.4727+3.2181 x	261.6	244.8 ± 292.8
4	20×10^4	3.068	Y= - 7.0000+4.7500 x	220.8	206.4 ± 242.4
5	25×10^4	0.176	Y= - 5.1348+5.3960 x	180.0	163.2 ± 196.8

*** : LT_{50} could not be calculated as 50 per cent larval mortalities were not resulted.**

Table 3 : Median Lethal Times (LT_{50}) for different concentrations of Bio-power, a commercial formulation of *B. bassiana* against the third instar larvae of *S. litura.*

Sl.No	*B. bassiana* Concentrations spores/ml	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	25×10^4	0.371	Y= - 1.3965+1.8103 x	*	*
2	50×10^4	0.127	Y= - 3.2068+4.3793 x	172.8	160.8 ± 196.8
3	75×10^4	0.005	Y= - 2.3448+5.4655 x	163.2	151.2 ± 187.2
4	100×10^4	0.310	Y= - 3.4482+8.1551 x	136.8	129.6 ± 141.6
5	125×10^4	0.248	Y= - 2.9137+8.7586 x	129.6	120.0 ± 136.8

*** : LT_{50} could not be calculated as 50 per cent larval mortalities were not resulted.**

Table 4 : Median Lethal Times (LT_{50}) for different concentrations of *M. anisopliae* as pure culture against the third instar larvae of *S. litura.*

Sl.No	*M. anisopliae* Concentrations spores/ml	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	100×10^4	1.532	Y= - 4.3054+3.1563 x	256.8	244.8 ± 273.6
2	150×10^4	3.074	Y= - 4.7054+3.9563 x	240.0	230.4 ± 252.0
3	200×10^4	0.353	Y= - 6.0799+4.1600 x	237.6	228.0 ± 247.2
4	250×10^4	4.261	Y= - 5.8539+5.5084 x	199.2	187.2 ± 211.2
5	300×10^4	4.014	Y= - 9.3483+5.9606 x	196.8	184.8 ± 211.2

Table 8 : Median Lethal Times (LT_{50}) of *B. bassiana* with and without the UV-protectants after 30 minutes exposure to UV-light against the third instar larvae of *S. litura.*

Sl.No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*B. bassiana* + Charcoal 1%	1.539	Y= - 6.2857+11.7857 x	91.2	84.0 ± 96.0
2	*B. bassiana* + Indian ink 1%	0.505	Y= - 5.6037+12.0566 x	93.6	86.4 ± 98.4
3	*B. bassiana* + Congo red 1%	0.111	Y= - 6.1132+10.1981x	105.6	100.8 ± 112.8
4	*B. bassiana* + Robin blue 0.5%	1.122	Y= - 9.7142+9.9142 x	112.8	105.6 ± 122.4
5	*B. bassiana* + Yeast extract 2%	1.362	Y= - 3.8433+3.8915 x	156.0	144.0 ± 177.6
5	*B. bassiana*	0.757	Y= - 3.3253+3.6867 x	160.8	146.4 ± 192.0

Table 9 : Median Lethal Times (LT_{50}) of *B. bassiana* with and without the UV-protectants after one hour exposure to UV- light against the third instar larvae of *S. litura.*

Sl.No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*B. bassiana* + Charcoal 1%	0.736	Y= - 7.6415+7.1226 x	134.4	127.2 ± 146.4
2	*B. bassiana* + Indian ink 1%	0.035	Y= - 3.7349+5.6626 x	144.0	134.4 ± 165.6
3	*B. bassiana* + Congo red 1%	0.099	Y= -3.3373+3.1566 x	160.8	148.8 ± 192.0
4	*B. bassiana* + Robin blue 0.5%	0.008	Y= 13.3361-0.0007 x	158.4	146.4 ± 187.2
5	*B. bassiana* + Yeast extract 2%	0.357	Y= - 2.1807+3.0481x	187.2	158.4 ± 309.6
6	*B. bassiana*	0.096	Y= - 1.7349+1.6626 x	194.4	165.6 ± 357.6

Table 10 : Median Lethal Times (LT_{50}) of *B. bassiana* with and without the UV-protectants after three hours exposure to UV-light against the third instar larvae of *S. litura.*

Sl.No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*B. bassiana* + Charcoal 1%	0.734	Y= - 4.8032+3.5377 x	235.2	213.6 ± 283.2
2	*B. bassiana* + Indian ink 1%	0.070	Y= - 5.7049+3.0065 x	242.4	220.8 ± 283.2
3	*B. bassiana* + Congo red 1%	0.406	Y= - 0.7300+0.5800 x	*	*
4	*B. bassiana* + Robin blue 0.5%	0.017	Y= -1.2500+1.0000 x	*	*
5	*B. bassiana* + Yeast extract 2%	0.217	Y= - 1.2400+1.0400 x	*	*
6	*B. bassiana*	0.064	Y= - 0.9400+0.7400 x	*	*

*** : LT_{50} S could not be calculated as 50 per cent larval mortalities were not resulted.**

Table 14 : Median Lethal Times (LT_{50}) of *M. anisopliae* with and without the UV-protectants after 30 minutes exposure to UV-light against the third instar larvae of *S. litura.*

Sl.No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*M. anisopliae* + Charcoal 1%	1.743	Y= - 1.8285+18.2571x	55.2	48.0 ± 60.0
2	*M. anisopliae* + Indian ink 1%	2.509	Y= 1.0857+15.6285 x	55.2	43.2 ± 62.4
3	*M. anisopliae* + Congo red 1%	0.226	Y= - 1.1714+16.7428 x	60.0	55.2 ± 64.8
4	*M. anisopliae* + Robin blue 0.5%	3.446	Y= -3.0857+17.3714 x	60.0	52.8 ± 64.8
5	*M. anisopliae* + Yeast extract 2%	0.257	Y= -4.8285+9.2571 x	98.4	88.8 ± 117.6
6	*M. anisopliae*	0.028	Y= - 4.0285+5.4571 x	117.6	103.2 ± 156.0

Table 15 : Median Lethal Times (LT_{50}) of *M. anisopliae* with and without the UV-protectants after one hour exposure to UV-light against the third instar larvae of *S. litura.*

Sl.No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*M. anisopliae* + Charcoal 1%	0.057	Y= - 2.5903+9.0240 x	115.2	105.6 ± 122.4
2	*M. anisopliae* + Indian ink 1%	0.054	Y= - 4.8674+7.8313 x	127.2	120.6 ± 134.4
3	*M. anisopliae* + Congo red 1%	0.067	Y= - 4.2771+5.8072 x	141.6	134.4 ± 158.4
4	*M. anisopliae* + Robin blue 0.5%	0.757	Y= -3.3253+3.6867 x	160.8	146.4 ± 192.0
5	*M. anisopliae* + Yeast extract 2%	1.549	Y= -3.6867+3.7831 x	156.0	146.4 ± 192.0
6	*M. anisopliae*	0.046	Y= - 2.1807+2.0481 x	182.4	160.8 ± 266.4

Table 16 : Median Lethal Times (LT_{50}) of *M. anisopliae* with and without the UV-protectants after three hours exposure to UV-light against the third instar larvae of *S. litura.*

Sl.No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	$\chi 2$ Value	Regression Equation (Y= a + bx)	LT_{50} (Hours)	Fiducial Limits (95%)
1	*M. anisopliae* + Charcoal 1%	0.057	Y= - 0.9719+4.3925 x	199.2	168.0 ± 307.2
2	*M. anisopliae* + Indian ink 1%	0.054	Y= - 3.6822+4.4485 x	196.8	168.0 ± 264.0
3	*M. anisopliae* + Congo red 1%	0.067	Y= - 3.4953+4.0654 x	213.6	177.6 ± 314.4
4	*M. anisopliae* + Robin blue 0.5%	0.757	Y= -2.8785+3.7009 x	230.4	184.8 ± 388.8
5	*M. anisopliae* + Yeast extract 2%	1.549	Y= -2.5607+3.1495 x	*	*
6	*M. anisopliae*	0.046	Y= - 1.8317+1.3551 x	*	*

*** : LT_{50} S could not be calculated as 50 per cent larval mortalities were not resulted.**

Table17: Growth response of *B. bassiana* with and without the UV-protectants exposed for specified durations to UV-light.

Sl. No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	Fungal Growth (diameter in cm)		
		Intervals of UV exposure		
		30 minutes	One hour	Three hours
1	*B. bassiana*	5.52 ^{d}	4.15 ^{c}	1.40 ^{b}
2	*B. bassiana* + Charcoal 1%	7.77 ^{a}	5.90 ^{a}	3.15 ^{a}
3	*B. bassiana* + Indian ink 1%	7.45 ^{a}	5.82 ^{a}	2.75 ^{a}
4	*B. bassiana* + Congo red 1%	6.77 ^{bc}	4.92 ^{b}	2.00 ^{b}
5	*B. bassiana* + Robin blue 0.5%	6.42 ^{cd}	4.55 ^{bc}	1.82 ^{b}
6	*B. bassiana* + Yeast extract 2%	6.10 ^{d}	4.55 ^{bc}	1.45 ^{b}
	F test	Sig.	Sig.	Sig.
	SED	0.29	0.29	0.34
	C.D (p=0.05)	0.63	0.62	0.73

In each column, values having common alphabet do not vary significantly.

Table 18 : Spore production of *B. bassiana* with and without the UV-protectants exposed for the specified durations to UV-light.

Sl. No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	Spore Production (×10^4 Spores/ml)		
		Intervals of UV exposure		
		30 minutes	One hour	Three hours
1	*B. bassiana*	422.5 ^{c}	110.5 ^{c}	8.75 ^{b}
2	*B. bassiana* + Charcoal 1%	617.5 ^{a}	222.5 ^{a}	20.0 ^{a}
3	*B. bassiana* + Indian ink 1%	557.5 ^{b}	170.0 ^{b}	15.0 ^{ab}
4	*B. bassiana* + Congo red 1%	532.5 ^{b}	125.0 ^{c}	11.25 ^{b}
5	*B. bassiana* + Robin blue 0.5%	447.5 ^{c}	120.0 ^{c}	8.75 ^{b}
6	*B. bassiana* + Yeast extract 2%	430.0 ^{c}	117.5 ^{c}	8.75 ^{b}
	F test	Sig.	Sig.	Sig.
	SED	12.9	12.9	3.91
	C.D (p=0.05)	27.5	27.6	8.33

In each column, values having common alphabet do not vary significantly.

Table 20 : Spore production of *M. anisopliae* with and without the UV-protectants exposed for the specified durations to UV-light.

Sl.No	*M. anisopliae* 98.9×10^4 spores/ml+ UV-protectants	Spore Production (×10^4 spores/ml)		
		Intervals of UV exposure		
		30 minutes	One hour	Three hours
1	*M. anisopliae*	315.0 ^{d}	85.0 ^{d}	7.50 ^{b}
2	*M. anisopliae* + Charcoal 1%	632.5 ^{a}	185.0 ^{a}	23.75 ^{a}
3	*M. anisopliae* + Indian ink 1%	530.0 ^{b}	160.0 ^{b}	12.50 ^{b}
4	*M. anisopliae* + Congo red 1%	500.0 ^{b}	150.0 ^{b}	10.00 ^{b}
5	*M. anisopliae* + Robin blue 0.5%	405.0 ^{c}	146.0 ^{bc}	10.00 ^{b}
6	*M. anisopliae* + Yeast extract 2%	355.0 ^{Cd}	125.0 ^{c}	8.75 ^{b}
	F test	Sig.	Sig.	Sig.
	SED	23.56	11.2	2.61
	C.D (p=0.05)	50.3	23.9	5.56

In each column, values having common alphabet do not vary significantly.

Table 19: Growth response of *M. anisopliae* with and without the UV-protectants exposed for the specified durations to UV-light.

Sl. No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	Fungal Growth (diameter in cm)		
		Intervals of UV exposure		
		30 minutes	One hour	Three hours
1	*M. anisopliae*	5.07 ^{c}	4.02 ^{d}	1.45 ^{d}
2	*M. anisopliae* + Charcoal 1%	7.45 ^{a}	6.20 ^{a}	2.92 ^{a}
3	*M. anisopliae* + Indian ink 1%	7.45 ^{a}	5.70 ^{b}	2.47 ^{b}
4	*M. anisopliae* + Congo red 1%	6.77 ^{b}	5.57 ^{b}	2.00 ^{c}
5	*M. anisopliae* + Robin blue 0.5%	6.50 ^{b}	5.40 ^{c}	1.87 ^{c}
6	*M. anisopliae* + Yeast extract 2%	6.22 ^{b}	5.32 ^{c}	1.47 ^{d}
	F test	Sig.	Sig.	Sig.
	SED	0.29	6.10	0.17
	C.D (p=0.05)	0.62	0.13	0.37

In each column, values having common alphabet do not vary significantly.

Table : 5 Effect of UV-protectants on the efficacy of *B. bassiana* as pure culture at its LC_{50} with 30 minutes exposure to UV-light on the mortality of the third instar larvae of *S .litura.*

Sl. No.	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	Larval Mortality %		
		2DAT	4DAT	6DAT
1	*B. bassiana*	0.00 (0.00)^b	3.23 (1.25)^d	34.54 (32.50)^c
2	*B. bassiana* + Charcoal 1%	21.26 (13.75)^a	45.72 (51.25)^a	71.20 (88.75)^a
3	*B. bassiana* + Indian ink 1%	8.92 (5.00)^b	45.00 (50.00)^a	66.41 (83.75)^a
4	*B. bassiana* + Congo red 1%	6.46 (2.50)^b	37.01 (36.25)^b	60.91 (76.25)^b
5	*B. bassiana* + Robin blue 0.5%	0.00 (0.00)^b	23.83 (18.75)^c	56.82 (70.00)^b
6	*B. bassiana* + Yeast extract 2%	0.00 (0.00)^b	6.46 (2.50)^d	36.24 (35.00)^c
	F test	Sig.	Sig.	Sig.
	SED	3.48	4.71	3.05
	C.D (p=0.05)	7.32	9.91	6.41

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

Table : 6 Effect of UV-protectants on the efficacy of *B. bassiana* as pure culture at its LC_{50} with one hour exposure to UV-light on the mortality of the third instar larvae of *S. litura.*

Sl. No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	Larval Mortality %		
		4DAT	5DAT	6DAT
1	*B. bassiana*	3.23 (1.25)^{c}	11.06 (5.00)^{d}	28.71 (24.75)^{d}
2	*B. bassiana* + Charcoal 1%	25.39 (18.75)^{a}	36.98 (36.25)^{a}	47.87 (55.00)^{a}
3	*B. bassiana* + Indian ink 1%	19.23 (11.25)^{ab}	31.33 (27.50)^{b}	44.28 (48.75)^{b}
4	*B. bassiana* + Congo red 1%	12.44 (6.25)^{bc}	21.55 (13.75)^{c}	35.44 (33.75)^{c}
5	*B. bassiana* + Robin blue 0.5%	6.46 (2.50)^{c}	19.23 (11.25)^{cd}	33.05 (30.00)^{cd}
6	*B. bassiana* + Yeast extract 2%	3.23 (1.25)^{c}	20.61 (12.50)^{d}	31.56 (27.5)^{d}
	F test	Sig.	Sig.	Sig.
	SED	4.15	3.56	2.79
	C.D (p=0.05)	8.74	7.50	5.88

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

Table : 7 Effect of UV-protectants on the efficacy of *B. bassiana* as pure culture at its LC_{50} with three hours exposure to UV-light on the mortality of the third instar larvae of *S. litura.*

Sl. No	*B. bassiana* 9.4×10^4 spores/ml + UV-protectants	Larval Mortality %		
		5DAT	7DAT	9DAT
1	*B. bassiana*	0.00 (0.00)	0.00 $(0.00)^b$	18.14 $(10.00)^c$
2	*B. bassiana* + Charcoal 1%	12.15 (6.25)	28.71 $(24.75)^a$	39.17 $(40.00)^a$
3	*B. bassiana* + Indian ink 1%	4.60 (2.50)	23.50 $(16.25)^a$	37.71 $(37.50)^a$
4	*B. bassiana* + Congo red 1%	3.23 (1.25)	6.46 $(2.50)^b$	23.58 $(16.25)^b$
5	*B. bassiana* + Robin blue 0.5%	0.00 (0.00)	3.23 $(1.25)^b$	23.58 $(16.25)^b$
6	*B.bassiana* + Yeast extract 2%	0.00 (0.00)	0.00 $(0.00)^b$	20.61 $(12.50)^{bc}$
	F test	N.S	Sig.	Sig.
	SED	-	4.04	2.36
	CD (p=0.05)	-	8.5	4.96

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

Table : 11 Effect of UV-protectants on the efficacy of *M. anisopliae* as pure culture at its LC_{50} with 30 minutes exposure to UV-light on the mortality of the third instar larvae of *S. litura.*

Sl. No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	Larval Mortality %		
		2DAT	3DAT	4DAT
1	*M. anisopliae*	3.23 (1.25)^{c}	18.14 (10.00)^{d}	33.17 (30.00)^{d}
2	*M. anisopliae* + Charcoal 1%	42.12 (45.00)^{a}	54.78 (66.25)^{a}	75.51 (91.25)^{a}
3	*M. anisopliae* + Indian ink 1%	38.47 (38.75)^{ab}	51.57 (61.25)^{ab}	73.05 (88.75)^{ab}
4	*M. anisopliae* + Congo red 1%	37.68 (37.50)^{ab}	49.34 (57.50)^{ab}	66.49 (83.49)^{ab}
5	*M. anisopliae* + Robin blue 0.5%	35.44 (33.75)^{b}	39.86 (43.75)^{b}	63.52 (80.00)^{b}
6	*M. anisopliae* + Yeast extract 2%	13.10 (7.50)^{c}	28.55 (23.75)^{c}	44.28 (48.75)^{c}
	F test	Sig.	Sig.	Sig.
	SED	3.67	6.51	4.79
	CD (p=0.05)	7.72	13.75	10.07

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

Table : 12 Effect of UV-protectants on the efficacy of *M. anisopliae* as pure culture at its LC_{50} with one hour exposure to UV-light on the mortality of the third instar larvae of *S. litura.*

Sl. No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	Larval Mortality %		
		4DAT	5DAT	6DAT
1	*M. anisopliae*	3.23 (1.25)^c	14.29 (6.25)^e	26.47 (20.00)^e
2	*M. anisopliae* + Charcoal 1%	34.74 (32.50)^a	46.44 (52.50)^a	57.74 (71.25)^a
3	*M. anisopliae* + Indian ink 1%	23.50 (16.25)^b	39.15 (40.00)^b	53.81 (65.00)^b
4	*M. anisopliae* + Congo red 1%	17.05 (8.75)^{bc}	32.31 (28.75)^c	45.00 (50.00)^c
5	*M. anisopliae* + Robin blue 0.5%	6.46 (2.50)^c	24.44 (17.50)^d	35.48 (33.75)^d
6	*M. anisopliae* + Yeast extract 2%	3.23 (1.25)^c	24.30 (17.50)^d	34.67 (32.50)^d
	F test	Sig.	Sig.	Sig.
	SED	3.60	2.90	2.62
	CD (p=0.05)	7.57	6.11	5.51

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

Table : 13 Effect of UV-protectants on the efficacy of *M. anisopliae* as pure culture at its LC_{50} with three hours exposure to UV-light on the mortality of the third instar larvae of *S. litura.*

Sl. No	*M. anisopliae* 98.9×10^4 spores/ml + UV-protectants	Larval Mortality %		
		3DAT	5DAT	7DAT
1	*M. anisopliae*	3.23 (1.25)	3.23 (1.25)^c	21.55 (13.75)^c
2	*M. anisopliae* + Charcoal 1%	16.76 (8.75)	28.99 (23.75)^a	38.47 (38.75)^a
3	*M. anisopliae* + Indian ink 1%	11.06 (5.00)	28.94 (23.75)^a	37.71 (37.50)^a
4	*M. anisopliae* + Congo red 1%	8.92 (5.00)	24.44 (17.50)^{ab}	36.90 (36.25)^a
5	*M. anisopliae* + Robin blue 0.5%	7.83 (3.75)	24.44 (17.50)^{ab}	34.67 (32.50)^{ab}
6	*M. anisopliae* + Yeast extract 2%	7.83 (3.75)	22.64 (15.00)^b	31.51 (27.50)^b
	F test	N.S	Sig.	Sig.
	SED	-	3.08	2.70
	CD (p=0.05)	-	6.48	5.68

DAT : Days After Treatment.
Values in parantheses are angular transformed values.
In each column, values having common alphabet do not vary significantly.

CHAPTER – V

DISCUSSION

The results obtained in the present investigation on the 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' are discussed in this chapter in the light of earlier findings.

5.1 MEDIAN LETHAL CONCENTRATIONS

The median lethal concentrations (LC_{50}) of *B. bassiana* as pure culture for nine and eleven days after treatment (DAT) till pupation against the third instar larvae of *S. litura* were $20.4x10^4$ and $9.4x10^4$ spores/ml, respectively. Whereas, LC_{50} values for *B. bassiana* as Bio-power, a commercial formulation were $94.6x10^4$ and $56.2x10^4$ spores/ml at six and seven DAT, respectively. This indicates that *B. bassiana* used as pure culture in the present study was more efficacious than the commercial formulation, Bio-power.

The present LC_{50} $20.4x10^4$ spores/ml for *B. bassiana* as pure culture at nine DAT obtained in the present investigation is in agreement with the earlier reports of Deva Prasad *et al.,* 1990 ($2.17x10^5$ spores/ml) against the second instar larvae of *Helicoverpa armigera* (Hub.) at eight DAT and Fluents and Carballo, 1995 ($2.2x10^5$ spores/ml) against the third instar larvae of *Plutella xylostella* L. at ten DAT. However, it differed with the report of Dayakar and

Kanaujia, 2003a ($14.85x10^5$ conidia/ml) at eight DAT against the third instar larvae of *S. litura.*

Similarly, the present LC_{50} of $9.4x10^4$ spores/ml for *B. bassiana* as pure culture after eleven DAT is also in agreement with the earlier findings of Sivasankaran *et al.,* 1990 (10^5 spores/ml) against the second and third instar larvae of *Chilo infuscatellus* Snellen and Manjula and Padmavathamma, 1999 (10^5 spores/ml) against the third instar larvae of *H. armigera.*

Whereas, with regard to *M. anisopliae* as pure culture, the LC_{50} values were $265.2x10^4$ and $98.9x10^4$ spores/ml at nine and eleven DAT, respectively. The LC_{50} of $265.2x10^4$ spores/ml obtained in the present investigation was almost nearer to the value reported by Ajay Kumar Pandey and Kanaujia, 2003 ($2.65x10^6$ conidia/ml) against the fourth instar larvae of *S. litura*.

Similarly, the present LC_{50} of *M. anisopliae* as pure culture after eleven DAT *i.e.,* $98.9x10^4$ spores/ml obtained in the present investigation is also in close agreement with the earlier findings of Dayakar and Kanaujia, 2001 ($12.53x10^5$ conidia/ml) against the second instar larvae of *S. litura*, Dayakar and Kanaujia, 2003b ($9.8x10^5$ conidia/ml) against the second instar larvae of *Ergolis merione* L. and Dayakar and Kanaujia, 2003a ($14.85x10^5$ conidia/ml) against the third instar larvae of *S. litura*.

The variations observed in the LC_{50} values of both the entomopathogenic fungi in the present studies compared to the earlier reports might be due to variation in the test insect, larval instars and strains of the fungi tested. Further, the LC_{50} values of both the entomopathogenic fungi tested decreased with increase in the period of exposure of the larvae to the

pathogens, *B. bassiana* and *M. anisopliae*. This might be due to nutritional stress because of reduced food consumption by the infected larvae that enhances the insect susceptibility to fungal diseases (Ramoska and Todd, 1985). The fungal metabolites produced during the early stages of infection process act initially to stimulate and then inhibit food consumption (Fargues *et al.,* 1994).

5.2 MEDIAN LETHAL TIMES

The median lethal times (LT_{50}) for *B. bassiana* as pure culture were in the range of 180.0 to 302.4 hours for the concentrations $25x10^4$ to $10x10^4$ spores/ml. Whereas, the calculated LT_{50} values for *B. bassiana* as Bio-power, the commercial formulation ranged between 129.6 to 172.8 hours for the concentrations $125x10^4$ to $50x10^4$ spores/ml. The lower concentrations $5x10^4$ spores/ml and $25x10^4$ spores/ml, of *B. bassiana* as pure culture and as Bio-power, respectively did not result in 50 per cent larval mortality. Lack of sufficient number of spores that adhere to the body of *S. litura* larvae to result in 50 per cent mortality might be the reason for not attaining LT_{50} values in the lower concentrations. The virulence of *B. bassiana* strain used in the marketed formulation, Bio-power is lower than that of *B. bassiana* pure culture. This might be the reason for not attaining LT_{50} at $25x10^4$ spores/ml in the formulation as against LT_{50} of 180.0 hours for *B. bassiana* pure culture at $25x10^4$ spores/ml concentration.

The present LT_{50} values of 180.0 to 302.4 hours for *B. bassiana* as pure culture and 129.6 to 172.8 hours as Bio-power, were in close proximity with the earlier reports of Sivasankaran *et al.,* 1990 (4.6 to 6.2 days) and

Dayakar and Kanaujia, 2003 (5.13 to 6.45 days) against the second and third instar larvae of *C. infuscatellus* (10^5 spores/ml) and third instar larvae of *S. litura* ($14.85x10^5$ spores/ml), respectively.

With regard to *M. anisopliae* the LT_{50} values at $300x10^4$, $250x10^4$, $200x10^4$, $150x10^4$ and $100x10^4$ spores/ml were 196.8,199.2, 237.6, 240.0 and256.8 hours, respectively.

The present LT_{50} values of 196.8 to 256.8 hours were in close proximity with the LT_{50} values of 5.13 to 6.45 and 7.01 days reported earlier by Dayakar and Kanaujia (2003a) and Ajay Kumar Pandey and Kanaujia (2003) against the third (1.73 to $6.42x10^6$ conidia/ml) and fourth (14.85 to $71.69x10^5$ conidia/ml) instar larvae of *S. litura*, respectively.

The differences in the LT_{50} values of the present studies compared to the earlier reports of both the pathogens might be due to variations in the test insects, concentrations tested and strains of entomopathogenic fungi used.

Entomopathogenic fungi can infect insects not only through the gut, but also through spiracles and particularly through the surface of the integument. This property leads directly to the theoretical possibility of infecting insects independently of their feeding activity (Ferron, 1978).

B. bassiana does not have known sexual cycle. Insects are infected by conidia (asexual propagules) which attach to the host cuticle. Conidia germinate in an environment with humidity. The germ tubes developing from the conidia penetrate the host cuticle and invade the haemocoel. A successful infection by *B. bassiana* is dependant primarily on various

enzymatic activities for degradation of proteins, chitin and lipids in the insect integument (Ferron *et al.,* 1991; Khachatourians, 1991). On invading the haemocoel, the fungus proliferates. Mycelia from the elongated germ tube are septate and release blastospores. Host insects are killed due to depletion of haemolymph nutrients and/or due to toxaemia caused by fungal toxic metabolites (Khachatourians, 1991; Roberts, 1981). Under moist conditions, the fungus emerges and produce a layer of aerial conidia on the surface of host cadavers (Plate 8).

Electron microscopic studies on the histopathology of fungal infections of wire worms by *M. anisopliae* showed that the fungus develops through at least six identifiable stages (1) External infective spores – produce the germ tube. (2) Appressorial cells formed from germ tube against the surface of the cuticle – produce the penetrant peg through the epicuticle. (3) Sub epicuticular penetrant plate – produces hyphae that give rise to the penetrant hyphal bodies. (4) Irregular walled and smooth walled penetrant hyphal bodies – give rise to hyphae that penetrate the procuticle to the coelom. (5) Detached coelomic hyphal bodies produced by the penetrant hyphae – circulate in the haemolymph, germinate to produce new hyphae and then spread the fungus in the body cavity and (6) Chlamydospores produced after the death of the host – maintain the fungus in viable state within the host cadaver (Plate 9). The spores subsequently germinate to form emergent hyphae that sporulate on the surface of the host to produce new external infective spores (Kish and Allen, 1976; Kish *et al.,* 1976; Zacharuk, 1973).

Several toxic compounds were isolated and identified from cultures of *B. bassiana* and *M. anisopliae.* The cyclodepsipeptide, beauvericin was found in *B. bassiana* (Hamill *et al.,* 1969). The cyclicdepsipeptides, such as destruxins A, B, C and D and desmethyl destruxin B were isolated from cultures of *M. anisopliae* (Roberts, 1966; Roberts, 1969).

Histological studies of insect tissues attacked by entomopathogenic fungi showed that toxins produced by the fungus incite progressive degeneration of the host tissues, with no appreciable swelling or shrinkage of host cells. The primary effects appeared to be on the structural integrity of the membranes of the various cytoplasmic organells. This and the serious dehydration of tissue cells, seemed to be primarily responsible for host mortality (Zacharuk, 1971).

5.3 EFFECT OF UV-PROTECTANTS ON THE PATHOGENICITY AND GROWTH OF *B. bassiana* AND *M. anisopliae*

The effect of UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% on the pathogenicity and growth of the two entomopathogenic fungi *viz., B. bassiana* and *M. anisopliae* were tested at their LC_{50} values ($9.4x10^4$ spores/ml and $98.9x10^4$ spores/ml, respectively).

The effect of UV-protectants on the pathogenicity of the test fungi, *B. bassiana* in the descending order of larval mortality was charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%. The larval mortality ranged between 71.20 to 39.17 per cent for charcoal 1%, 66.41 to 39.17 per cent for indian ink 1%, 60.91 to 23.58 per cent for congo red 1%,

56.82 to 23.58 per cent for robin blue 0.5% and 36.24 to 20.61 per cent for yeast extract 2% as against 34.54 to 18.14 per cent larval mortality in case of *B. bassiana* without any UV-protectant with 30 minutes to three hours exposure of the fungal spores to UV-light.

For both the fungi the descending order of the UV-protectants that improved their pathogenicity by reducing the median lethal times against the third instar larvae of *S. litura* were charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%. The reduction in LT_{50} values because of the addition of UV-protectants compared to *B. bassiana* without any UV-protectant with 30 minutes and one hour of UV exposure ranged between 43.28 to 30.86 per cent for charcoal 1%, 41.79 to 25.92 per cent for indian ink 1%, 34.32 to 17.28 per cent for congo red 1%, 29.85 to 18.51 per cent for robin blue 0.5% and 2.98 to 3.70 per cent for yeast extract 2%.

The effect of UV-protectants on the pathogenicity of the test fungi, *M. anisopliae* in the descending order of larval mortality was charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%. The larval mortality ranged between 75.51 to 38.47 per cent for charcoal 1%, 73.05 to 37.71 per cent for indian ink 1%, 66.49 to 36.90 per cent for congo red 1%, 63.52 to 34.67 per cent for robin blue 0.5% and 44.28 to 31.51 per cent for yeast extract 2% as against 33.17 to 21.55 per cent in case of *M. anisopliae* without any UV-protectant with 30 minutes to three hours exposure of the fungal spores to UV-light.

Whereas, the reduction in LT_{50} values because of the addition of the UV-protectants compared to *M. anisopliae* without any UV-protectant with

30 minutes and one hour of UV exposure ranged between 53.06 to 36.84 per cent for charcoal 1%, 53.06 to 30.26 per cent for indian ink 1%, 48.97 to 23.36 per cent for congo red 1%, 48.97 to 11.84 per cent for robin blue 0.5% and 16.32 to 14.47 per cent for yeast extract 2%.

However, the UV-protectants did not give any protection to the pathogenicity of the entomopathogenic fungi to result 50 per cent larval mortality, if the fungi were exposed to five hours of UV-light, hence the LT_{50} values were not obtained. Similar was the case for three hours of UV exposure with congo red 1%, robin blue 0.5% and yeast extract 2% for *B. bassiana* and yeast extract 2% for *M. anisopliae*.

With regard to the effect of UV-protectants on the fungal growth exposed for the specified durations to UV-light, in the descending order was charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2%. The increase in *B. bassiana* fungal growth (diameter in cm) because of addition of the UV-protectants compared to *B. bassiana* without any UV-protectant for 30 minutes to three hours UV exposure ranged between 40.76 to 125.00 per cent for charcoal 1%, 34.96 to 96.42 per cent for indian ink 1%, 22.64 to 42.85 per cent for congo red 1%, 16.30 to 30.00 per cent for robin blue 0.5% and 10.50 to 3.57 per cent for yeast extract 2%.

Similarly, the increase in spore production of *B. bassiana* because of the protection given by UV-protectants compared to *B. bassiana* without any UV-protectant with 30 minutes to three hours UV exposure ranged between 46.15 to 128.57 per cent for charcoal 1%, 31.95 to 71.42 per cent for Indian

ink 1%, 26.03 to 28.57 per cent for congo red 1%, 5.91 to 0.00 per cent for robin blue 0.5% and 1.77 to 0.00 per cent for yeast extract 2%.

Whereas, the increase in *M. anisopliae* fungal growth because of addition of the UV-protectants compared to *M. anisopliae* without any UV-protectant with 30 minutes to three hours UV exposure ranged between 46.94 to 101.37 for charcoal 1%, 46.96 to 70.34 per cent for indian ink 1%, 33.53 to 37.93 per cent for congo red 1%, 28.20 to 28.96 per cent for robin blue 0.5% and 22.68 to 1.37 per cent for yeast extract 2%.

With regard to *M. anisopliae,* the increase in spore production because of the addition of UV-protectants compared to *M. anisopliae* without any UV-protectant with 30 minutes to three hours UV exposure ranged between 100.79 to 216.66 per cent for charcoal 1%, 68.25 to 66.66 per cent for indian ink 1%, 58.73 to 33.33 per cent for congo red 1%, 28.57 to 33.33 per cent for robin blue 0.5% and 12.69 to 16.66 per cent for yeast extract 2%.

Thus, it is evident that the lethal effect of UV-light on the growth and spore production is increasing with increase in period of exposure to UV-light. Hence, the LT_{50} values were not obtained for the fungi either with or without some of the UV-protectants from three hours of exposure onwards. However, charcoal 1% and indian ink 1% can be stated as the successful UV-protectants for the entomopathogenic fungi, *B. bassiana* and *M. anisopliae,* which increased the larval mortality and reduced the LT_{50} values through sustained fungal growth and spore production of the respective fungi.

The products used as UV-protectants in the present study were reported to be efficacious as UV-screening compounds and improved the bioefficacy of microbials like NPV against *S. litura* (Ramakrishnan and Chaudhary, 1991) and *H. armigera* (Reddy *et al.,* 2001), and *Bacillus thuringiensis* var. *kurstaki* against *S. litura* (Srivastava and Prasad, 2000) and *P. xylostella* (Gailce Leo Justin *et al.,* 1999).

Considerable work has been done on UV-protection of insect baculoviruses but relatively very little information on entomopathogenic fungi was published despite the fact that UV-light is recognized as a key factor limiting the impact of these pathogens (Gardner *et al.,* 1977; Feng *et al.,* 1994; Inglis *et al.,* 1995). The UV-B portion of solar radiation (290-320 nm) represents the UV-light of greatest biological interest, while negative effects on microorganisms have been noted for wavelengths outside this range (Bullock *et al.,* 1970). It is with in the UV-B range that most damage occurs (Roberts and Campbell, 1977; Killick, 1987; Fargues *et al.,* 1997).

UV exposure can have two effects, (1) Direct damage to DNA, creating strand breaks or cross linkages between bases, which can block the synthesis of normal DNA and create high levels of mutations and (2) the production of highly reactive and deleterious radicals such as peroxides (Pearlman *et al.,* 1985; Ignoffo and Garcia, 1994). The result of both is a rapid reduction in the stability of the microorganism and a limit to its insecticidal activity (Moore *et al.,* 1993; Inglis *et al.,* 1995).

Rapid inactivation of spores of entomopathogenic fungi occurred following exposure to UV-light. The rate of inactivation was reduced through

the addition of UV-protectants. The present study confirmed the very high sensitivity of fungal spores to UV-radiation and demonstrated how, cheap and simple UV-protectants could enhance spore persistence even under intense exposure to UV-light.

CONCLUSIONS:

The sailent findings of the present laboratory investigation on the 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' are as follows.

- The LC_{50} values were $94.6x10^4$ and $56.2x10^4$ spores/ml after six and seven DAT for *B. bassiana* as Bio-power, a commercial formulation, 20.4×10^4 and $265.2x10^4$ spores/ml and $9.4x10^4$ and $98.9x10^4$ spores/ml at nine and eleven DAT for *B. bassiana* and *M. anisopliae* as pure cultures, respectively.

- The LT_{50} values for *B. bassiana* as pure culture @ $25x10^4$ to $10x10^4$ spores/ml and as Bio-power @ $125x10^4$ to $50x10^4$ spores/ml, and *M. anisopliae* as pure culture @ $300x10^4$ to $100x10^4$ spores/ml were ranged between 180.0 to 302.4 hours , 129.6 to 172.8 hours and 196.8 to 256.8 hours, respectively.

- For *B. bassiana* as pure culture @ $5x10^4$ spores/ml and *B. bassiana* as Bio-power @ $25x10^4$ spores/ml did not result in 50 per cent larval mortalities. Hence, LT_{50} values were not obtained.

- The LT_{50} values for *B. bassiana* @ $9.4x10^4$ spores/ml (LC_{50}) and *M. anisopliae* @ $98.9x10^4$ spores/ml (LC_{50}) with and without UV-protectants for 30 minutes and one hour of UV exposure ranged between 91.2 to 194.4 hours and 55.2 to 182.4 hours, respectively.

- Whereas, the LT_{50} values of *B. bassiana* at its LC_{50} with charcoal 1% and indian ink 1% were 235.2 and 242.4 hours and LT_{50} values for *M. anisopliae* at its LC_{50} with charcoal 1%, indian ink 1%, congo red 1% and robin blue 1% ranged between 199.2 to 230.4 hours with three hours exposure to UV-light, respectively.

- The LT_{50} values could not be calculated with three hours exposure to UV-light in case of *B. bassiana* at its LC_{50} with or without congo red 1%, robin blue 0.5% and yeast extract 2%, and for *M. anisopliae* without any UV-protectants and with yeast extract 2% as 50 per cent larval mortalities were not resulted.

- With five hours of exposure to UV-light, for both the fungi either with or without the UV-protectants, 50 per cent larval mortalities were not resulted. Hence, LT_{50}s could not be calculated.

- *B. bassiana* and *M. anisopliae* at their LC_{50}s with the UV-protectants resulted in significantly higher larval mortalities, which ranged between 20.61 to 71.20 and 31.51 to 75.51 per cent as against 18.14 to 34.54 and 21.55 to 33.17 per cent in *B. bassiana* and *M. anisopliae* without any UV-protectant with 30 minutes to three hours exposure to UV-light, respectively.

- The fungal growths (diameter in cm) of *B. bassiana* and *M. anisopliae* at their LC_{50}s with UV-protectants were significantly protected (1.40 to 7.77 cm and 1.45 to 7.45 cm) when compared to *B. bassiana* (1.40 to 5.52 cm) and *M. anisopliae* (1.45 to 5.07 cm) without any UV-protectant with 30 minutes to three hours exposure to UV-light, respectively.

- Spore productions of *B. bassiana* and *M. anisopliae* at their LC_{50}s with UV-protectants were significantly higher (8.75 to $617.5x10^4$ spores/ml and 7.50 to $632.5x10^4$ spores/ml), compared to *B. bassiana* (8.75 to $422.5x10^4$ spores/ml) and *M. anisopliae* (7.50 to $315.0x10^4$ spores/ml) without any UV-protectant with 30 minutes to three hours exposure to UV-light, respectively.

CHAPTER VI

SUMMARY

The present investigation entitled 'Effect of UV-protectants on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin against *Spodoptera litura* (Fab.)' was carried out under laboratory conditions in the Department of Entomology, Agricultural College, Bapatla during 2004-05. The experiments were conducted in completely randomized block design with each treatment replicated four times.

The LC_{50} values of the two entomopathogenic fungi were $20.4x10^4$ and $9.4x10^4$ spores/ml after nine and eleven days after treatment (DAT) for *B. bassiana* as pure culture, $94.6x10^4$ and $56.2x10^4$ spores/ml at six and seven DAT for *B. bassiana* as Bio-power, a commercial formulation, and $265.2x10^4$ and $98.9x10^4$ spores/ml at nine and eleven DAT for *M. anisopliae* as pure culture, respectively.

The LT_{50} values for *B. bassiana* as pure culture @ $25x10^4$ to $10x10^4$ spores/ml and as Bio-power @ $125x10^4$ to $50x10^4$ spores/ml, and *M. anisopliae* as pure culture @ $300x10^4$ to $100x10^4$ spores/ml ranged between 180.0 to 302.4 hours, 129.6 to 172.8 hours and 196.8 to 256.8 hours, respectively.

The LT_{50} values were not obtained for *B. bassiana* as pure culture @ $5x10^4$ spores/ml and as Bio-power @ $25x10^4$ spores/ml as 50 per cent larval mortalities were not resulted.

B. bassiana at its $LC_{50,}$ $9.4x10^4$ spores/ml with UV-protectants *viz.,* charcoal 1%, indian ink 1%, congo red 1%, robin blue 0.5% and yeast extract 2% resulted in significantly higher larval mortalities (36.24 to 71.20 per cent at six DAT, 31.56 to 47.87 per cent at six DAT and 20.61 to 39.17 per cent at nine DAT) with 30 minutes, one hour and three hours exposure to UV-light compared to those without any UV-protectant (34.54, 28.71 and 18.14 per cent, respectively).

Similarly, *M. anisopliae* at its $LC_{50,}$ $98.9x10^4$ spores/ml with the UV-protectants resulted in significantly higher larval mortalities (44.28 to 75.51 per cent at four DAT, 34.67 to 57.74 per cent at six DAT and 31.51 to 38.47 per cent at seven DAT) with 30 minutes, one hour and three hours exposure to UV-light compared to those without any UV-protectant (33.17, 26.47 and 21.55 per cent, respectively).

The LT_{50} values for *B. bassiana* at its LC_{50} either with or without the UV-protectans with 30 minutes and one hour exposure to UV-light ranged between 91.2 to 160.8 hours and 134.4 to 194.4 hours, respectively. Whereas, the LT_{50} values of *B. bassiana* at its LC_{50} with charcoal 1% and indian ink 1% as UV-protectans with three hours of exposure to UV-light were 235.2 and 1242.4 hours, respectively.

The LT_{50} values for *M. anisopliae* at its LC_{50} either with or without the UV-protectants with 30 minutes and one hour exposure to UV-light were in the range of 55.2 to 117.6 hours and 115.2 to 182.4 hours, respectively. Whereas, the LT_{50} values for *M. anisopliae* at its LC_{50} with charcoal 1%,

indian ink 1%, congo red 1% and robin blue 0.5% with three hours exposure to UV-light were in the range of 199.2 to 230.4 hours.

The LT_{50} values could not be calculated with three hours exposure to UV-light in case of *B.bassiana* at its LC_{50} without any UV-protectant and with congo red 1%, robin blue 0.5% and yeast extreact 2%, and *M. anisopliae* at its LC_{50} with without any UV-protectant and with yeast extract 2%.

Similarly, the LT_{50} values could not be calculated for both the fungi either with or without any UV-protectant as 50 per cent larval mortality was not resulted with five hours exposure.

The fungal growth (diameter in cm) of *B. bassiana* at its LC_{50} with the UV-protectants ranged between 6.10 to 7.77 cm, 4.55 to 5.90 cm and 1.45 to 3.15 cm as against *B. bassiana* without any UV-protectant 5.52, 4.15 and 1.40 cm with 30 minutes, one hour and three hours exposure to UV-light, respectively.

Spore production of *B. bassiana* at its LC_{50} with the UV-protectants ranged between 430.0 to 617.5×10^4 spores/ml, 117.5 to 225.5×10^4 spores/ml and 8.75 to 20.0×10^4 spores/ml as against 422.5×10^4, 110.5×10^4 and 8.75×10^4 spores/ml in case of *B. bassiana* without any UV-protectant with 30 minutes, one hour and three hours of exposure to UV-light, respectively.

The fungal growth (diameter in cm) of *M. anisopliae* at its LC_{50} with the UV-protectants ranged between 6.22 to 7.45 cm, 5.32 to 6.20 cm and 1.47 to 2.92 cm as against *M. anisopliae* without any UV-protectant 5.07, 4.02 and 1.45 cm with 30 minutes, one hour and three hours exposure to UV-light, respectively.

Spore production of *M. anisopliae* at its LC_{50} with the UV-protectants ranged between 355.0 to 632.5x10^4 spores/ml, 125.0 to 185.0x10^4 spores/ml and 8.75 to 23.75x10^4 spores/ml as against *B. bassiana* 315.0x10^4, 85.0x10^4 and 7.50x10^4 spores/ml in case *M. anisopliae* without any UV-protectant with 30 minutes, one hour and three hours exposure to UV-light, respectively.

LITERATURE CITED

Ajay Kumar Pandey and Kanaujia K R 2003 Effect of larval extract medium on pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin against tobacco caterpillar, *Spodoptera litura* (F.) Journal of Entomological Research 27 (3): 175-180.

Baskaran R K M, Venugopal M S and Janarthanan R 1998 Ultraviolet protection of the nuclear polyhedrosis virus of the groundnut red hairy caterpillar, *Amsacta albistriga* Wlk. (Lepidoptera : Arctiidae) through congo red. Journal of Entomological Research 22 (4): 343-347.

*Bullock H R, Hollingsworth J P and Harstack A W 1970 Virulence of Heliothis nuclear polyhedrosis virus exposed to monochromatic ultraviolet radiation. Journal of Invertebrate Pathology 16: 419-422.

*Cohen E, Rozen H, Joseph T, Braun S and Margulies L 1990 Photoprotection of *Bacillus thuringiensis* Kurstaki from ultraviolet irradiation. Journal of Invertebrate Pathology 57: 343-351.

Dayakar S and Kanaujia K R 2001 Susceptibility of tobacco caterpillar, *Spodoptera litura* (F.) (Lepidoptera : Noctuidae) to different isolates of entomopathogenic fungi (Deuteromycotina : Hyphomycetes). Indian Journal of Plant Protection 29 (1, 2): 62-67.

Dayakar S and Kanaujia K R 2003a Evaluation of the pathogenicity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Nomuraea rileyi* on different larval stages of tobacco caterpillar, *Spodoptera litura* (F.). Indian Journal of Plant Protection 31 (2): 9-12.

Dayakar S and Kanaujia K R 2003b Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to different development stages of castor spiny caterpillar, *Ergolis merione* L. Journal of Oilseeds Research 20 (2): 312-314.

Delarosa W, Alatorre R, Trujillo J and Barrera J F 1997 Virulence of *Beauveria bassiana* (Deuteromycetes) strains against the coffee berry borer (Coleoptera: Scolytidae). Journal of Economic Entomology 90 (6): 1534-1538.

Desh Mukh P G and Mathai A T 1991 Efficacy of *Bacillus thuringiensis* against larvae of *Spodoptera litura.* Pestology 15: 34-36.

Deva Prasad V, Jayaraj S and Rabindra R J 1989 Susceptibility of tobacco caterpillar, *Spodoptera litura* (F.) (Noctuidae : Lepidoptera) to certain entomogenous fungi. Journal of Biological Control 3 (1): 53-55.

Deva Prasad V, Jayaraj S and Rabindra R J 1990 Susceptibility of gram Caterpillar, *Heliothis armigera* (Hub.) (Noctuidae : Lepidoptera) to certain entomogenous fungi. Journal of Biological Control 4 (1): 44-47.

Dunkle R L and Shasha B S 1989 Response of starch encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. Environmental Entomology 18 (6): 1035-1041.

*Edgington S, Segura H, Delarosa W and Williams T 2000 Photoprotection of *Beauveria bassiana* **:** testing simple formulation for control of the coffee berry borer. International Journal of Pest Management 46 (3): 169-176.

*Ekesi S, Adamu R S and Maniania N K 2002 Ovicidal activity of entomopathogenic hyphomycetes to the legume pod borer, *Maruca vitrata* and the pod sucking bug, *Clavigralla tomentosicollis*. Crop Protection 21:589-595.

Fargues J , Rouiger M, Goujet R, Smits N, Coustere C and Hier B 1997 Inactivation of conidia of *Paecilomyces fumosoroseus* by near ultra violet (UV B and UV A) and visible radiation. Journal of Invertebrate Pathology 69:70-78.

Fargues J, Delmas J C and Lebrum R A 1994 Leaf consumption of the Colorado potato beetle (Coleoptera: Chrysomelidae) infected with the entomopathogen, *Beauveria bassiana*. Journal of Economic Entomolgy 87: 67-71.

Feng M G, Poprawski T J and Khachatourians G G 1994 Production, formulation and application of the entomopathogenic fungus, *Beauveria bassiana* for insect control **:** current status. Biocontrol Science and Technology 4: 3-34.

Ferron P 1978 Biological control of insect pests by entomogenous fungi. Annual Review of Entomology 23: 409-42.

*Ferron P, Fargues J and Riba G 1991 Fungi as microbial insecticides against pests. Hand Book of Applied Mycology Vol 2, pp: 665-706.

*Finney D J 1984 Probit analysis. Cambridge University Press, London pp. 333.

*Fluents R G and Carballo V M 1995 Evaluation of isolates of *Beauveria bassiana* (Bals.) Vuill. for the control of *Plutella xylostella* (L.) (Lepidoptera : Yponomeutidae). Manejo Integrado de Plagas 35: 14-18.

Gailce Leo Justin C, Rabindra R J and Swamiappan M 1999 Screening of substances as UV protectants for *Bacillus thuringiensis* Berliner against *Plutella xylostella* (L.) on cauliflower. Pest Management and Economic Zoology 7 (1): 67-72.

Gardner W A, Sutton R M and Noblet R 1977 Persistence of *Beauveria bassiana, Nomuraea rileyi* and *Nosema necatrix* on soybean foliage. Environmental Entomology 6: 616-618.

Gaugler R and Boush G M 1979 Laboratory tests on ultraviolet protectants of entomogenous nematode. Environmental Entomolgy 8: 810-813.

*Glorina A S, Raja N, Seshadri S, Janarthanan S and Ignacimuthu S 2000 Pathogenicity of entomopathogens, *Bacillus thuringiensis* sub sp. kurstaki and *Beauveria bassiana* to the larvae of *Spodoptera litura* (F.) and *Pericalia ricini* (F.). Biological Agriculture and Horticulture 18 (3): 235-242.

*Gomez K A and Gomez A A 1984 Statistical procedures for Agricultural Research, 2nd Edition. International Rice Research Institute, Philippines pp. 680.

Gopalakrishnan C and Narayanan K 1988 Occurrence of two entomofungal pathogens, *Metarhizium anisopliae* (Metsch.) Sorokin var. minor Tulloch and *Nomuraea rileyi* (Farlow) Samson on *Heliothis armigera* (Hub.) (Noctuidae: Lepidoptera).Current Science 5(57): 867-868.

Gopalakrishnan C and Narayanan K 1989 Studies on the susceptibility of *Helicoverpa armigera* (Hub.) (Lepidoptera : Noctuidae) to the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin var. Anisopliae Tulloch Entomon 14 (3, 4): 191-197.

Gopalakrishnan C and Narayanan K 1990 Studies on the dose mortality relationship between the entomofungal pathogen, *Beauveria bassiana* and *Helicoverpa armigera* (Hub.). Journal of Biological Control 4: 115-122.

*Hamill R L, Higgens C E, Boaz H I and Gorman M 1969 The structure of Beauvericin, a new depsipeptide antibiotic toxin *Artemia salina*. Tetrahedron Letters 49: 4255-58.

Haseeb M Murad H and Sharma S 2001 Dose mortality relationship between the entomogenous fungus, *Beauveria bassiana* and larvae of *Spilosoma obliqua* Walker (Lepidoptera : Arctiidae). Bioved 12 (1, 2): 85-89.

*Hoffmann W C, Kirk I W,Dugger P and Richter D 2000 Sunlight protection for ULV malathion. 2000 Proceedings Beltwide Cotton Conferences, San Antonia, USA 4-8: 1356-1358.

Ignoffo C M and Garcia C 1994 Antioxidant and oxidative enzyme effects on the inactivation of inclusion bodies of the Heliothis baculovirus by simulated sunlight – UV. Environmental Entomology 6: 411-415.

Ignoffo C M, Hostetter D L, Sikorowski P P, Sutter G and Brooks W M 1977 Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus and protozoan by an ultraviolet light source. Environmental Entomolgy 6: 411-415.

*Im D J, Jin B R, Choi K N and Kong B K 1990 Microbial control of the tobacco cut worm, *Spodoptera litura* (F.) using *S. litura* nuclear polyhedrosis virus III. Field evaluation of the viral insecticides. Korean Journal of Applied Entomology 29 (4): 252-256.

*Inglis G D, Goettel M S and Johnson D L 1995 Influence of ultraviolet light on persistence of the entomopathogenic fungus, *Beauveria bassiana.* Biological Control 5: 581-590.

Kamalajayanthi P D and Padmavathamma K 1996 Effect of microbial agents on different developmental stages of tobacco caterpillar *Spodoptera litura* (F.). Indian Journal of Plant Protection 24(1 &2): 102-109.

Khachatourians G G 1986 Production and use of biological pest control agents. Trends in Biotechnology 4: 120-124.

*Khachatourians G G 1991 Physiology and genetics of entomopathogenic fungi. Hand Book of Applied Mycology Vol 2, pp: 613-663.

*Killick H J 1987 Ultraviolet and Panolis nuclear polyhedrosis virus **:** A non-problem? in S.R. leather, population biology and control of the pine beauty moth. Forestry Commission Bulletin No.67, pp: 69-75.

*Kish L P and Allen G E 1976 Conidial production of *Nomuraea rileyi* on *Pseudoplusia includens*. Mycologia 68: 436-39.

*Kish L P, Greene G L and Allen G E 1976 A method of determining the number of potential conidia forming cadavers of *Anticarsia gemmatalis* infected with *Nomuraea rileyi* in a soybean field. Fla Entomolgy 59: 103-5.

*Koa S S and Huang L H 1992 Effectiveness of UV protectants for *Spodoptera exigua* (Hub.) nuclear polyhedrosis virus. Chinese Journal of Entomology 12 (1): 31-40.

Kulat S S, Patil P R, Nimbalkar S A, Patil P S and Sarnaik P K 2003 Bioassay of *Metarhizium anisopliae* against pigeonpea pod borer, *Helicoverpa armigera.* International Journal of Pest Management 10: 55-56.

*Li z z 1988 A list of insect hosts of *Beauveria bassiana*, in study and application of entomogenous fungi in China. Academic Periodical Press, Beijing pp: 241-255.

Manjula K and Padmavathamma K 1999 Effect of insect pathogens on the larvae of *Helicoverpa armigera* (Hub.) (Lepidoptera : Noctuidae). Entomon 24 (1): 71-74.

Michael R, Guire M C, Douglas A S and Shasha B S 1991 Evaluation of starch encapsulation for formulation of grasshopper (Orthoptera : Acrididae) entomopox viruses. Journal of Economic Entomology 84 (6): 1652-1656.

Michael R, Guire M C, Shasha B S, Lewis L C, Bartelt R J and Kinney K 1990 Field evaluation of granular starch formulations of *Bacillus thuringiensis* against *Ostrinia nubilalis* (Lepidoptera : Pyralidae). Journal of Economic Entomology 83 (6): 2207-2210.

Mondal P, Bhattacharya A K, Tewary S C and Biswas P G 2001 A new computer programme for probit analysis **:** Effect of *Beauveria bassiana* on *Helicoverpa armigera*. Indian Journal of Entomology 63 (3): 295-308.

Moore D, Bridge P D, Higgins P M, Bateman R P and Prior C 1993 Ultra-violet radiation damage to *Metarhizium flavoviride* conidia and the protection given by vegetable and mineral oils and chemical sunscreens. Annals of Applied Biology 122: 605-616.

Padmavathamma K and Veeresh G K 1995 Effect of sunlight protectants and time of application on the virulence of nuclear polyhedrosis virus of *Plutella xylostella* (L.). Current Research 24 (5): 92-98.

*Pearlman D A, Holbrook S R, Pirkle D H and Kim S H 1985 Molecular models for DNA damaged by photoreaction. Science 227: 1304-1308.

*Pishokha N P and Timchenko G A 1988 The effect of photo protective additives in preserving the virulent properties of Gomelin.Lesovodstvo I Agrolesomelioratsiya 76 (4): 48-51.

Prabakaran G, Padmanabhan V and Balaraman K 2000 Comparative efficacy of UV- screens in protecting the activity of a *Bacillus thuringiensis* formulation. Journal of Biological Control 14 (2): 63-66.

Prasad T V, Srivastava K L and Kumar J 2003 Stabilization of *Bacillus thuringiensis* in WDP formulation by UV protectants. Annals of Plant Protection Science 11 (2): 189-191.

Rabindra R J and Jayaraj S 1988 Larval extracts and other adjuvants for increased efficacy of nuclear polyhedrosis virus against *Helicoverpa armigera* larvae. Journal of Biological Control 2 (2): 102-105.

Rabindra R J, Sathiah N, Muthiah C and Jayaraj S 1989 Controlled droplet application of nuclear polyhedrosis virus with adjuvants and UV-protectants for the control of *Helicoverpa armigera* (Hbn.) on chickpea. Journal of Biological Control 3 (1): 37-39.

Ramakrishnan N and Chaudhary S 1991 Effectiveness of some ultraviolet protectants on the activity of nuclear polyhedrosis virus of *Spodoptera litura* (F.). Indian Journal of Entomology 53 (4): 548-551.

Ramesh K, Murali Mohan Ch, Lakshmi K A, Padmavathi J and Uma Devi K 1999 *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes, Moniliales) in cotton pest management **:** A field trial on the cotton leaf roller, *Sylepta derogata* Fab. (Lepidoptera : Pyraustidae). Journal of Entomological Research 23 (3): 267-271.

Ramoska W A and Todd T 1985 Variation in the efficacy and viability of *Beauveria bassiana* in the chinch bug (Hemiptera: Lygaeidae) as a result of feeding activity on selected host plants. Environmental Entomology 14: 146-148.

*Reddy A R, Divakar B J, Reddy S M, Rao D and Vidyavati 2001 Effect of robin blue on the efficacy of nuclear polyhedrosis for the management of *Helicoverpa armigera* (Hub.) in groundnut crop. Perspectives in Biotechnology (6): 41-43.

Roberts D W 1966 Toxins from the entomogenous fungus, *Metarhizium anisopliae* II. Symptoms and detection in moribund hosts. Journal of Invertebrate Pathology 8: 222-27.

Roberts D W 1969 Toxins from the entomogenous fungus, *Metarhizium anisopliae* **:** isolation of destruxins from submerged cultures. Journal of Invertebrate Pathology 14: 82-88.

Roberts D W 1981 Toxins of entomopathogenic fungi **:** microbial control of pests and plant diseases. Academic Press, London pp: 441-464.

*Roberts D W and Campbell A S 1977 Stability of entomopathogenic fungi in C.M. Ignoffo and D.L Hostetter (eds) Environmental stability of microbial insecticides (USA: American Entomological Society) Miscellaneous Publications No.10, pp: 19-76.

*Sandhu S S 1993 Mode of entry of *Beauveria bassiana* in *Helicoverpa armigera* larvae. National Academy Science Letters 16 (4): 133-135.

Shapiro M 1989 Congo red as an ultraviolet protectant for the gypsy moth (Lepidoptera : Lymantriidae) nuclear polyhedrosis virus. Journal of Economic Entomology 82 (2): 548-550.

Sivasankaran P, Easwaramoorthy S and David H 1990 Pathogenicity and host range of *Beauveria nr. bassiana*, a fungal pathogen of *Chilo infuscatellus* Snellen. Journal of Biological Control 4 (1): 48-51.

Sood A K, Mehta P K, Kashyap N P and Ramesh Lal 2001 Bio efficacy of *Beauveria bassiana* against diamond back moth, *Plutella xylostella* (L.) (Lepidoptera : Yponomeutidae). Journal of Entomological Research 25 (4): 267-271.

Srivastava K L and Prasad T V 2000 Testing of “Pusa – BT” – A new water dispersible powder formulation of *Bacillus thuringiensis* against last instar larvae of *Spodoptera litura* (Fab.). Bioved 11 (1, 2): 11-12.

The Hindu Survey of Indian Agriculture 2003 pp: 200-202.

Wadyalkar S R, Wasule D L, Bhoyte S J and Wadskar R M 2003 Mass multiplication and formulation of *Metarhizium anisopliae* (Metsch.) Sorokin. Journal of Biological Control 17 (2): 141-146.

*Yendol W G and Robert D W 1971 Is microbial control with entomogenous fungi possible? Journal Series of the Pennsylvania State University Agriculture Experimental Station 28-42.

Yoon C S, Sung G H, Park H S, Lee S G and Lee J O 1999 Potential of the entomopathogenic fungus, *Beauveria bassiana* strain CS-1 as a biological control agent of *Plutella xylostella* (Lepidoptera : Yponomeutidae). Journal of Applied Entomology 123 (7): 423-425.

*Zacharuk R Y 1971 Ultra structural changes in tissues of larval elateridae infected with the fungus, *Metarhizium anisopliae.* Canadian Journal of Microbiology 17: 281-89.

*Zacharuk R Y 1973 Electron microscope studies of the histopathology of fungal infections by *Metarhizium anisopliae.* Miscellaneous Publications of Entomological Society of America 9: 112-19.

***Originals not seen**

Note: The literature is cited as per the “Thesis Guide lines” prescribed by Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.