STUDY ON STORABILITY OF *Metarhizium anisopliae* METSCHNIKOFF (SOROKIN)

By

Mr. RATHOD GOKUL PUNDLIK
Reg. No. 09/111

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI – 413722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA)

In partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL ENTOMOLOGY

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH, RAHURI-413722
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2012

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part there of has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place :- M.P.K.V., Rahuri.
Date :- / /2012 (Rathod G. P.)

Dr. J. R. Kadam
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Maharashtra, India.

CERTIFICATE

This is to certify that the thesis entitled, **"STUDY ON STORABILITY OF *Metarhizium anisopliae* METSCHNIKOFF (SOROKIN)"** submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist-Ahmednagar, Maharashtra, India, in partial fulfillment of the requirement for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL ENTOMOLOGY** embodies the results of a piece of *bonafide* research work carried out by **Mr. RATHOD GOKUL PUNDLIK,** under my guidance and supervision and no part of the thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation has been duly acknowledged.

Place :- MPKV, Rahuri (J. R. Kadam)
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CERTIFICATE

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Place :- MPKV, Rahuri

Dated :-   /   /2012   (R. S. Patil)

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Place : MPKV, Rahuri.

Date : *(Rathod G. P.)*

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LIST OF ABBREVIATIONS

@	:	at the rate of
a.i.	:	active ingredient
AS	:	Aqua Suspension
^{0}C	:	Degree Celsius
Bb	:	*Beauveria bassiana*
CD	:	Critical difference
Cfu	:	Colony Forming Units
et al.	:	et alia (and others)
g	:	gram
J.	:	Journal
i.e.	:	Id est (that is)
kg	:	kilogram
m	:	meter
mm	:	millimeter
M.a.	:	*Metarrhizium anisopliae*
µg	:	microgram
µl	:	microliter
ml	:	milliliter
ml-1	:	per milliliter
S.E.	:	Standard Error
viz.	:	Videlicet (Namely)
V.l.	:	*Verticillium lecanii*
WP	:	Wettable Powder
Wt	:	Weight
%	:	per cent
h	:	hour

ABSTRACT

"STUDY ON STORABILITY OF *Metarhizium anisopliae* METSCHNIKOFF (SOROKIN)"

By

Mr. RATHOD GOKUL PUNDLIK
A Candidate for the Degree
of
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Department of Agricultural Entomology,
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2012

Research Guide	:	**Dr. J.R.Kadam**
Department	:	**Agricultural Entomology**

The present investigations on "Studies on storability of *M.anisopliae* METSCHNIKOFF (Sorokin)" were carried out at Biocontrol Research Laboratory, Department of Entomology, M.P.K.V., Rahuri during 2010-11. storability was studied for 18 months of *M.anisopliae* 3% AS and 1.15% WP under refrigerator and ambient conditions. The bioefficacy of *M.anisopliae* against *S. litura* was also studied. The cfu count of both the formulations was determined. It was 3.9×10^9 cfu/ml for the 3% AS and 3.0×10^9 cfu/ml for the 1.15% WP.

Storability study showed that on the basis of growth of the micoagent and viability the safe storage period of

M.anisopliae in refrigerator condition for 3% AS was 17 months and that for 1.15% WP was 16 months against the under ambient conditions *M.anisopliae* 3% AS could be stored upto 15 months and 1.15% WP upto 14 months against the storability.

The bioefficacy study revealed that the mortality of one week old larvae of *S. litura* with *M.anisopliae* 3% AS and 1.15% WP either stored under refrigerator and at ambient conditions for1 to 18 months and applied at 0.6, 0.5 and 0.4% were more promising against one week old larvae of *S. litura.* The bioefficacy decreased from 75.56 to 38.89% in *M.anisopliae* 1.15% WP and 86.67 to 44.44% in *M.anisopliae* 3% AS when stored under refrigerator conditions. The corresponding figures for the fungus stored at ambient conditions were 73.77 to 35.56% and 81.11 to 36.67%, respectively.Maximum mortality was observed on 14th day of treatment for both of these formulations. But all the treatments were significantly superior to untreated control in reducing the population of larvae.

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1. INTRODUCTION

Integrated pest management is gaining importance in recent years in view of risk of synthetic chemical insecticides oriented environmental pollution and health hazards. Biological control is an important, effective, ecofriendly and economical component of IPM for almost all important pest of major crops for the development of sustainable cropping systems. The fungal pathogens causing disease to the insect are one of the potential microbial bioagents. Entomopathogenic fungi are practically more significant as they are epizootic in nature. Also they have the advantage of ease in production and contact action, which allow direct penetration in host cuticle without ingestion (Payne,1988). Out of 700 species of entomopathogenic fungi currently known, the viable microbial chemical insecticide formulations of only 10 species are presently being developed for insect pest control Keller (1992), Kerwin (1992), MeCoy *et al.* (1988) and Rath (1992). It implies scope for the subject. *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Verticillium lecani,* (Zimmarman) viegas are some of the frontline entomopathogenic fungi. These microbial agents are attractive alternative to the use of many chemical insecticides.

Green muscardine fungus, *M. anisopliae* Sorokin is one of the potential entomopathogenic fungus. (Order: Monillales, Family: Monilliacae). Metschnikoff (1879) was first to isolate fungus, *M. anisopliae* from the larvae of grain weevil and also first to demonstrate entomopathognic nature of the fungus against chrysomelid, curculonid and scarabaeid beetles. It is capable of infecting more than 100 species of insect pests belonging to a variety of insect orders. These are Orthoptera (Grasshopper and Cockroaches), Homoptera (Spittle bug, *Nilapervata lugens*) and Lepidoptera (*Helicoverpa armigera, Spodoptera litura*) (MeCoy *et al.*, 1988).

M. anisopliae is characterized as green muscardine fungus due to green colour of the sporulating colonies. It forms a mycelia mat on cuticle of insects. The infective unit is conidia or blastospores which germinate and forms short germ tube bearing appresoria with infective peg attach to cuticle. The infective pegs penetrate in layer of integument by enzymatic dissolution of chitin and protein. It reaches the haemocoel and internal organs and insect is filled with fungus. The death of insects occurs due to obliteration of tissues, production of toxins (destruxin A, B, C, D, E) and proteolytic enzymes secreted by the fungus. Infected insects show symptoms like loss of appetite, decreased irritability, general or partial paralysis, loss of mobility, discolouration and mummification. Thus fungus infect by contact action grow in body, release toxins (destruxin A, B, C, D, E).

Richard and Donald (1983) reported that *M. anisopliae* were stored at four temperatures *viz.*, 37; 26; 19 and 4 ^{0}C at each of six RH *viz.*, 0, 12, 33, 53, 75 and 98 %. Conidia were found to survive longest when moderate temperature, high RH (26 ^{0}C – 97 % RH or 19 ^{0}C – 97 % RH) or low temperature, low RH (4 ^{0}C – 0 % RH) were used while intermediate RH values between 33 and 75 % decreased conidial viability and temprature 37 to 4 ^{0}C increased conidial viability upto 18 month.

Considering the failure of chemical insecticides to control *S.litura* on one hand and health hazards on the other hand it is necessary to develop sustainable pest management strategy. Biochemical insecticides are the best solution as they are ecofriendly. *M. anisopliae* was found to be pathogenic to *S.litura* (Dayakar and Kanaujia, 2001).Recently MPKV, Rahuri developed 1.15% WP and 3% AS formulations of *M. anisopliae.* So far there is no research on Storability, pathogenicity and bioefficacy of *M. anisopliae* in Maharashtra. Hence, due to ecofriendly properties of *M.anisopliae* the investigation was undertaken on storability of *M. anisopliae* with following objectives:

1) To study effect of storability on viability of *M.anisopliae.*
2) To find out shelf life of *M.anisopliae* under refrigerated and ambient conditions storage.

2. REVIEW OF LITERATURE

Microbial control of crop pests offers an environmentally acceptable strategy with lower cost and effects in longer run. Entomopathogenous fungi played a significant role in the history of insect pathology and specifically in microbial control (Steinhaus, 1956). The general review on entomopathogenous fungi has been presented by Narishmna (1970), Kamat and Raw (1975), Ferron (1981) and Wolf (1981).

Green muscardine fungus, *Metarhizium anisopliae* (Mitschnikoff) Sorokin is a cosmopolitan fungus infecting about 200 spp of insects from 7 orders.

2.1 Classification and morphology of *M. anisopliae*

Tulloch *et al.* (1976) observed that the entomopathogenic fungus, *M.anisopliae* occurs in two varieties. These are separated primarily on conidial length, *M. anisopliae* var. anisopliae (3.5 – 9.0 µm) and *M. anisopliae* var. Major (9.0 – 18 µm).

MeCoy *et al.* (1998) reported that *M. anisopliae* was capable of infecting more than 100 species of insect pests belonging to variety of insect orders.

Milner *et al.* (2002) observed variation in colony characters of *M.anisopliae* var. anisopliae collected from different geographical locations and host insects.

Scoco *et al.* (2007) studied the survival of *B. bassiana* and *M. anisupliae* in a faceville series soil (stem sterilized, oven dried and brought to a moisture content of 10

%) over 28 days period. Germ tube were initially observed at 36 – 48 h after placement and mycelial growth was evident there after *M. anisopliae* produced a more extensive mycelial mat than the *B. bassiana.*

Rachappa *et al.* (2009) noted that *M. anisopliae* collected from different geographical regions was studied for various parameters like growth and sporulation on PDA medium Ma_3 and Ma_4 isolates took 6 days to initiate sporulation while Ma_1 and Ma_2 isolates 2-3 days on PDA. Mycelial growth in Ma_1 and Ma_2 was significantly higher (38.60 and 42.30 mm, respectively) than Ma_3 (32.30 mm) and Ma_4 (33.19 mm).

2.2 Versatility of *M. anisopliae*

Ferron (1981) reported that *M.anisopliae* is an entomopathogenic hyphomycetes fungus with potential practical value for the control of agriculturally important insect pests.

Aguda *et al.* (1984) reported that *M.anisopliae* and *B.bassiana* is host specific and virulent.

Prior and Arura (1985) isolated *M.anisopliae* from the rhinocerous beetle, *Scapines australis* and *Oryctes rhinocerous*, the black palm weevil, *Rhynchophorus bilineatus* (coleopteran), and three other insect orders (Hemiptera, Lepidoptera and Dermaptera). The 32 per cent of dead *Scapines* adults collected during the 48 weeks from released palms compared to 1 per cent in the non release palms.

Singha *et al.* (2006) reported that two strain of *M. anisopliae* and two strains of *B. bassiana* for potential use of biological control against *Microcerotermes beesoni (*snyder). The strains were screened for relative patogenicity and the mediuml lethal time eliciting 50 per cent mortality (LT_{50}) was calculated.All isolates exhibited the ability to grow, sporulate and produce an epizootic in treated pests.

2.3 Persistivity of *M. anisopliae*

Quesada Moraga *et al.* (2008) evaluated the horizontal transmission capacity of the autochthonous *M. anisopliae* strain EAM_a 01/58-54 among *Ceratitis capitata* in laboratory tests. Male and females inoculated either with dry conidia or with wet conidia became infected exhibiting 95.0 – 100 per cent, mortality rates with mycosis and average survival time values of 8.30 – 9.30 days.

Roberts (1970) reported that *M. anisopliae*, a hypomycetes fungus has a biological control agent of agricultural pests for more than 100 years but recently proposed as a mosquito larvicide agent.

Garcia *et al.* (1981) observed that *M. anisopliae* infecting adult of the Mediterranean fruit fly, *Ceratitis capitata*, under laboratory condition.

Milner and Prior (1994) noted high pathogenicity in certain isolates of *M. anisopliae* to previously uncontrolled host and observed three times grater susceptibility of *Chortoicetes terminifera* to isolates of *M.anisopliae*.

Milner *et al.* (2003) suggested that *M. anisopliae* is used in Australia as a biochemical insecticide for control of sugarcane white grubs in soil. The biocane formulation was effective for maximum period of 3 years.

2.4 Storability of *M. anisopliae*

Bell and Hamalle (1974) studied 14 species and 6 strains of fungus and found some could be stored upto 3 years without measurable loss of viability, but other could be stored for few weeks only. *B. bassiana*, *M. anisopliae* and *Spicaria rilevi* retained their pathogenicity to insects after 3 years also.

Bell (1975) observed that conidiospore of *M. anisopliae*, *N. rileyi* and *B. bassiana* stored outdoor for 12 months continuous in shate culture tube alone or with silica gel crystals (loose or in porous cloth bag) and tested quarterly in laboratory for pathogenicity to larvae of *Heliothis zea* (Boddie),spore of *B. bassiana* were unable to survive any of the test conditions. But those of *N. rileyi* retained 12 months and *M. anisopliae* for 6 months stored in bag with silica gel for both spp. some activity was retained for 6 months without silica gel. These results are discussed in relation to pathogenicity of fungi in the field.

Muller-Kogler *et al.* (1980) reported that storage at 18^{0}C proved the most statisfactory for fungus imperfecti (including *M. anisopliae*, *B. bassiana* and *N. rileyi*) and some of the groups were still alive after a year. Sporulation and growth of subculturing after storage were comparable to those of the original cultures.

Muller-Kogler and Zimmermann (1980) evaluate 12 isolates each of *M. anisopliae*, *B. bassiana*, *B. brongniartii* and *Hirsutella* spp. were stored at 4^{0}C all of the isolates survived for 3 years.

Couch and Ignoffo (1981) observed biological and physiological properties of the formulation must remain stable at least for 1 year, but preferably for greater than 18 months of commercialization to occure.

Richard and Donald (1983) reported that unformulated conidia of three strains of *M. anisopliae* were stored at four temperatures (*viz.*, 37, 26, 19 and 4^{0}C) at each of six RH (*viz.*, 0, 12, 33, 53, 75 and 98%) and at -20^{0}C at 0 % RH. Conidial viability and virulence against two species of mosquitoes were determined over 2 years period. Conidia were found to survive longest when moderate temperature high RH (26^{0}C – 97% RH or 19^{0}C – 97% RH) or low temperature low RH (4^{0}C – 0% RH), while intermediumte RH values between 33 and 75 % depending upon the strain, were most lethal as the temperature declined from 37 to 4^{0}C. Conidia remained highly virulent under favourable storage conditions for at least 18 months against *Culex pipiens* or 12 months against *Anophelus stephengi* larvae.

Couch and Ignoff (1981) reported that water may be inappropriate for long term storage of some entomopathogenic fungus.

Daoust and Roberts (1983a) reported that conidia of *M. anisopliae* were found to survive longest when moderate temperature and high RH (26^{0}C – 97% RH or 19^{0}C – 97% RH) or low temperature low RH (4^{0}C – 0% RH) were used.

Daoust and Roberts (1983b) reported that the best storage conditions of retention of both spore viability and virulence of *M. anisopliae* against mosquitoes were 19^{0}C – 97% RH and 4^{0}C – 0% RH.

Alives *et al.* (1987) studied the viability of 64 formulations of the entomopathogenious fungus, *M. anisopliae* under two storage conditions (at room temperature and refrigerated) and for pure conidia of the fungus and one formulation of the pure conidia plus silica gel under deep frozen conditions. The time for which the fungus could be stored increased by upto 33 per cent at room temperature and upto 52 per cent in refrigerator (at 2 – 3^{0}C), depending on formulation and formulation kept in the freezer were viable for upto 660 days (70 % viability).

Wandersch *et al.* (1990) reported that liquid culture of *M. anisopliae* can induced to form aggregates of hypal cell under certain conditions during fermentation increased in diameter from 0.1 to 1.5 mm granule dried at 50^{0}C. The final product. Was known as BIO-1020. The cell remained viable for 8 months at 4^{0}C, but the shelf life was increased to 18 months at this temperature when the granules were stored in a vacuum. The biological activity tested against *Otiorhynchus sulcatus* at a rate of 0.1, 0.5 and 1.0 gm granules liter $^{-1}$ water. Pots were infested with One week old larvae in the green house at 23^{0}C and 80% RH. The number of formed conidia was constant for about 6 months and biological activity was 60 to100 %.

Stathers *et al.* (1993) observed that fungi mixed with oil of high aromatic content may damage conidia. Soya oil and groundnut oil were slightly better than kerosene but rancid oil inhibit germination of conidia.

Kleepsir and Zimaram (1994) studied blastospores of 3 strains of *M. anisopliae* stored in liquid solution at 4, 20 and 35^{0}C for 18, 12 and 8 days respectively. Viability was quantified by determination of their germination. The blastospores survived best at 4^{0}C in 10 per cent hydroxyethyl starch, germination of *M. anisopliae* more than 80 per cent after storage for 18 weeks. The blastospores survived best at 20^{0}C in 25 per cent Ringer's solution. Germination 43 per cent after storage for 12 weeks. The blastospores survived at 35 ^{0}C in 25 per cent glycerol, 45 per cent germination after storage 7 days.

Jackson *et al.* (1995) enumerate that the storage of fungus at 4^{0}C after air drying lead to 25 per cent survival for 7 months and improved to 60 per cent by freezer drying and storage under vacuum.

Moore *et al.* (1997) investigated that effect of moisture content and temperature in the medium term (3-4 months) storage of conidia of *M. anisopliae*. The optimal moisture content for storage was 4-5 per cent. Germination level of upto 96 per cent and 80 per cent after 80 days at 10 – 14 and 28 – 32^{0}C, respectively. In another experiment, dried conidia maintained greater than 90 per cent germination over 128 days, with or without silica gel at 10 – 14^{0}C.

Guillon (1997) concluded that the possibility of using additives was really protecting *M. anisopliae* conidia at 4 ^{0}C and *V. lecanii* at 2 ^{0}C from the deleterious effect of cold.

Faria *et al.* (1999) reported that conidial viability of 24 strain of entomopathogenic fungi (hyphomycetes: *B. bassiana, M. anisopliae, N. rileyi, Pacilomyces farinosus, P. fumosorosus* and *P. lilacinus*) presented by Lyphilization and in liquid nitrogen. Germination rate of 16 – 24 months old culture stored in liquid nitrogen decreasedd on an average less than 13.3 to 94.5 per cent for 29 – 49 months old culture. The viability loss on an average from 28.6 – 94.5 per cent.

Marques (1990) studied the preparation of experimental formulation with mycelia of *B. bassiana* and *M. anisopliae*. The formulations were prepared with sodium alginate and pre-gelatinized corn starch and stored at 25, 30 and 35^{0}C for 120 days. Pre-gelatinized corn starch was more suitable than sodium alginate for the formulation with mycelia of *B. bassiana* and *M. anisopliae*.

2.5 Congeniality of *M. anisopliae*

2.5.1 Light

Moore *et al.* (1993) reported that delay in germination following exposure to stimulated sunlight.It may be the result of damage to protein and nucleic acids leading to slower growth rate.

Fargues *et al.* (1996) reported the longest exposure time (8 hr) survival of *M. anisopliae* and *M. flavoviride* isolates ranged from 0.1 to 11 per cent and from 0 to 1 per cent, respectively.

Alves *et al.* (1998) studied the survival of *M. anisopliae* var. acridum (IMI 330189) at 1, 2, 4 and 8 hours of exposure to stimulated sunlight, respectively. In addition to causing spore mortality, exposure to UV inhibits the germination rate of *M. anisopliae.*

Samson *et al.* (1988) reported that some limiting factor like ultraviolet (UV), temperature and humidity greatly affect the activity of entomopathogenic fungi.

Horaczek and Virenstein (2004) observed the variability in UV tolerance between *M. anisopliae* and *M. flavoviride* strains. Reported UV-B exposure reduced survival of *M. anisopliae* conidia.

2.5.2 Temperature

Walstad *et al.* 1970 reported that conidia of both entomopathogenous and plant pathogenic fungi generally remain viable longer as the temperature decline to near freezing.

Bell and Hamalle (1974) reported that *M. anisopliae* spore remain viable for upto 3 years at -20 ^{0}C on silica gel crystal.

Roberts and Campbel (1977) reported that temperature is one of the environmental factors that influence fungal growth and disease development in insects.

Patel *et al.* (1990) denoted that *M. anisopliae* var anisopliae grown on Zapeck's Dox medium was the most suitable for redial growth, sporulation and biomass production. The optimum temperature was growth at 25 ^{0}C.

Tanada and Kaya (1993) reported that infection, growth and sporulation of survival entomopathogenic fungi are influenced by environmental factors, especially temperature and humidity.

Hedgecock *et al.* (1995) observed that drying *M. anisopliae* aerial conidia improves their thermal stress tolerance.

Hong *et al.* (1997) reported that a negative logarithmic relationship between the moisture content of *M. anisopliae* var. acridum aerial conidia and their ability to survive thermal stress at 50^0C.

Hong *et al.* (2000) reported that method of drying *M.anisopliae* var. acridum aerial conidia influences the thermal stress tolerance of dried spores.

Arthurs and Thomas (2001) studied the infection of *M.anisopliae* var. acridum on desert locust. The mortality rate at constant incubation temperature between 15 and 30 ^{0}C broadly reflected the temperature related vegetative growth profile of the fungus, with higher dose causing quickest mortality upto 80 per cent.

Strasser *et al.* (2003) studied the nine strains of *M. anisopliae* var. anisopliae (KVL-275, V-245, V-208, IMBST-9601, IMBST-9609, IMBST-9631, FAL-376 and FAL-538). All strains lost the ability to germinate at temperature higher than 30^0C.

Tadele and Ken (2003) studied the effect of temperature regimes on conidial germination, redial growth and virulence of *B. bassiana* and *M. anisopliae* a suitable temperature range at 20 – 30^0C and at 25 – 30^0C induced mortality 100 per cent to *Chilo partellus* larvae in 6 days. The LC_{50} decreased for the isolates with increasing temperature.

Dimbi *et al.* (2004) studied the temperature effects on conidial germination, mycelial growth suseptibility of adult of three tephritid fruit fly, *Ceratitis capitata*, *C. fasciventris* and *C. cosyra* to six isolates of *M. anisopliae* in laboratory. Observed over 80 per cent conidia growth at 20, 25 and 30 ^{0}C. The LT_{50} value decreased at increasing temperature more than 30^{0}C.

2.5.3 Relative humidity

Kawakami and Mikuni (1985) showed that relative humidity was very important to the survival of both *B. bassiana* and *P. farinose.* Most favorable humidity was 75 to 80 per cent.

Clerk and Madelin (1965) attempted a correlation of temperature, humidity and light on the survival of conidia of three insect pathogenic fungi in *M. anisopliae, B. bassiana* and *N. rileyi* over a 635 days period.

Lord (2005) evaluated that entomopathogenic fungi are effective against insect in high humidity but there also report of low humidity favouring the efficacy of *M. anisopliae* and *B. bassiana* on entomopathogenus fungi.

Ihra *et al.* (2008) evaluated the effects of temperature and relative humidity (RH) on the pathogenicity of *M. anisopliae* strain FRM 515 to *Plautia crossota* Stali. Low temperature decreased the pathogenicity of the fungal strain. Its pathogenicity at 15^{0}C was lower than that at higher temperatures. Mortality rates differed little among stink bugs kept at 80, 60 and 40 per cent RH after treatment with a suspension containing 16 x 10^8 conidia^{-ml}, however, when 1 x 10^7 conidia ml^{-1} was used, the LT_{50} value at 40 per cent RH was 1.7 times that at 80 per cent RH.

Luz *et al.* (2008) studied the effects of relative humidity (43%, 75%, 86% and 98%) on *Aedes aegupti* egg treated with *M. anisopliae* or water only for upto a six months exposure at 25^0C. Survival of larvae inside egg was clearly affected by the lowest humidity 43% tested and conclusion diminished at all humidity after increasing period of exposure. *M. anisopliae* showed to have a strong ovicidal activity only at humidity close to saturation.

2.6 Formulation of *M. anisopliae*

Wadyalkar *et al.* (2003) reported that for mass multiplication of *M. anisopliae* yeast phosphate soluble starch agar (YPSSA) medium and 27^0C temperature was the best for germination, growth and sporulation.

Kulat *et al.* (2002) observed eight culture medium (Emerson YPSS, Sabouraud's dextrose agart yeast, Asthana and Hawker's, Barher's (Zapek's, potato Dextrose agar, corn meal and plain agar) evaluated for the growth of sporulation of *M. anisopliae* after inoculation sporulation initiated at 5.66 and 7.66 days on Emerson YPSS and barner's medium respectively. The highest spore (9.43 x 10^6 spores ml^{-1}) of fungal suspension was observed in barners medium followed by Emerson YPSS medium (8.29 x 10^6) and SDA+Y medium (7.16 x 10^6) at 10 days after inoculation.

Gupta *et al.* (2002) grown *M.anisopliae* on the different liquid medium viz., sabaraud dextrose yeast extract (SDY), sabauraud's dextrose malt extract (SDM) 1 per cent molasses yeast extract (MY-II) and 0.5 per cent molasses yeast extract (MY-I) and found spacially SDY medium best for growth and sporulation of *M. anisopliae*.

Song *et al.* (2000) cultured *M.anisopliae* in liquid medium containing various amino acid, vitamin, KNO_3 and $(NH_4)_2$ SO_4 to determine their growth and sporulation. The results showed that amino acid and vitamin has significant effects on growth and sporulation of submerged conidia. L-asporagine or L-alanine produced large number of submerged conidia (5.1 x 10^9 and 4.7 x 10^9) spore litres^{-1}. Medium containing vitamin B_6 (pyridoxine) + vitamin B produced large number of conidia with yield 17.8 x 10^9 and 16.4 x 10^9 spore litres^{-1}, respectively.

Li and Holdom (1995) found that soluble starch and soya peptone was a source of carbon and nitrogen in a agar medium and supported highest growth and sporulation of *M. anisopliae*.

2.7 Bioefficacy of *M.anisopliae* against *S.litura*

Siddaramaiah *et al.* (1986) recorded the per centage incidence of infection of larvae of *S. litura* with *M. anisopliae* on groundnuts in Karnataka, India, averaged 2.23, 8.59, 8.19, 12.72, 7.60, 7.20, 5.66 and 6.23 in the 8 years from 1977 to 1984, respectively. Infection began to appear in the II fortnight of June, was high in mid-August and fell to nil in November.

Dayakar and Kanaujia (2001) investigated the virulence of *B. bassiana*, *M. anisopliae* and *N. rileyi* isolates against the second instar of *S. litura* in laboratory bioassays. The most aggressive isolates were *B.bassiana* isolates MUCL-38502, *M. anisopliae* isolate MUCL-8237 and *N. rileyi* isolate MUCL-8217 with LC_{50} values of 5.23, 12.53 and 16.11×10^5 conidia ml^{-1}, respectively.

Dayakar and Kanaujia (2003) applied conidial suspensions (10^4-10^{10} conidia ml^{-1}) of the entomopathogenic fungi *B. bassiana*, *M. anisopliae* and *Nomuraea rileyi* to II-IV instar larvae of *S. litura* infesting tobacco to determine the pathogenicity of the fungi to the pest. The LC_{50} values of *B. bassiana*, *M. anisopliae* and *N. rileyi* against the II instar larvae were 6.43, 13.97, 11.33×10^5 conidia ml^{-1} and the LT_{50} values were 4.34, 5.33 and 4.85 days, respectively. The LC_{50} values for the III and IV instar larvae of the three fungi range from 14.85 to 71.69×10^5 conidia ml^{-1}, whereas the LT_{50} values recorded for the III and IV instar larvae ranged from 5.13 to 6.45 days. The results of the bioassays indicated that susceptibility of the pest decreased with age of the larvae in terms of both LC_{50} and LT_{50}.

Purwar and Sachan (2005) studied toxicity of two isolates of *B. bassiana* and *M. anisopliae* on tobacco caterpillar, *S. litura* and *Spilarctia obliqua* found that the activity decreased with the aging of the larvae.

Pandey and Kanaujia (2008) studied influence of different cereal grains as solid substrates on sporulation, viability and pathogenicity of *M.anisopliae* under laboratory condition. Among maize, sorghum, fingermillet, barley and wheat, sorghum as solid substrate resulted in highest spore (6.36×10^{7} conidia ml^{-1}) production with spore viability of 86.6%. Conidia produced from fingermillet showed highest virulence against one week old larvae of *S. litura* with LC_{50} value of 1.72×10^{6} conidia ml^{-1} compared to conidia from other sources. However, conidia produced from SDA medium showed highest spore production, viability and virulence to *S. litura* larvae compared to conidia produced from solid substrates.

Sree and Padmaja (2008) investigated that the cyclodepsipeptidic mycotoxin, destruxin produced by *M.anisopliae* has larvicidal properties. Destruxin causes serious damage to the epithelial cells of the midgut.The ultrastructural effects of crude destruxin on the salivary glands of 9 day old *S. litura* larva after 24 h of treatment with the mycotoxin at a dosage of 0.147 µg body wt. LD_{50} were observed.This investigation focused that salivary glands are also affected by destruxin apart from the already known midgut, Malphigian tubules and haemocytes.

Rajesh (2009) treated the pupae of *S. litura* (Fab.), with different concentrations of conidia *M.anisopliae* (ARSEF 7487), under laboratory conditions. Suspensions (10^{8} ml^{-1}) of conidia harvested from Sabouraud dextrose agar yeast extract (SDAY) plates resulted in the highest mortality (85.8%). The values of LC_{50} and LC_{90} suggested that *M. anisopliae* was the most virulent fungal strain. In soil bioassays, drenching the soil with conidial suspensions of ARSEF 7487 (10^8 conidia g^{-1} of soil) reduced the adult emergence from pupa by 81.3%, while premixing the sterile soil with conidia killed lesser number of pupae 62.9%.

3. MATERIAL AND METHODS

During the course of the present investigations storability of *Metarhizium anisopliae* (Metschnikoff) was studied at two conditions, under room temperatures/ambient and freezing at 4^{0}C with two solutions i. e. liquid solution and of wettable powder in Laboratory at Biocontrol Research Laboratory, Department of Agriculture Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeth, Rahuri, Maharashtra.

The material and methods employed for these studies are presented in this chapter.

3.1 Material

3.1.1 Taxonomic status of the test mycoagent, *M.anisopliae* (courtecy: compendium of mycology)

Kingdom: Mycota
Division: Eumycota
Sub-division: Deuteromycotina
Class: Deuteromycetes
Order: Moniliales
Family: Moniliaceae
Genus: *Metarhizium*
Species: *anisopliae*

3.1.2 Taxonomic position of test insect *S. litura*

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Lepidoptera
Family: Noctuidae
Genus: *Spodoptera*
Species: *litura*

3.1.3 Bio-insecticides used

The bio-insecticides, *M.anisopliae* 1.15% WP (Minimum 1×10^8 cfu g^{-1}) and 3% AS (Minimum 2×10^8 cfu ml^{-1}) were formulated and supplied by Biocontrol Research Laboratory, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri. Details of biochemical insecticides used for the experiment with their formulations and source are given in the following Table.

Details of bio-insecticides used for the experiment

Sr. No.	Bioinsecticide	Bioingredient content	cfu $ml^{-1}g^{-1}$	Source
1.	Phule Metarhizium 3% AS	*Metarhizium anisopliae* 3% V/V	2×10^8	Biocontrol Research Laboratory, Dept. of Agril. Entomology, MPKV, Rahuri.
2.	Phule Metarhizium 1.15% WP	*Metarhizium anisopliae* 1.15% V/W	1×10^8	Biocontrol Research Laboratory, Dept. of Agril. Entomology, MPKV, Rahuri.

3.1.4 Chemical insecticide used

Bioefficacy of *M.anisopliae* was compared with Triazophos 0.05%. Details of chemical insecticides used are given in the following Table.

Sr. No.	Chemical insecticide	Trade name	Source
1.	Triazophos 40EC	Hostathion	Bayer Co.Ltd. Mumbai

3.1.5 Medium for multiplication

The medium used for multiplication of the fungus was autoclaved potato dextrose agar (PDA) broth medium.

3.1.6 Other material

Aluminium trays, inoculation needles, laminar flow cabinet, petridishes, glass slides, plastic tubs, glass jars, tissue paper, sterilized cotton, test tubes, pipettes, B.O.D incubator, polythene bags, pins, ocular and stage micrometer, binocular microscope, binocular microscope with photographic facility, spirit, spirit lamp, disinfectants were made available in Biocontrol Laboratory of Dept. of Agril. Entomology, PGI, M.P.K.V., Rahuri.

3.2 Methodology

3.2.1 Morphological and Biometrical characters of *M. anisopliae*

Mycelial, conidiospore and conidial characters were studied by observing the petriplate culture under binocular microscope in laboratory conditions. The culture was grown under ambient condition (average temperature of 27 $\pm$ 2^0C with RH of 85 per cent) in laminar flow cabinet. Autoclaved potato dextrose agar in petri plates was inoculated with *M. anisopliae* culture. The inoculation was made in the center of the pertidishes and incubated at 20^0C in the incubator. In the same manner 10 petridishes were prepared and utilized for recording the biometrical and morphological characters of *M. anisopliae*.

3.2.2. Determination of colony forming units/ml

Volume of 60 ml of the already prepared potato dextrose cultured *M. anisopliae* inoculums was made upto 100 ml and grinded homogenously with the help of mechanical blender. The grinded material was filtered through UV sterilized muslin cloth. A drop of suspension was released in between a graduated portion of 'Neubaeur hematocytometer' and the cover slip. The number of conidia in each of the 100 randomly selected squares were counted. The CFU/ml were determined with the help of following formula.

$$\text{Standardization of CFU/ml} = \frac{\text{Number of CFU observed x dilution factor}}{\text{Number of squares observed x } 1/1/(4 \times 10^8)}$$

3.2.3 Viability of CFUs

The method used for the purpose was as suggested by Ming Guage Feng *et al.,* 1990. The autoclaved potato dextrose agar medium in petridishes (100 mm diameter) was inoculated with the help of micropipette by releasing 0.1 ml *M. anisopliae* suspension, other petridishes with medium were prepared in similar manner and inoculated with various dilution in the series (10^1 to 10^{10} CFU/ml) and incubated at 27 ± 1^0C. After 48 hrs colony count was recorded.

3.2.3.1 Storability of *M. anisopliae*

3.2.3.1.1 Study on storability of *M. anisopliae* under ambient condition.

3.2.3.1.1.1 Stydy on storability of *M.anisopliae* 3% AS.

i)	Design	:	CompletelyRandomized Design
ii)	Replication	:	3
iii)	Treatments	:	36 sample of formulated *M.anisopliae* 3% AS formulation each drawn tested for the growth and viability at fortnight interval starting from 01.09.2009 to 28.02.2011.
iv)	Quantity of *M. anisopliae*	:	1 Liters
v)	Storage temperature	:	Ambient temperature (27 ± 2 ^{0}C)
vi)	Storage R.H.	:	Ambient RH (85 %)
vii)	Growth testing conditions of the samples	:	Average temperature 22.5 ^{0}C (12.5–24.4^{0}C) Average RH 92% (90 – 94%)

viii)	Medium used	:	Potato dextrose agar sterilized in autoclave
ix)	Ranged of serial dilution	:	3% AS formulation 10^{-1} to 10^{-10}
x)	Special care	:	All the operations of these were carried out in laminar flow.
xi)	Quantity of sample used	:	1 ml/40 ml PDA.
xii)	Observation	:	After 24 hr cfu count and after 8 days biomass weight and surface area covered (per centage) recorded. The growth pattern of *M. anisopliae* in each replication was recorded.

3.2.3.1.1.2 Study on storability of *M.* anisopliae 1.15 % WP.

i)	Design	:	Completely Randomized Design
ii)	Replication	:	3
iii)	Treatments	:	36 sample of formulated *M.anisopliae* 1.15 % WP formulation each drawn tested for the growth and viability at fortnight interval starting from 01.09.2009 to 28.02.2011.
iv)	Quantity of *M. anisopliae*	:	1 Kg.
v)	Storage temperature	:	Ambient temperature (27±2 ^{0}C)
vi)	Storage R.H.	:	Ambient RH (85 %)
vii)	Growth testing conditions of the samples	:	Average temperature 22.5 ^{0}C (12.5–24.4^{0}C) Average RH 92% (90 – 94%)
viii)	Medium used	:	Potato dextrose agar sterilized in autoclave
ix)	Ranged of serial	:	3% AS formulation 10^{-1} to 10^{-10}

	dilution		
x)	Special care	:	All the operations of these were carried out in laminar flow.
xi)	Quantity of sample used	:	1gm/40 ml PDA.
xii)	Observation	:	After 24 hr cfu count and after 8 days biomass weight and surface area covered (per centage) recorded. The growth pattern of *M. anisopliae* in each replication was recorded.

3.2.3.1.2 Study on storability of *M.* anisopliae under refrigerator condition.

3.2.3.1.2.1 Study on storability of *M. anisopliae* 3% AS.

i)	Design	:	Completely Randomized Design
ii)	Replication	:	3
iii)	Treatments	:	36 sample of formulated *M.anisopliae* 3% AS formulation each drawn tested for the growth and viability at fortnight interval starting from 01.09.2009 to 28.02.2011.
iv)	Quantity of *M. anisopliae*	:	1 Liter
v)	Storage temperature	:	Refrigerator temperature (6±1 ^{0}C)
vi)	Storage R.H.	:	Refrigerator RH (98%)
vii)	Medium used	:	Potato dextrose agar sterilized in autoclave
viii)	Ranged of serial dilution	:	3% AS formulation 10^{-1} to 10^{-10}
ix)	Special care	:	All the operations of these were carried out in laminar flow.
x)	Quantity of sample used	:	1 ml/40 ml PDA.
xi)	Observation	:	After 24 hr cfu count and after 8 days

biomass weight and surface area covered (per centage) recorded. The growth pattern of *M. anisopliae* in each replication was recorded.

3.2.3.1.2.2 Study on storability of *M.* anisopliae 1.15 % WP.

i)	Design	:	Completely Randomized Design
ii)	Replication	:	3
iii)	Treatments	:	36 sample of formulated *M.anisopliae* 1.15 % WP formulation each drawn tested for the growth and viability at fortnight interval starting from 01.09.2009 to 30.02.2011.
iv)	Quantity of *M. anisopliae*	:	1 Kg.
v)	Storage temperature	:	Refrigerator temperature (6±1 ^{0}C)
vi)	Storage R.H.	:	Refrigerator RH (98%)
vii)	Medium used	:	Potato dextrose agar sterilized in autoclave
viii)	Ranged of serial dilution	:	3% AS formulation 10^{-1} to 10^{-10}
ix)	Special care	:	All the operations of these were carried out in laminar flow.
x)	Quantity of sample used	:	1gm/40 ml PDA.
xi)	Observation	:	After 24 hr cfu count and after 8 days biomass weight and surface area covered (per centage) recorded. The growth pattern of *M. anisopliae* in each replication was recorded.

3.2.3.2 Bioefficacy of *M.anisopliae* formulations against *S.litura* storage sample upto 18 months.

The Bioefficacy of *M.anisopliae* against one week old larvae of *S.litura* after storage 1, 6, 12 and 18 months was checked.

3.2.3.2.1 *M.anisopliae* 3% AS stored under ambient and refrigeratr condition.

Design : Completely Randomized Design

Replication : 3

Treatment : 8

Treatment Details:

Sr. No.	Treatments	Concentration based on		
		Product (%)	BAI %	cfu count
1.	*M.anisopliae* AS	0.1	0.003	1.69×10^5
2.	*M.anisopliae* AS	0.2	0.006	3.38×10^5
3.	*M.anisopliae* AS	0.3	0.009	5.07×10^5
4.	*M.anisopliae* AS	0.4	0.012	6.76×10^5
5.	*M.anisopliae* AS	0.5	0.015	8.45×10^5
6.	*M.anisopliae* AS	0.6	0.018	1.014×10^6
7.	Triazophos 40EC	0.2	0.08	-
8.	Untreated control	-	-	-

3.2.3.2.2 *M.anisopliae* 1.15% WP stored under ambient and refrigeratr condition.

Design : Completely Randomized Design

Replications : 3

Treatment : 8

Treatment Details:

Sr. No.	Treatments	Concentration based on		
		Product (%)	BAI %	cfu count
1.	*M.anisopliae* 1.15 % WP	0.1	0.0015	1.01×10^5
2.	*M.anisopliae* 1.15 % WP	0.2	0.0030	2.02×10^5
3.	*M.anisopliae* 1.15 % WP	0.3	0.0045	3.03×10^5
4.	*M.anisopliae* 1.15 % WP	0.4	0.0060	4.04×10^5
5.	*M.anisopliae* 1.15 % WP	0.5	0.0075	5.05×10^5
6.	*M.anisopliae* 1.15 % WP	0.6	0.0090	6.06×10^5
7.	Triazophos 40EC	0.2	0.08	-
8.	Untreated control	-	-	-

Date of release of larvae : 1st September 2009,
1st March 2010,
1st September 2010
1st March 2010.

No. of larvae released : 30/polybag/ replication/treatment.

3.2.3.2.3 Method of treatment

The surface sterilized castor leaves were treated with respective, solution as given under treatment details. In each treatment of each replication thirty, one week old larvae were released on the respective, concentration treated castor leaves using fine hair brush. These were placed in 30×20 cm sized perforated polythene bags. The respective, AS concentration of the test formulations of *M.anisopliae* was sprayed on the *S. litura* larvae. The bags used were uniformly perforated (100 perforations/ bag) and bags were sealed at open end with fine sterile pins. Water filled trays kept inside the wooden cage and sticks placed over the trays to ensure high humidity. Then bags were kept on the sticks. The observations on mortality of larvae and mycoses were taken at 7th and 14th day of treatment.

3.2.3.2.4 Method of recording observation

The dead larvae were counted daily. The observations were taken for larval mortality at 7th and 14th day of treatment. The per cent mortality was worked out on the basis of total number of live and dead larvae.

4. RESULTS AND DISCUSSION

The results of the studies on storability of *M.anisopliae* with ancillary study on bioefficacy of the entomopathogen against *Spodoptera litura* are presented in this chapter and discussed in light of available literature.

4.1 Morphological characters of *M.anisopliae*

M.anisopliae is characterized as a green muscardine fungus due to green colour of the sporulating colonies. It forms mycelial mat on cuticle of insects. The conidia are 3.5-9 μm long celled, long-ovoid to cylindrical forming long basipetal chain/columns. Conidiophores are short, erect, and septate, branched single or cluster of phialides, which abruptly taper towards apex (Figure1). It Infect insects through the mouthparts (food) or the host cuticle. The infective unit is conidia or blastospores. The spore germinates and forms short germ tube bearing appresoria which get attached to cuticle. The infective pegs penetrate in layer of integument.

4.2 Determination of cfu count of the test formulations of *M.anisopliae*

The data on the colony forming units of the propagules inoculated through serial dilution of *M.anisopliae* formulations are presented in Table 1. The colony count ml^{-1} of *M.anisopliae* 3% AS for serial dilutions of 10^{-8} and 10^{-7} was 39 and 323, respectively. Similarly, the colony count ml^{-1} of *M.anisopliae* 1.15% WP for 10^{-8} and 10^{-7} serial dilutions was 30 and 143, respectively. On an average the count was 3.9×10^{9} cfu ml^{-1} in *M.anisopliae* 3% AS against 3.0×10^{9} cfu ml^{-1} in the 1.15 % WP formulation.

Table1.Colony count of serial dilutions for *M.anisopliae* 3% AS and *M.anisopliae* 1.15% WP upto 72 hrs

Serial Dilution	*M.anisopliae* 3% AS colony count	*M.anisopliae* 1.15% WP colony count
10^{-8}	39	30
10^{-7}	323	143
10^{-6}	Uncountable	Uncountable

4.3 Storability of *M.anisopliae*

The data on the growth, development and viability of *M.anisopliae* formulations stored upto 18 months under refrigerator and ambient conditions are presented in Table 2 to 5.

4.3.1 Refrigerator condition.

4.3.1.1 Effect on storability of *M.anisopliae* 3% AS.

Surface area of *M.anisopliae*

The data on per cent medium surface covered by the green muscardine fungus are presented in Table 2. The surface coverage ranged from 25 to100 % during the 18 months of storage period. It was cent percent up to 9 months storage. There after, it declined gradually during 9 to 15 months from 98 to 70 %. After 16 months decreased in the surface coverage was some what rapid from 70 to 25 %.

Biomass weight of *M.anisopliae*

The data on weight of biomass of *M.anisopliae* 3% AS are presented in the Table 2. The biomass decreased with increasing storage period. The biomass range from 6.8 to3.2 gm 40 ml^{-1} medium during 18 months of storage period.It was range from 6.8 to 6.2 gm 40 ml^{-1} medium during 1^{st} to 9^{th} months of storage, respectively. There after, it decline gradually upto 5.2 gm 40 ml^{-1} medium at 14 months of

Table 2. Effect of storability of *M.anisopliae* 3% AS growth and viability under RF,Temp. 6 ±1^{0}C and RH 98±1 %

Month of storage	Mean cfu g^{-1} ($\times 10^{-8}$)	Biomass wt. g 40 ml^{-1} medium	Medium surface coverage (%)
01 Sep 2009	4.4	6.8	100
15 Sep 2009	4.4	6.8	100
01 Oct 2009	4.3	6.7	100
15 Oct 2009	4.4	6.7	100
01 Nov 2009	4.3	6.8	100
15 Nov 2009	4.2	6.8	100
O1 Dec 2009	4.2	6.7	100
15 Dec 2009	4.3	6.7	100
01 Jan 2010	4.1	6.7	100
15 Jan 2010	4.1	6.6	100
01 Feb 2010	4.0	6.6	100
15 Feb 2010	4.0	6.5	100
01 Mar 2010	3.9	6.5	100
15 Mar 2010	3.9	6.4	100
01 Apr 2010	3.8	6.4	100
15 Apr 2010	3.8	6.4	100
01 May 2010	3.6	6.3	100
15 May 2010	3.6	6.2	100
01 Jun 2010	3.4	6.2	100
15 Jun 2010	3.2	5.9	100
01 Jul 2010	3.0	5.8	98
15 Jul 2010	3.1	5.9	98
01 Aug 2010	3.0	5.7	97
15 Aug 2010	2.9	5.6	94
01 Sep 2010	2.8	5.6	92
15 Sep 2010	2.9	5.5	90
01 Oct 2010	2.7	5.5	88
15 Oct 2010	2.6	5.4	82
01 Nov 2010	2.6	5.2	86
15 Nov 2010	2.5	4.7	75
01 Dec 2010	2.3	4.3	72
15 Dec 2010	2.1	4	70
01 Jan 2011	1.9	3.8	60
15 Jan 2011	1.8	3.7	45
01 Feb 2011	1.5	3.4	30
15 Feb 2011	0.9	3.2	25
Grand mean	17.39	13.82	77.66
S.E.±	0.76	0.45	1.55
C.D. at 5%	2.30	1.36	4.77

storage. At the end of 18 months, it was drastically reduced to 3.2 gm 40 ml^{-1} medium.

Viability of *M.anisopliae*

The viability decreased with increasing storage period of the *M.anisopliae 3 %* AS. The storage was carried out in refrigerator at 6 ±1^{0}C and RH 98±1 % for the period of 18 months. The viability ranged from 4.4 ×10^{-9} to 0.9 ×10^{-9} cfu gm^{-1} of medium during the 18 months of storage period. It was range form 4.4 ×10^{-9} to 3.2 ×10^{-9} cfu gm^{-1} of medium during 1st to 8 th months of storage, respectively. There after, it declined gradually upto 2.1 ×10^{-9} cfu gm^{-1} of medium at 15 month of storage. At the end of 18 months it was drastically reduced to 0.9 ×10^{-9} cfu gm^{-1} of medium.

4.3.1.2 Effect on storability of *M.anisopliae* 1.15% WP.

Surface area of *M.anisopliae*

The data on percent medium surface covered by the green muscardine fungus are presented in Table 3.The surface coverage ranged from 18 to100 % during the 18 months of storage period. It was cent percent up to 8.5 months storage. There after, it declined gradually during 8.5 to 13 months from 99 to 86 %. After 14 months, decreased in the surface coverage was some what rapid from 60 to 18 %.

Biomass weight of *M.anisopliae*

The data on weight of biomass of *M.anisopliae* 1.15% WP are presented in Table 3. The biomass decreased with increasing storage period. The biomass ranged from 6.5 to 2.7 gm 40 ml^{-1} medium during 18 months of storage period. The biomass ranged from 6.5 to 6.0 gm 40 ml^{-1} medium during 1st to 8.5th month of storage, respectively. There after, it decline gradually upto 4.0 gm 40 ml^{-1} medium at 15 months of storage. At the end of 18 months it was drastically reduced to 2.7 gm 40 ml^{-1} medium.

Table 3.Effect of storability of *M.anisopliae* 1.15% WP growth and viability under RF, Temp. 6 ±1⁰C and RH 98±1 %

Month of storage	Mean cfu g^{-1} ($\times 10^{-8}$)	Biomass wt. g 40 ml^{-1} medium	Medium surface coverage (%)
01 Sep 2009	3.6	6.5	100
15 Sep 2009	3.6	6.5	100
01 Oct 2009	3.6	6.5	100
15 Oct 2009	3.5	6.4	100
01 Nov 2009	3.6	6.4	100
15 Nov 2009	3.5	6.3	100
O1 Dec 2009	3.5	6.4	100
15 Dec 2009	3.4	6.3	100
01 Jan 2010	3.5	6.2	100
15 Jan 2010	3.4	6.3	100
01 Feb 2010	3.4	6.3	100
15 Feb 2010	3.3	6.1	100
01 Mar 2010	3.2	6	100
15 Mar 2010	3.3	6	100
01 Apr 2010	3.3	5.9	100
15 Apr 2010	3.2	5.9	100
01 May 2010	3.2	6	100
15 May 2010	3.1	5.8	99
01 Jun 2010	3.0	5.7	98
15 Jun 2010	3.0	5.8	94
01 Jul 2010	3.1	5.8	95
15 Jul 2010	2.8	5.7	93
01 Aug 2010	2.8	5.5	92
15 Aug 2010	2.6	5.5	91
01 Sep 2010	2.5	5.3	88
15 Sep 2010	2.4	5.1	86
01 Oct 2010	2.2	4.9	60
15 Oct 2010	2.1	4.6	65
01 Nov 2010	2.0	4.5	56
15 Nov 2010	1.9	4.3	52
01 Dec 2010	1.6	4	50
15 Dec 2010	1.4	3.6	49
01 Jan 2011	1.2	3.2	40
15 Jan 2011	0.9	3	35
01 Feb 2011	0.8	2.9	30
15 Feb 2011	0.5	2.7	18
Grand mean	12.88	13.35	73.44
S.E.±	0.82	0.49	1.80
CD@ 5%	2.50	1.51	5,54

Viability of *M.anisopliae*

The viability decreased with increasing storage period of the *M.anisopliae* 1.15 % AS. The storage was carried out in refrigerator at 6 ±1^{0}C and RH 98±1 % for the period of 18 months. The viability ranged from 3.6 ×10^{-9} to 0.5 ×10^{-9} cfu gm^{-1} of medium during the 18 months of storage period. It was rangedd form 3.6 ×10^{-9} to 3.3 ×10^{-9} cfu gm^{-1} of medium during 1st to 7.5 th months of storage respectively. There after it decline gradually upto 1.9 ×10^{-9} cfu gm^{-1} of medium at 15 month of storage. At the end of 18 months it was drastically reduced to 0.5 ×10^{-9} cfu gm^{-1} of medium.

4.3.2 Ambient condition

4.3.2.1 Effect on storability of *M.anisopliae* 3% AS.

Surface area of *M.anisopliae*

The data on per cent medium surface covered by the green muscardine fungus are presented in Table 4. The surface coverage ranged from 21 to100 % during the 18 months of storage period. It was cent percent up to 8 months storage. There after, it declined gradually during 9 to 13.5 months from 99 to 88 %. After 14 months, decreased in the surface coverage was some what rapid from 63 to 21 %.

Biomass weight of *M.anisopliae*

The data on weight of biomass of *M.anisopliae* 1.15% WP are presented in Table 4. The biomass decreased with increasing storage period. The biomass ranged from 6.6 to 3.0 gm 40 ml^{-1} medium during 18 months of storage period.The biomass ranged from 6.6 to 6.1 gm 40 ml^{-1} medium during 1 to 8.5 months of storage, respectively, there after it declined gradually upto 4.4 gm 40 ml^{-1} medium at 15 months of storage. At the end of 18 months, it was drastically reduced to 3.0 gm 40 ml^{-1} medium.

Table 4. Effect of storability of *M.anisopliae* 3 % AS on growth and viability under Ambient **condition.**

Month of storage	Temperature ^0C		Min RH (%)		Mean cfu gm^{-1} $(\times 10^{-8})$	Biomass wt. gm 40 ml^{-1} medium	Surface area (%)
	Min.	Max.	Morning	Afternoon			
01 Sep 2009	20.8	30.3	93.5	67.5	3.9	6.6	100
15 Sep 2009	21	32.7	91.5	52.5	3.9	6.6	100
01 Oct 2009	21.1	31.7	93	55	3.9	6.6	100
15 Oct 2009	16.7	32.4	90.5	35	3.8	6.5	100
01 Nov 2009	18.5	27.6	91.5	64.5	3.7	6.5	100
15 Nov 2009	12.8	28.7	89.5	41	3.8	6.4	100
O1 Dec 2009	12.2	29.9	88.5	37	3.8	6.5	100
15 Dec 2009	10.8	28.2	90.5	42	3.7	6.4	100
01 Jan 2010	11.8	28.3	90.5	42.5	3.7	6.3	100
15 Jan 2010	8.9	28.1	90.5	36	3.6	6.4	100
01 Feb 2010	14.1	30.5	90	42.5	3.6	6.4	100
15 Feb 2010	13.7	33.2	88.5	32	3.5	6.2	100
01 Mar 2010	15.3	34.8	88.5	30.5	3.4	6.1	100
15 Mar 2010	17.2	37.7	87.5	22.5	3.4	6.1	100
01 Apr 2010	19.6	38.8	88	18	3.4	6.0	100
15 Apr 2010	21.7	40.9	86.5	21.5	3.4	6.0	100
01 May 2010	21.2	40.4	87.5	22.5	3.3	6.1	99
15 May 2010	24.2	39.3	90	31	3.3	5.9	97
01 Jun 2010	22.8	34.2	93	49	3.3	5.9	98
15 Jun 2010	22.9	33.3	93.5	55.5	3.2	5.9	96
01 Jul 2010	22.2	31.1	93	62	3.1	5.8	95
15 Jul 2010	22.3	30.2	93.5	64.5	3.1	5.8	94
01 Aug 2010	21.5	29.9	93	65	3.0	5.6	93
15 Aug 2010	21.3	28.9	93	72.5	2.9	5.6	90
01 Sep 2010	21.1	30.1	91	60.5	2.8	5.5	91
15 Sep 2010	21.5	30.8	94	63	2.7	5.2	86
01 Oct 2010	20.4	31.3	93	49	2.6	5.0	88
15 Oct 2010	20.7	30.1	92	51.5	2.4	4.9	63
01 Nov 2010	18.1	27.2	92	65.0	2.2	4.7	61
15 Nov 2010	12.3	28.9	89	4.01	2.0	4.4	58
01 Dec 2010	12.4	30.1	88.5	38.0	1.8	4.0	55
15 Dec 2010	10.9	28.6	90.5	42.5	1.7	3.9	45
01 Jan 2011	11.9	28.5	90.5	42.5	1.5	3.5	40
15 Jan 2011	9.1	28.2	90	36.5	1.4	3.3	35
01 Feb 2011	14.2	30.6	90	42.5	1.0	3.1	25
15 Feb 2011	13.8	33.3	88	32.5	0.8	3	21
Grand mean					16.41	13.49	73.85
S.E.±					0.69	0.46	1.99
C.D. at 5%					2.11	1.39	6.16

Viability of *M.anisopliae*

The viability decreased with increasing storage period of the *M.anisopliae* 3% AS. The storage was carried out in ambient for the period of 18 months. The viability ranged from 3.9 $\times 10^{-9}$ to 0.8 $\times 10^{-9}$ cfu gm^{-1} of medium during the 18 months of storage period.

It was rangedd form 3.9 $\times 10^{-9}$ to 3.3 $\times 10^{-9}$ cfu gm^{-1} of medium during 1 to 9.5 months of storage, respectively. There after, it declined gradually upto 2.0 $\times 10^{-9}$ cfu gm^{-1} of medium at 15 months of storage. At the end of 18 months it was drastically reduced to 0.8 $\times 10^{-9}$ cfu gm^{-1} of medium.

4.3.2.2 Effect on storability of *M.anisopliae* 1.15% WP.

Surface area of *M.anisopliae*

The data on per cent medium surface covered by the green muscardine fungus are presented in Table 5. The surface coverage ranged from 20 to100 % during the 18 months of storage period. It was cent percent up to 7.5 months storage. There after, it declined gradually during 8.0 to 12 months from 98 to 88 %. After 14 months, decreased in the surface coverage was some what rapid from 60 to 20 %.

Biomass weight of *M.anisopliae*

The data on weight of biomass of *M.anisopliae* 1.15% WP are presented in Table 5. The biomass decreased with increasing storage period. The biomass ranged from 6.1 to 2.5 gm 40 ml^{-1} medium during 18 months of storage period.The biomass ranged from 6.1 to 5.3 gm 40 ml^{-1} medium during 1^{st} to 8^{th} months of storage, respectively. There after, it declined gradually upto 4.5 gm 40 ml^{-1} medium at 14 months of storage. At the end of 18 months it was drastically reduced to 2.5 gm 40 ml^{-1} medium.

Table 5. Effect of storability of *M.anisopliae* 1.15% WP growth and viability under Ambient **condition.**

Month of storage	Temperature (^{0}C)		Min RH (%)		Mean cfu g^{-1} ($\times 10^{-8}$)	Biomass wt. g 40 ml^{-1} medium	Surface area (%)
	Min.	Max.	Morning	Afternoon			Min.
01 Sep 2009	20.8	30.3	93.5	67.5	3.0	6.1	100
15 Sep 2009	21	32.7	91.5	52.5	2.8	6.1	100
01 Oct 2009	21.1	31.7	93	55	2.6	6.1	100
15 Oct 2009	16.7	32.4	90.5	35	2.7	5.9	100
01 Nov 2009	18.5	27.6	91.5	64.5	2.8	5.9	100
15 Nov 2009	12.8	28.7	89.5	41	2.8	5.8	100
O1 Dec 2009	12.2	29.9	88.5	37	2.8	5.9	100
15 Dec 2009	10.8	28.2	90.5	42	2.7	5.8	100
01 Jan 2010	11.8	28.3	90.5	42.5	2.7	5.7	100
15 Jan 2010	8.9	28.1	90.5	36	2.6	5.7	100
01 Feb 2010	14.1	30.5	90	42.5	2.5	5.7	100
15 Feb 2010	13.7	33.2	88.5	32	2.6	5.5	100
01 Mar 2010	15.3	34.8	88.5	30.5	2.6	5.4	100
15 Mar 2010	17.2	37.7	87.5	22.5	2.4	5.4	100
01 Apr 2010	19.6	38.8	88	18	2.5	5.3	100
15 Apr 2010	21.7	40.4	86.5	21.5	2.5	5.3	98
01 May 2010	21.2	40.9	87.5	22.5	2.4	5.3	97
15 May 2010	24.2	39.3	90	31	2.1	5.2	95
01 Jun 2010	22.8	34.2	93	49	2.1	5.2	94
15 Jun 2010	22.9	33.3	93.5	55.5	2.0	5.2	93
01 Jul 2010	22.2	31.1	93	62	1.8	5.1	93
15 Jul 2010	22.3	30.2	93.5	64.5	1.6	5	91
01 Aug 2010	21.5	29.9	93	65	1.5	5	91
15 Aug 2010	21.3	28.9	93	72.5	1.4	5.1	88
01 Sep 2010	21.1	30.1	91	60.5	1.4	5	60
15 Sep 2010	21.5	30.8	94	63	1.3	4.9	65
01 Oct 2010	20.4	31.3	93	49	1.4	4.7	61
15 Oct 2010	20.7	30.1	92	51.5	1.3	4.5	60
01 Nov 2010	18.1	27.2	92	65	13	4	58
15 Nov 2010	12.3	28.9	89	41	1.1	3.8	55
01 Dec 2010	12.4	30.1	88.5	38	1.0	3.6	35
15 Dec 2010	10.9	28.6	90.5	42.5	0.8	3.4	25
01 Jan 2011	11.9	28.5	90.5	42.5	0.7	3.2	21
15 Jan 2011	9.1	28.2	90	36.5	0.6	3	21
01 Feb 2011	14.2	30.6	90	42.5	0.5	2.9	20
15 Feb 2011	13.8	33.3	88	32.5	0.4	2.5	20
Grand mean					19.3	12.86	69.87
S.E.±					0.85	0.45	1.84
C.D. at 5%					2.60	1.39	5.67

with increasing storage period. The biomass ranged from 6.1 to 2.5 gm 40 ml^{-1} medium during 18 months of storage period.The biomass ranged from 6.1 to 5.3 gm 40 ml^{-1} medium during 1 to 8 months of storage, respectively. There after it declined gradually upto 4.5 gm 40 ml^{-1} medium at 14 months of storage. At the end of 18 months it was drastically reduced to 2.5 gm 40 ml^{-1} medium.

Viability of *M.anisopliae*

The viability decreased with increasing storage period of the *M.anisopliae* 3% AS. The storage was carried out in ambient for the period of 18 months. The viability ranged from 3.0 $\times 10^{-9}$ to 0.4 $\times 10^{-9}$ cfu gm^{-1} of medium during the 18 months of storage period. It was rangedd form 3.0 $\times 10^{-9}$ to 2.4 $\times 10^{-9}$ cfu gm^{-1} of medium during 1^{st} to 8.5^{th} months of storage, respectively. There after, it declined gradually upto 1.3$\times 10^{-9}$ cfu gm^{-1} of medium at 14.5 months of storage. At the end of 18 months it was drastically reduced to 0.4 $\times 10^{-9}$ cfu gm^{-1} of medium.

Bell and Hamalle (1974)reported that *B. bassiana*, *M. anisopliae* and *Spicaria rilevi* retained their pathogenicity to insects after 3 years also.

Muller-Kogler *et al.* (1980) reported that storage at 18^0C proved the most statisfactory for fungus imperfecti (including *M. anisopliae*, *B. bassiana* and *N. rileyi*) and some of the group were still alive after a year. Sporulation and growth of subculturing after storage were comparable to those of the original cultures.

Muller-Kogler and Zimmermann (1980) evaluate 12 isolates each of *M. anisopliae*, *B. bassiana*, *B. brongniartii* and

Hirsutella spp. which were stored at 4⁰C, all of the isolates survived for 3 years.

Alives *et al.* (1987) reported that the time for which the *M. anisopliae* could be stored increased by upto 33 per cent at room temperature and upto 52 per cent in refrigerator (at 2 – 3⁰C), depending on formulation and formulation kept in the freezer were viable for upto 660 days (70 % viability).

The findings reported by above worker are, in agreement with the present study. More or less due to variability in test conditions and formulations.

4.4 Bioefficacy of *M.anisopliae* against *S. litura*

The results of the bioefficacy study are presented in Table 2 to 5.The I and II instar of the pest feed in congregation while one week old disperses in the field. *M.anisopliae* 3% AS and 1.15% WP was tested for determining the biological activity on the one week old larvae of *S. litura*. In congregation while one week old disperses in the field. Both the formulations were effective to cause disease in *S.litura*. The infected caterpillars became sluggish and ceased to feed. The fungus multiplied rapidly inside the body of larvae and spreads throughout the insect haemoceol. The pest mortality ranged from 25.56 per cent to 75.56 per cent for *M.anisopliae* 1.15% WP formulation on 14th day; whereas, that was 31.11 to 86.67 per cent for *M.anisopliae* 3% AS. Amer *et al.* (2008) reported that mortality of *S.litura* II instar larvae started on day 2. It was 60% on day 7 in *M.anisopliae*. It confirmed that *M.anisopliae* is effective against *S.litura*

4.4.1 Effect of *M.anisopliae* on behavior of *S. litura*

When the larvae were contaminated with 0.6 per cent *M.anisopliae* 1.15% WP and 3 % AS the feeding was ceased in 48 hours and their movement became slower. On 3rd and 4th day the larvae became dull and sluggish and tend to gather at the side of polythene bags closed by folding the open end with pins. At 6th and 7th day haemocoelic fluid was oozing from burst part of posterior side of larval body, just before and after the death. The growth of green, fluffy mycelium of fungus on dead larvae was observed between 9th to 14th days of treatment. Some larvae succeeded in pupating showed mycosis on the pupal case. Early instar larvae when infected with the fungus showed profuse fungal growth than advanced instar larvae. Some bigger larvae burst and only showed internal mycosis in body fluid.

Rabie (2002) reported relationship between fungal pathogenicity and production of entomotoxin destruxin E.

The cyclodepsipeptidic mycotoxin, destruxin produced by *M.anisopliae* has larvicidal properties. Destruxin E causes serious damage to the epithelial cells of the midgut of larves (Sree and Padmaja, 2008).

In present study, the larval brust might be related to damage of not only to midgut epithelium but also to endocuticle and chitin due to enzymatic action as suspected by St. Leger *et al.* (1994). These reports are in support of the present findings.

The data on mortality of one week old larvae of *S. litura* as influenced by the treatments recorded on 7th and 14th days after treatment are presented in the Tables 6 to 9

4.4.1.1 Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S.litura* after 1 month.

4.4.1.1.1 Refrigerator condition

At 7th day

The mortality in the treatments ranged from 12.22 to 41.11 per cent for 3% AS whereas, it was 11.11 to 40.40 per cent for the 1.15% WP against zero per cent in untreated control. At 7th day oozing of haemocoelic fluid from the burst posterior part of larval cadavers was observed. Chemical insecticide Triazophos 0.5 % shown 100 per cent mortality. Only *M.anisopliae* 0.5% and 0.6% AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 35.56 and 41.11% AS and 34.44 and 40.00 % WP.

At 14th day

The mortality ranged from 31.11 to 86.67 in 3% AS and 25.56 to 75.56 in 1.15% WP. *M.anisopliae* 0.6 per cent caused the maximum (86.67 and 75.56%) and significantly more mortality of the pest than rest of the treatments of both of the formulations. The extent of kill by the pathogen was moderate since the efficacy was tested against one week old larvae. In some larvae, complete body fluid was oozed out. Some dead larvae were covered with growth of green fluffy mycelium of the fungus. Many survived larvae after 14th days entered in pupation which also showed mycosis. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 43.33, 52.22, 58.89 and 71.11 in 0.2, 0.3, 0.4 and 0.5 per cent 3% AS and 36.67, 41.11, 51.11, and 63.33 per cent in 0.2, 0.3 0.4 and 0.5 per cent 1.15% WP could be considered suitable for long term effect on the pest life cycle.

Table 6. Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 1 month **under two storage condition.**

Treatments	Mortality of *S. litura* (%)							
	Refrigerator				Ambient			
	3% AS		1.15% WP		3% AS		1.15% WP	
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
***M*.a. 0.1%**	12.22 (20.42)	31.11 (33.89)	11.11 (19.43)	25.56 (30.36)	11.11 (19.43)	26.67 (31.09)	10.00 (18.43)	24.44 (29.62)
***M*.a. 0.2%**	15.56 (23.20)	43.33 (41.17)	13.33 (21.32)	36.67 (37.27)	14.44 (22.31)	41.11 (39.87)	12.22 (20.42)	36.67 (37.27)
***M*.a. 0.3%**	18.89 (25.74)	52.22 (46.27)	16.67 (24.03)	41.11 (39.88)	16.67 (24.03)	52.22 (46.27)	15.56 (23.20)	40.00 (39.23)
***M*.a. 0.4%**	28.89 (32.48)	58.89 (50.16)	27.78 (31.80)	51.11 (45.64)	26.67 (30.97)	57.78 (49.48)	25.56 (30.36)	47.78 (43.72)
***M*.a. 0.5%**	35.56 (36.60)	71.11 (57.52)	34.44 (35.93)	63.33 (52.73)	35.56 (36.60)	65.56 (54.07)	34.45 (35.92)	58.89 (50.13)
***M*.a. 0.6%**	41.11 (39.87)	86.67 (68.59)	40.00 (39.22)	75.56 (60.38)	42.22 (40.51)	81.11 (64.31)	38.89 (38.57)	73.33 (58.91)
Triazophos 0.5%	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm	0.90	1.02	1.01	0.62	1.31	0.83	0.83	0.80
CD@5%	(2.79)	(3.15)	(3.10)	(1.93)	(4.03)	(2.56)	(2.64)	(2.46)

4.4.1.1.2 Ambient condition

At 7 th day

Highest concentration of both formulations of *M.anisopliae* 0.6 % gave highest 42.22 and 38.57 % mortality. The mortality in the treatment ranged from 11.11 to 42.22 per cent for 3% AS whereas, it was 10 to 38.57 per cent for the 1.15% WP against zero per cent in untreated control. At 7th day oozing of haemocoelic fluid from the burst posterior part of larval cadavers was also observed. Chemical insecticide Triazophos 0.5% showed 100 per cent mortality.

At 14 th day

The pest mortality was 26.67 to 81.11% in 3% AS and 24.44 to 73.33 in 1.15% WP. *M.anisopliae* 0.6 per cent caused the maximum (81.11 and 73.33 %) and significantly more mortality of the pest than rest of the treatments of both of the formulations. The mycoses observations were almost similar as those given under 4.3.2.1.1. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 41.11, 52.22, 57.78 and 65.56 in 0.2, 0.3, 0.4 and 0.5 per cent 3% AS and 36.67, 40.00, 47.78 and 58.89 per cent in 0.2, 0.3 0.4 and 0.5 per cent 1.15% WP could be considered suitable for long term effect on the pest life cycle. The observed effectiveness of *M.anisopliae* against *S. litura* in present study is supported by the reports in available literature.

4.4.1.2 Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 6 months **storage.**

4.4.1.2.1 Refrigerator condition

At 7 th day

Highest concentration of both formulations of *M.anisopliae* 0.6 % gave highest 40.00 and 38.89 % mortality. The mortality in the treatments varied from 11.11to 40.00 per cent for 3% AS. It was 10.00 to 38.89 per cent for the 1.15% WP against zero per cent in untreated control. Chemical insecticide Triazophos 0.5% showed 99 per cent mortality.

At 14 th day

Mortality rangedd from 27.78 to 76.67% in 3% AS and 24.44 to 67.78 in WP at 14th day. *M.anisopliae* 0.6 % caused the maximum (76.67 and 67.78%) and significantly more mortality of the pest than rest of the treatments of both of the formulations. The extent of kill by the pathogen was moderate since the efficacy was tested against one week old larvae. In some larvae, complete body fluid was oozed out. Some dead larvae were covered with growth of green fluffy mycelium of the fungus. Many survived larvae after 14th days entered in pupation which also showed mycosis. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 42.22, 50.00, 54.44 and 60.00 in 0.2, 0.3, 0.4 and 0.5 per cent AS and 33.33, 37.78, 44.44, and 51.11 per cent in 0.2, 0.3 0.4 and 0.5 per cent WP could be considered suitable for long term effect on the pest life cycle.

Table 7. Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 6 months **under two storage conditions.**

Treatments	Mortality of *S. litura* (%)							
	Refrigerator				Ambient			
	3% AS		1.15% WP		3% AS		1.15% WP	
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
***M*.a. 0.1%**	11.11 (19.43)	27.78 (31.80)	10.00 (18.27)	24.44 (29.62)	13.33 (21.41)	25.56 (30.36)	8.89 (17.28)	22.22 (28.11)
***M*.a. 0.2%**	14.44 (22.31)	42.22 (40.52)	13.33 (21.32)	33.33 (35.25)	15.56 (23.20)	41.11 (39.87)	11.11 (19.43)	32.22 (34.56)
***M*.a. 0.3%**	17.78 (24.92)	50.00 (45.00)	15.55 (23.13)	37.78 (37.92)	25.56 (30.15)	48.89 (44.36)	14.44 (22.31)	36.67 (37.27)
***M*.a. 0.4%**	27.78 (31.75)	54.44 (47.55)	26.67 (31.06)	44.44 (41.81)	32.22 (34.54)	50.00 (45.00)	23.33 (28.85)	43.33 (41.17)
***M*.a. 0.5%**	34.44 (35.93)	60.00 (50.78)	33.33 (35.25)	51.11 (45.64)	38.89 (38.57)	58.89 (50.14)	31.11 (33.86)	50.00 (45.00)
***M*.a. 0.6%**	40.00 (39.22)	76.67 (61.15)	38.89 (38.57)	67.78 (55.46)	42.00 (43.52)	74.44 (59.64)	35.56 (36.51)	66.67 (54.80)
Triazophos 0.5%	99.00 (89.00)	100.00 (90.00)	99.00 (89.00)	100.00 (90.00)	97.00 (87.00)	100.00 (90.00)	97.00 (87.00)	100.00 (90.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm	O.98	0.83	1.31	0.90	1.43	1.24	1.45	1.07
CD@5%	(3.02)	(2.56)	(4.04)	(2.77)	(4.42)	(3.81)	(1.46)	(3.30)

4.4.1.2.2 Ambient condition

At 7 th day

The mortality in the treatment ranged from 13.33 to 42.00 per cent for AS whereas, it was 8.89 to 35.56 per cent for the WP formulation against zero per cent in untreated control. Chemical insecticide Triazophos 0.5% showned 97 per cent mortality. Appreciable mortality of the pest was observed in *M.anisopliae* 0.6% 42.00 per cent in AS and 35.56 per cent in WP.

At 14 th day

Mortality ranged from 25.56 per cent to 74.44 per cent in AS and 22.22 per cent to 66.67 per cent in WP at 14^{th} day. *M.anisopliae* 0.6 per cent caused the maximum (74.44 and 66.67%) and significantly more mortality of the pest than rest of the treatments of both of the formulations. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 41.11, 48.89, 50.00 and 58.89 in 0.2, 0.3, 0.4 and 0.5 per cent AS and 32.22, 36.67, 43.33, and 50.00 per cent in 0.2, 0.3 0.4 and 0.5 per cent WP could be considered suitable for long term effect on the pest life cycle.

4.4.1.3 Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 12 months storage.

4.4.1.3.1 Refrigerator condition

At 7 th day

The larval mortality kill in the treatments was 8.89 to 35.56 per cent for 3% AS whereas, 1.15% WP formulation caused 6.67 to 31.11 per cent lethal effect against the zero per cent in untreated control. At 7^{th} day oozing of haemocoelic fluid from burst posterior part of larval cadavers was observed. Chemical insecticide Triazophos 0.5% showned 94 per cent mortality.

Table 8. Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 12 months **under two storage condition.**

Treatments	Mortality of *S. litura* (%)							
	Refrigerator				Ambient			
	3% AS		1.15% WP		3% AS		1.15% WP	
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
***M*.a. 0.1%**	8.89 (17.280	25.56 (30.36)	6.67 (14.97)	24.44 (29.62)	7.78 (16.12)	24.44 (29.62)	7.78 (16.12)	21.11 (27.34)
***M*.a. 0.2%**	11.11 (19.43)	35.56 (36.60)	8.89 (17.28)	33.33 (35.25)	8.89 (17.28)	34.44 (35.93)	10.00 (18.43)	31.11 (33.89)
***M*.a. 0.3%**	14.44 (22.31)	46.67 (43.09)	11.11 (19.26)	37.78 (37.92)	13.33 (21.41)	43.33 (41.15)	11.11 (19.43)	35.56 (36.60)
***M*.a. 0.4%**	23.33 (28.85)	51.11 (45.64)	22.22 (28.11)	44.44 (41.81)	21.11 (27.25)	46.66 (43.08)	20.00 (26.51)	42.22 (40.52)
***M*.a. 0.5%**	31.11 (33.87)	53.33 (46.91)	28.89 (32.48)	51.11 (45.64)	30.00 (33.19)	52.22 (46.27)	27.78 (31.80)	48.89 (44.36)
***M*.a. 0.6%**	35.56 (36.60)	61.11 (51.49)	31.11 (33.87)	60.11 (50.84)	34.44 (35.93)	55.56 (48.19)	30.00 (33.19)	57.78 (49.48)
Triazophos 0.5%	94.00 (84.00)	98.00 (88.00)	94.00 (84.00)	98.00 (88.00)	92.00 (82.00)	96.00 (86.00)	92.00 (82.00)	96.00 (86.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm	0.95	1.23	1.14	0.76	1.05	1.14	0.88	0.70
CD@5%	(2.93)	(3.79)	(3.52)	(2.33)	(3.23)	(3.52)	(2.71)	(2.17)

At 14 th day

The fungus caused moderate to heavy mortality (25.56 to 61.11 per cent) in the AS and the WP (24.44 to 60.11 per cent). *M.anisopliae* 0.6 per cent caused the maximum (74.44 and 66.67%) and significantly more mortality of the pest than rest of the treatments of both of the formulations. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 35.56, 46.67, 51.11 and 53.33 in 0.2, 0.3, 0.4 and 0.5 per cent AS and 33.33, 37.78, 44.44 and 51.11 per cent in 0.2, 0.3 0.4 and 0.5 per cent WP could be considered suiable for long term effect on the pest life cycle.

4.4.1.3.2 Ambient condition

At 7 th day

The mortality was moderate in the treatment.It ranged from 7.78 to 34.44 per cent for AS whereas, it was 7.78 to 30.00 per cent for the WP against zero per cent in untreated control. Chemical insecticide Triazophos 0.5% showned 92 per cent mortality.

At 14 th day

The mortality was 24.44 per cent to 55.56 per cent in AS and 21.11 per cent to 57.78 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum (55.56 and 53.78 %) and significantly more mortality of the pest than rest of the treatments of both of the formulations. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 34.44, 43.33, 46.66 and 52.22 in 0.2, 0.3, 0.4 and 0.5 per cent AS and 31.11, 35.56, 42.22 and 48.89 per cent in 0.2, 0.3 0.4 and 0.5 per cent WP could be considered suitable for long term effect on the pest life cycle.

4.4.1.4 Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 18 months of storage.

4.4.1.4.1 Refrigerator condition

At 7 th day

The mortality in the treatment ranged from 6.67 to 30.00 per cent for 3% AS whereas, it was 3.33 to 24.44 per cent for the 1.15% WP formulation against zero per cent in untreated control. Chemical insecticide Triazophos 0.5% showned 92 per cent mortality on 7th day of treatment.

Table 9. Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 18 month **under two storage conditions.**

Treatments	Mortality of *S. litura* (%)							
	Refrigerator				Ambient			
	3% AS		1.15% WP		3% AS		1.15% WP	
	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
***M*.a. 0.1%**	6.67 (14.97)	21.11 (27.34)	3.33 (10.51)	18.89 (25.740	2.59 (9.22)	17.78 (24.92)	2.22 (8.57)	16.67 (24.10)
***M*.a. 0.2%**	8.89 (17.28)	31.11 (33.89)	4.44 (12.00)	25.56 (30.36)	3.33 (10.51)	22.22 (28.11)	2.59 (9.22)	22.22 (28.07)
***M*.a. 0.3%**	11.11 (19.43)	35.56 (36.60)	7.78 (15.79)	31.11 (33.86)	7.78 (16.12)	27.78 (31.800	6.67 (14.97)	28.89 (32.48)
***M*.a. 0.4%**	18.89 (25.74)	37.89 (38.58)	15.56 (23.20)	32.22 (34.58)	14.45 (22.21)	31.11 (33.89)	12.22 (20.42)	31.11 (33.89)
***M*.a. 0.5%**	23.33 (28.88)	38.89 (38.57)	21.11 (27.34)	36.67 (37.27)	18.89 (25.74)	34.45 (35.92)	17.78 (24.92)	34.44 (35.93)
***M*.a. 0.6%**	30.00 (33.21)	44.44 (41.81)	24.44 (29.62)	38.89 (38.58)	23.33 (28.88)	36.67 (37.27)	22.22 (28.11)	35.56 (36.60)
Triazophos 0.5%	92.00 (82.00)	96.00 (84.00)	92.00 (82.00)	96.00 (84.00)	88.00 (78.00)	92.00 (82.00)	88.00 (78.00)	92.00 (82.00)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm	0.61	0.71	1.18	0.82	0.87	0.71	0.58	0.83
CD@5%	(1.89)	(2.19)	(3.63)	(2.53)	(2.67)	(2.20)	(1.78)	(2.57)

At 14 th day

Mortality rangedd from 21.11 per cent to 44.44 per cent in AS and 18.89 per cent to 38.89 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum (55.56 and 53.78 %) and significantly more mortality of the pest than rest of the treatments of both of the formulations. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 35.56, 38.89 and 38.89 in 0.3, 0.4 and 0.5 per cent AS and 31.11, 32.32, and 36.67 per cent in 0.3 0.4 and 0.5 per cent WP could be considered suitable for long term effect on the pest life cycle.

4.4.1.4.2 Ambient condition

At 7 th day

Highest concentration of both formulation of *M.anisopliae* 0.6 % gave highest 23.33 and 22.22 % mortality. The mortality in the treatment varied from 2.59 to 23.33 per cent for AS whereas, it was 2.22 to 22.22 per cent for the WP against zero per cent in untreated control. Chemical insecticide Triazophos 0.5% showned 88 per cent mortality.

At 14 th day

The pest mortality 17.78 to 36.67 per cent in AS and 16.67 to 35.56 per cent in WP. *M.anisopliae* 0.6 per cent caused the maximum (36.67 and 35.56 %) and significantly more mortality of the pest than rest of the treatments of both of the formulations.Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for the lethal effect. However, the treatments showing mortality of 27.78, 31.11 and 34.45 in 0.3, 0.4 and 0.5 per cent AS and 28.89, 31.11, and 34.44 per cent in 0.3 0.4 and 0.5 per cent WP could be considered suitable for long term effect on the pest life.

5. SUMMARY AND CONCLUSIONS

The use of chemical insecticide is a crucial aspect for pest management due to fear of residual hazards, destruction of natural enemies and damage to ecosystem. Thus, developing economically viable, ecofriendly and sustainable pest management strategies are getting impetus in recent years. In this direction biological control, especially microbial control is desirable as compared to insect natural enemies. It has the advantage of higher host specificity, virulence, shelf life, ease in production, farmers and producers friendly, safety to natural enemies and compatibility with other pest management methods.

Among the microbial agents, entomopathogenic fungi are more significant. Because they have unique characteristic of killing pest by disease and toxic action, epizootic in nature, ease of production and contact action which allow direct penetration of host cuticle. Among the entomopathogenic fungi, *M. anisopliae* is characterized as green muscardine fungus due to green colour of the sporulating colonies. Considering importance of *S.litura* and uniqueness of entomopathogenous fungi, the present investigation was undertaken on storability and bioefficacy of *M.anisopliae*.

5.1 Summary

5.1.1 Morphology of *M.anisopliae*

M.anisopliae is a green muscardine fungus due to green colour of the sporulating colonies. It form mycelial mat over cuticle of insects. Its conidia are 3.5-9 µm long, celled, long-ovoid to cylindrical forming long basipetal chain/columns. Conidiophores short, erect, septate, branched single or cluster of phialides, which abruptly taper towards apex. It Infects insects through the mouthparts (food) or the hosts exterior.

5.1.2 Determination of cfu count of test formulations

The serial dilutions of *M.anisopliae* 3% AS and 1.15% WP were prepared. One ml of aqua suspension from each of these dilutions was poured on PDA in petridishes. It was found that the AS contained 3.9×10^{9} cfu ml^{-1} as against 3.0×10^{9} cfu ml^{-1} in WP.

5.1.3 Storability of *M.anisopliae*

5.1.3.1 Refrigerator condition.

The *M.anisopliae* two formulatons are stored under refrigerator condition.

5.1.3.1.1 Storability of *M.anisopliae* 3 % AS.

The viability decreasedd gradually from 4.4×10^{-9} at beginning of storage to 4.0×10^{-9} cfu after 6 months. Similliarly, it declined at 12 month of storage while it becomes (2.9×10^{-9} cfu/ gm^{-1}). At 18 month of storage, it becomes rapidly declined at 0.9×10^{-9} cfu/ gm^{-1}. The Cfu count at the end of 18 months of storage was more than that specified for the product. The biomass weight decreased gradually from 6.8 to 1.5 gm/40ml medium. It was 6.8 gm/40ml medium at 1 month storage and 1.5gm/40ml medium after 18 months storage period. The surface coverage ranged from 25 to100 % during the 18 months of storage period. It was cent percent up to 9 months storage. There after, it declined gradually at 9 to 15 months from 98 to 70 %. After 16 months, decrease in the surface coverage was some what rapid from 60 to 20 %.

5.1.3.1.2 Storability of *M.anisopliae* 1.15 % WP.

The viability decreased gradually from 3.6×10^{-9} at the beginning of storage to 3.3×10^{-9} cfu after 6 months. Similliarly, it declined at 12 month of storage and it becomes 2.6

$\times 10^{-9}$ Cfu/ gm^{-1}. At 18 month of storage it becomes rapidly declined (0.5 $\times 10^{-9}$ Cfu/ gm^{-1}). The Cfu count at the end of 18 months of storage was more than that specified for the product. The biomass weight decreased gradually from 6.5to 2.7 gm/40ml medium. It was 6.8 gm/40ml medium at 1 month storage and 2.7 gm/40ml medium after 18 months storage period. The surface coverage ranged from 18 to100 % during the 18 months of storage period. It was cent percent up to 8.5 months storage. There after it declined gradually at 9 to 13 months from 99 to 86 %. After 14 months, decrease in the surface coverage was some what rapid from 60 to 18 %.

5.1.3.2 Ambient condition.

The *M.anisopliae* two formulatons are stored under ambient condition.

5.1.3.2.1 Storability of *M.anisopliae* 3 % AS.

The viability decreased gradually from 3.9 $\times 10^{-9}$ to 3.9 $\times 10^{-9}$ at beginning of storage to 3.5 $\times 10^{-9}$ cfu after 6 months. Similliarly, it declined at 12 month of storage it becomes 2.9 $\times 10^{-9}$ Cfu/ gm^{-1}. At 18 month of storage, it rapidly declined (0.8 $\times 10^{-9}$ Cfu/ gm^{-1}). The Cfu count at the end of 18 months of storage was more than that specified for the product. The biomass weight decreased gradually from 6.6 to 3 gm/40ml medium. It was 6.6 gm/40ml medium at1 month storage and 3 gm/40ml medium after 18 months storage period. The surface coverage ranged from 21 to100 % during the 18 months of storage period. It was cent percent up to 8 months storage. There after, it declined gradually at 9 to 13.5 months from 99 to 88 %. After 14 months, decrease in the surface coverage was some what rapid from 63 to 21 %.

5.1.3.2.2 Storability of *M.anisopliae* 1.15 % WP.

The viability decreased gradually from 3.0 $\times 10^{-9}$ at beginning of storage to 2.6 $\times 10^{-9}$ Cfu after 6 months. Similliarly, it declined at 12 months of storage while it becomes 1.4 $\times 10^{-9}$

Cfu/ gm^{-1}. At 18 month of storage it becomes rapidly declined at 0.4 $\times 10^{-9}$ Cfu/ gm^{-1}. The Cfu count at the end of 18 months of storage is more than that specified for the product. The biomass weight decreased gradually from 6.1 to 2.5 gm/40ml medium.It was 6.1 gm/40ml medium at1 month storage and 2.5 gm/40ml medium after 18 months storage period. The surface coverage ranged from 20 to100 % during the 18 months of storage period. It was cent percent up to 7.5 months storage. There after, it declined gradually 8 to 12 months from 98 to 88 %. After 14 months, decrease in the surface coverage was some what rapid from 60 to 20 %.

5.1.4 Bioefficacy of *M.anisopliae* against one week old larvae of *S. litura.*

The data on mortality of one week old larvae of *S. litura* as influnced by various treatments of both formulations of *M.anisopliae* and observation recorded on 7th and 14th days after treatment are summary followes.

5.1.4.1 Bioefficacy of *M.anisopliae* 3% AS and 1.15% WP against *S. litura* after 1 month storage.

When the larvae were treated with 0.6 per cent *M.anisopliae* 1.15% WP and 3 % AS, the larvae ceased feeding in 48 hours and their movement became slower. On 3rd and 4th day, the larvae became dull and sluggish and tend to gather at the side of polythene bags. At 6th and 7th day haemocoelic fluid was oozing from burst part of posterior side of larval body, just before and after the death. The growth of green, fluffy mycelium of fungus on dead larvae was observed between 9th to 14th days of treatment. Some larvae succeeded in pupating showed mycosis on the pupal case.

The 6 treatments at 0.1% to 0.6% each of *M.anisopliae* 3% AS and 1.15% WP were tested seperately for determining bioefficacy against one week old larvae of *S. litura.*

Mortality of larvae was recorded on 7th and 14th day of treatment.

5.1.4.1.1 For sample stored under refrigerator condition.

The mortality rangedd from 31.11 per cent to 86.67 per cent in AS and 25.56 per cent to 75.56 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality of 86.67% for 3% AS and 75.56 % for 1.15% WP of the pest than rest of the treatments of both of the formulations. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect. However, the treatments showing mortality of 43.33, 52.22, 58.89 in 0.2, 0.3 and 0.4 per cent AS and 41.11, 51.11 and 63.33 per cent in 0.4 and 0.5 per cent WP could be considered sutable for long term effect on the pest life cycle. The extent of kill by the pathogen was moderate since the efficacy was tested against one week old. In some larvae, complete body fluid was oozed out. Some dead larvae were covered with growth of green fluffy mycelium of fungus. Many survived larvae after 14th days entered in pupation which also showed mycosis.

5.1.4.1.2 For sample stored under ambient condition.

The mortality in the treatment rangedd from 26.67 per cent to 81.11 per cent in AS and 24.44 per cent to 73.77 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality of 81.11 for 3% AS and 73.77 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect. However, the treatments showing mortality of 41.11, 52.22 , 57.78 and 65.56 in 0.2, 0.3,0.4 and 0.5 per cent AS and 36.67,40.00,47.78 and 58.89 per cent in 0.2, 0.3,0.4 and 0.5 per cent WP could be considered sutable for long term effect on the pest life cycle.

5.1.4.2 Bioefficacy of *M.anisopliae* 3% AS **and** 1.15% **WP against *S. litura* after 6 month storage.**

5.1.4.2.1 For sample stored under refrigerated condition.

The pest mortality was 27.78 per cent to 76.67 per cent in AS and 24.44 per cent to 67.78 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality of 86.67% for 3% AS and 75.56 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.1.4.2.2 For sample stored under ambient condition.

Mortality ranged from 25.56 per cent to 74.44 per cent in AS and 22.22 per cent to 66.67 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality of 81.11 for 3% AS and 73.77 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.1.4.3 Bioefficacy of *M.anisopliae* 3% AS **and** 1.15% **WP against *S. litura* after 12 month storage.**

5.1.4.3.1 For sample stored under refrigerator condition.

The fungus caused moderate to heavy mortality from 25.56 to 61.11 per cent in AS and 24.44 to 60.11 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality of 86.67% for 3% AS and 75.56 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.1.4.3.2 For sample stored under ambient condition.

The mortality in the treatment rangedd from 24.44 to 55.56 per cent in AS and 21.11 to 53.78 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality 81.11 for 3% AS and 73.77 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.1.4.4 Bioefficacy of *M.anisopliae* 3% AS **and** 1.15% **WP against *S. litura* after 18 month storage.**

5.1.4.4.1 For sample stored under refrigerator condition.

The mortality rangedd from 21.11 to 44.44 per cent in AS and 18.88 to 38.89 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality 86.67% for 3% AS and 75.56 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.1.4.3.2 For sample stored under ambient condition.

The mortality was moderate in the treatment and ranged from 17.78 per cent to 36.67 per cent in AS and 16.67 per cent to 35.56 per cent in WP at 14th day. *M.anisopliae* 0.6 per cent caused the maximum and significantly more mortality 81.11 for 3% AS and 73.77 % for 1.15% WP of the pest than rest of the treatments of both of the formulations in separate experiments. Only *M.anisopliae* 0.5% and 0.4 % AS were the next promising treatments for lethal effect.

5.2 CONCLUSIONS

1. *M.anisopliae* **3% AS** could be stored safely for 17 months and **1.15% WP** for 16 months under refrigerator condition at 6^0C for the fungal growth and viability and bioefficacy against *S. litura.*
2. *M.anisopliae* 3% AS could be stored safely for 15 month and **1.15% WP** for14 months under ambient conditions for the fungal growth and viability and bioefficacy against *S. litura.*
3. *M.anisopliae* **3% AS** and **1.15% WP** either stored under refrigerator and at ambient conditions 1 to 18 months and applied at 0.6,0.5 and 0.4% were more promising against one week old larvae of *S. litura.*
4. The bioefficacy decreased from 75.56 to 38.89% in *M.anisopliae* **1.15% WP** and 86.67 to 44.44% in *M.anisopliae* **3% AS** when stored under refrigerator conditions. The corresponding figures for the fungus stored at ambient conditions were 73.77 to 35.56% and 81.11 to 36.67%, respectively.

6. LITERATURE CITED

Adamek, L. 1949. *Metarhizium* spore surface antigens are correlated with pathogenicity.Folia microbial (Prague),10: 255 – 257.

Aguda, R. M., Litsinger, J. A. and Roberts, D. W. 1984. Pathogenicity of *Beauveria bassiana* on Brown Plant Hopper (BPH), white Backed Plant Hopper (WPH) and Green Leaf Hopper (GLH). International Rice Research Newsletter, 9 (6) : 20.

Alves, R. T., Prior, C. and S. R. Leather. 1987. Evasion of host defense by in vivo-produced protoplast like cells of the insect mycopathogen *Beauveria bassiana*. J. Biocontrol. Sep, 175 (18) : 5962 – 5969.

Alves, R. T., Bateman, R. P., Prior, C. and S. R. Leather. 1998. Effects of stimulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. Crop Protection. 17 (8) : 675 – 679.

Arthurs, S. and Thomas, M.B. 2001. Effect of dose, pre-mortem host incubation temperature and thermal behavior on host mortality, mycosis and sporulation of *Metarhizium anisopliae* var. acridium in *schistocera gregaria*. Biocontrol Science and Technology. 11:3, 411 – 420.

Bell, J. V. 1975. Viability of entomopathogenic fungi stored outside. Journal of the Georgia Entomological Society. 4, 357 – 358.

Bell, J.V. and Hamalle, R. J. 1974. Viability and pathogenicity of entomogenus fungi after prolonged storage on silica gel at -20 ^{0}C and J. Microbiol. 20 (5) : 639 – 642.

Braga, G. U. l., Flint, S. D., Messias, C. L., Anderson, A. J. and Roberts, D. W. 2001. Effects of UV-B irradiance on conidia and germinants of the entomopathogenic hypomycete *Metarhizium anisopliae*: a study of reciprocity and recovery. Photochemistry and photobiology, 73: 2, 140 – 146.

Clerk, G. C. and Madelin, M. F. 1965. The longevity of conidia of three insect parasitizing hypomecetes. Trans. Briti, Mycol. Soc., 48 (2) : 193 – 209.

Couch, T. L. and Ignoffo, C. M. 1981. Formulation of insect pathogens, In "Microbial control of pests and plant disease 1970 – 1980" (H.D. Burgel, ed.) pp. 621 – 634. Academic press, New York.

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

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

Daoust, R. A. and Roberts, D. W. 1983a. Studies on prolonged storage of *Metarhizium anisopliae* conida effects of temperature and relative humidity on conidial viability and virulence against mosquitoes. J. Inverte. Pathol. 41: 143- 150.

Daoust, R. A. and Roberts, D. W. 1983b. Studies on prolonged storage of *Metarhizium anisopliae* conidia: effect of growth substrate on conidial survival and virulence against mosquitoes. J. Invert. Path., 41: 161 – 169.

Dimbi, S., Maniania, N. K., LUX, S. A. and Mueke, J. M. 2004. Effect of constant temperatures on germination, radial growth and virulences of *Metarhizium anisopliae* to three species of African tephritid fruit flies. Biocontrol 49: 1, 83 – 94.

Fargues, J., Goettel, M. S., Smits, N., Ouedraogo, A., vidal, C., Lacy, L. A., Lomer, C. J. and M. Rougier. 1996. Variability in susceptibility to stimulated sunlight of conidia among isolates of entomopathogenic hyphomycetes. Mycopathologia. 165: 171 – 181.

Faria M. R-de; Martins, I., Mello, R, Tigano, M. S. and de-Faria-MR. 1999. Entomopathogenic fungal (hypomycetel) collection: assessment of conidial viability. *Pesquisaagropecuaria brasileira*. 34: 8, 1497 – 1503.

Feron, P. 1981. Pest control by the *Beauveria* and *Metarhizium* in microbial control of pests and plant diseases, 1970 - 1980. H (H.D. Burges) Academic Press, Ney York, 465 – 482.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge Univ. Press, London.

Garcia, A. S., Messias, C. L., Souza, H. M. L., de, and Piedrabuena, A. E. 1981. Pathoginicide *Metarhizium anisopliae var. anisopliae* a *ceratitis capitata* (widemann) (Diptera: Tephritidae). Rev. Bras. Entomol. 28 : 421 – 424.

Guerrero, C. J., Carrillo, L. R. and Aguilera, P. A. 1999. Morphological characteristic and conidia germination of entomopathogenic gunfus *Metarhizium anisopliae var. anisopliae* isolated of *scarabaeidae* and *curculionidae* larvae, Agro. Sur. 1999, 27: 2, 23 – 34.

Guillon, M. 1997. Production of biochemical insecticides. Scale up and quality assurance. In Microbial insecticides, Novelty or Necessity. Symposium proceeding No.68 (ed. H. F. Evans) British Crop Protection Council, Farnhan: 151 – 162.

Gupta, R. B. L., Shashi, S., Yadava, C. P. S. and Sharma, S. 2002. Mass mycelia production of an entomopathogen *Metarhizium anisopliae*. Indian phytopathology, 55: 4, 503 – 504.

Gupta, R. B. L., Shashi-Sharma, Yadava, C.P.S. and Sharma, S. 2000. Determination of shelf life and virulence of talk formulations of *Metarhizium anisopliae* against *Holotrichia consanguinea*. Indian Journal of Agricultural Science, 70: 6, 422 – 429.

Hedgecock, S., Moore, D., Higgins, P. M. and Prior, C. 1995. Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. Biocontrol Science and Technology, 5: 371 – 377.

Hong, T. D., Jenkin, N. E. and Ellis, R. H. 2000. The effect of duration of development and drying regime on the longevity of conidia of *Metarhizium flavoviride*. Mycological Res. 104 (6) : 662 – 665.

Hong, T. D., Ellis, R. H. and Moore, 1997. Development of a model to predict the effect of temperature and moisture on fungal spore longevity. Ann. Botany, 79: 121 – 128.

Horaczek, A. and Virernstein, H. 2004. Comparison of three commonly used drying technologies with respect to activity and longevity of arial conidia of *Beauveria* *bassiana* and *Metarhizium anisopliae*. Biol. Control., 31: 65 – 71.

Ihra, T. D., Jenkin, N. E. and Ellis, R. H. 2008. The effect of duration of development and drying regime on the longevity of conidia of *Metarihizium flavoviride*. Mycological Res. 104 (6) : 662 – 665.

Jackson, M. S., Cliquet, S. and Bothast, R. J. 1995. Effect of the relative humidity of drying air on survival of liquid culture produce blastospores of *Paecilomyces fumosoroseus* conditions. Proc. Abstract of the 28th Annual meeting of the Society of Invertebrate Pathology, Cornell University, Ithaca, pp. 30.

Kamat, V.K. and Raw, M.R. 1975. Mass multiplication and formulation of entomopathogenic fungi and their efficacy against white grub. J. Mycol. Pl. Path., 29 (4\3) : 299 – 305.

Kawakami, K and Mikuni, T. 1965. Effect of relative humidity and temperature on the viability of conidia of some muscardine. Acta Sericol., 56: 42 – 46 (In Japanese).

Keller, P.N. 1992. Selection of a highly virulent fungal isolates *metarhizium anisopliae* MaTVB 101 for control fo taro beetle, *papuana uninodis* (Coleoptera: scarabaeidas). Biocontrol Science and Technolgy, 8: 187-195.

Kerwin, W.M. 1992. Concurrence of losing and chromosome and the ability to reduce destruxins in a mutant of *metarhizium anisopliae*. Microbiology Letters, 226 : 2, 373 – 378, 24 ref.

Kleepsir, M.D., and Zimaram T.I. 1994. Response of Chilean *Metarhizium anisopliae* (Metschnikoff) Sorobin isolate to the addition of a solar sunscreen. Agriculture technia. 63: 4, 339 – 346.

Kulat, S. S., Zade, M. N., Deshkar, L. N. and Varhode, S. S. 2002. Influence different culture medium on growth and sporulations of *Metarhizium anisopliae* (Metschnikoff) Sorobin. Journal of Biological Control, 16 : 2, 177 – 179.

Li, D. P. and. Holdom, D. G. 1995. Effects of nutrient on colony formation, growth and sporulation of *Metarhizium anisopliae*. Journal of Invertebrate Pathology, 65: 253 – 260.

Lord, C. J. 2005. Low humidity, moderate temperature and descant dust favour efficacy of *Beauveria bassiana* for their lesser grain borer, *Rhinopertha dominica*. Biological control, 34 (1) : 155-159.

Luz, S., Maniania, N. K. and Ampong, Nyarko, K. 2008. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjosteddi.* Biocontrol Science and Technology, 9: 177 – 185.

Marques, G. E. 1990. The production of destruxin by the entomogenous fungi *Metarhizium anisopliae var. major*. Journal of Invertebrate Pathology, 47: 120 – 122.

MeCoy, C.W. 1988. The entomopathogenic fungal *metarhizium anisopliae* and its potential as a biological agent. Pest Sci., 37 : 375 – 379.

MeCoy, C. W., Samson, R. A. Boucias, D. G. 1998. Entomogenous fungi, In Ignoffo, C. M., Mandava, N. B. (Eds), Handbook of Natural Chemical insecticides: Microbial Insecticides, Vol. V, Part A, CRC Press, Inc, Boca Raton, FI pp. 151 – 236.

Metschnikoff, E. 1879. Disease of larva of grain weevil insects harmfull to agriculture (seriel); issue III, the grain weevil, published by Commission attached to the Odessa Zemstvo Office for the investigation of the problem of insects harmful to agriculture, Odessa, 32 pp.

Milner, R. J., Samson, P. R. and Bullard, G. K. 2002. FI-1045: A profile of commercially useful isolate of *Metarhizium anisopliae var. anisopliae*. Biocontrol Science and Technology, 12: 43 – 58.

Milner, R. J. and Prior, C. 1994. Susceptability of the Australia Plague locust, *Chorloicetes terminifera* and the wingless grasshopper, *Phaulacridium vittalum* to the fungi *Metarhizium spp*. Biological control, 4: 132 – 137.

Milner, R. J., Samson, P. and Morten, R. 2003. Persistence of conidia of *Metarhizium anisopliae* in sugarcane fields effects of isolates and formulation on persistence over 3.5 year, Biocontrol Science and Technology, 13: 5, 507 – 516: 18.

Ming-guage Feng, L. R., Johnso, J. B. and Kish, L. P. 1990. The virulence of *Verticillium lecanii* and an aphid derived isolate of *Beauveria bassiana* for six species of cereal infesting aphid. J. Environ. Entomol. 19 (3) : 815 820.

Moore, D., Bridge, P. D., Higgins, P. M., Bateman, R. P., and C. Prior. 1993. Ultra-violet radiation damage to

Metarhizium flavoviride conidia and the protection given by vegetable and mineral oils and chemical sunscreens. Ann. Appl. Biol. 122: 605 – 616.

Moore, D., Langewald, J., and F. Obogon. 1997. Effects of rehydration on the conidial viability of *Metarhizium flavoviride* mycochemical insecticide formulations. Biocontrol Science Techno. 7: 87 – 94.

Muller-Kogler and Zimmermann, G. 1980. Zur aufbewahrung Entomologier pilkulturen. Entomophaga. 25, 301 – 311.45-47.

Muller-Kogler, E., Zimmermann, G. and Mortin, P. A.. 1980. On the storage of cultures of entomopathogenic fungi. Entomophaga, 25: 3, 301 – 311, 2 ref.

Narishmana, K.V. 1970. Attachment of *Metarhizium anisopliae* to southern green stink bug *Nezara viridula* cuticle and fungistatic effect of cuticular lipids and aldehydes. J. Invert. Pathol. 69: 31 – 39.

Pandey,A.K.,Kanaujia,K.R.2008. Effect of different grains as solid substrates on sporulation ,viability and pathogenicity of *Metarhizium anisopliae* (Metschnikoff) Sorokin. Journal of Biological Control., 22(2):369-374.

Patel, K. C., Yadav, D. N., Dube, H. C. Patel, R. J. 1990. Laboratory and mass production studies with *Metarhizium anisopliae*. Annals of Biology. Ludhiana, 6: 2, 135 – 140.

Prior, C. and Arura, M. 1985. The infectivity of *Metarhinium anisopliae* to two insect pests of coconuts. Journal of Invertebrates Pathology, 45: 187 – 194.

Purwar,J.P.,Sachan,G.C.2005.Biotoxicity of *B.bassiana* and *Metarhizium anisopliae* against *S.litura* and *Spilarctia obliqua.* Annals of Plant Protection Sciences., 13(2): 360-364.

Payne, K.T. 1988.Production and properties of *Beauveria bassiana* conidia cultivated in submerged culture. Can Jicrobiol. 33 : 12 – 20.

Quesada, W., 1995. New technologies for termite control. IPM Practitioner, 17, 1 – 9.

Quesada , W., Yadav, G. P. and Shrma, S. 2008. Effect of relative humidity of growth of eight isolates of entomofungal pathogens in vitro. Indian phytopathology. 54: 2, 256 – 257, 9 ref.

Rath, M.B. 1992. BIO1020 granular *metarhizium* a new product of biocontrol of soil pests. Brighton Crop Protection Conference, Pest and Disease, 363 – 368; 7 ref.

Rabie,M.M.2002.Relationship between pathogenicity of the entomopathogenic fungus *M.anisopliae* to *S.litura* and fungal production of destruxin E. Egyption. Journal of Bological pest control.,12(2):115-117.

Rachappa, V., Lingappa, S. and Patil, R. K. 2009. Growth characteristics and bio-efficacy of different isolates of *Metarhizium anisopliae* against certain key insect pests, J. Biological control, 23 (3) : 271 – 276, 2009.

Rajesh, A . D. 2009. Studies on certain entomopathogenes of *Heliothis armigera* and *Spondoptera litura* and their interaction with some botanicals. Ph.D. Thesis. Tamil Nadu Agriculture University, Coimbatore, 187 p.

Richard, A. D. and Donald, W. R. 1983. Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of temperature and relative humidity on conidial viability and virulence against mosquitoes. J. Invertebrate Patho. 41 : 143 – 150.

Roberts, D. W. 1970. *Coelomomyces, Entomophora, Beauveria and Metarhizium* as parasites of mosquitoes. Misc. Publication. Entomal. Soc. Amer., 7 (1) : 140 – 155.

Roberts, D. W. and Campbel, A. S. 1977. Stability of entomopathogenic fungi. Miscellaneous publication of the entomological society of America, 10 : 1-8.

Samson, R. A., Evans, H. C. and Latge, J. P. 1988. Atlas entomopathogenic fungi, springer-veriag, The Netherland, 187.

Scoco,C.M., Hosttter, D. L. Sikorowski, P. P., Sutter, G. and Brooks, W. M. 2007. Inactivation of representative species of entomopathogenic viruses, fungi, bacteria and protozoan by an ultraviolet light source. Environmental Entomology, 6: 3, 411 – 415.

Singha, D., Singha, B. and Dutta, B. K. 2006. Virulence of isolates of *Metarhizium anisopliae* (Mitschnikof) Sorokin and *Beauveria bassiana* (Ballsamo) vuillemin on tea termite *Microceroterm bessoni* snyder Barck valley of Assam. Indian Journal of Entomology, 68 (1) : 66 – 70.

Siddaramaiah.A.L., Narendrappa,T., Panchabhavi,K.S. 1986. Occurrence and severity of *M.anisopliae* on *S.litura* Fb. in groundnut field. Current Research, University of Agricultural Sciences, Bangalore.,15:5-6,48-49.

Sree,K.S. and Padmaja,V.2008. Oxidative stress induced by destruxin from *M.anisopliae* (Metch.) involves changes glutathione and ascorbate metabolism and instigate ultrastructural changes in the salivary glands of *S.litura* larvae. Toxicon., 51(7): 1140-1150.

Song, Z., Jiang, Y. C. and Raw, R. C. 2000. Sporulation of *Metarhizum anisopliane* on liquid medium containing various amino acid and vitamin. Journal of Fusian College of Forestry, 20: 3, 251 – 254.

St. Leger, R. J., Bidochka, M. J., and D. W. Roberts. 1994. Germinatin triggers of *Metarhizium anisopliae* conidia are related to host species. Microbiol. 140 : 1651 – 1660.

Stathers, T. E., Moore, D. and Prior, C. 1993. The effect of different temperature on the stability of *Metarhizium flavouride* conidia stored in vegetable and mineral oils, J. Invert. Patho. 62: 111 – 115.

Steinhaus, M.N. 1956. A model to explain differentiation of appressoria by germlings of *Metarhizium anisopliae*. J. Invert. Pathol. 57: 299 – 31.

Strasser, H., Eraschbamber, M. and Papierok, B. 2003. Effect of temperature on conidia germination and vegetation growth of *Metarhizium anicopliae*. Bulletin OILB-SROP, 26: 1, 117 – 120.

Tadele Tefera and Ken Pringle, 2003. Germination, radial growth and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates and their virulence to C*hilo partellus* (Lepidoptera: Pyralidae) at different temperature. Biocontrol Science and Technology. 13 (7): 699 – 704.

Tanada, Y. and Kaya, H. K. 1993. Insect pathology. Academic Press, San Diego, USA. 666 pp.

Tulloch, M and Trans. 1976. The studies on conidial length of *Metarhizium anisopliae*, British mycological society, 66: 407 – 411.

Wadyalkar, S. R., Wasule, D. L., Bhoyte, S. G., Tondon, R. L. (ed.), Ballal, C. R. (ed.), Jalali, S. K. (ed.) and Rabindra, R. J. 2003. Studies on mass multiplication and formulation of *Metarhizium anisopliae* (Metsch). Sorokin.

Walstad, J. D., Anderson, R. F. and Stambaugh, W. J. 1970. Effect of environmental condition of two species of muscardine fungi *Beauveria* and *Metarhizium anisopliae*. J, Invertebrate Pathology, 16 (2) ; 221 – 226.

Wandersch, W. Hartwig, J., Reinecke, P. and Stenzel, K. 1990. Production of mycelia granules of the entomopathogenic fungal *Metarhizium anisopliae* for biological control of soil pest. Vth International Colloguium on Invertebrate Pathology and Microbial Control, Adelaide, Austrialia, 2-5.

Wolf, D.M. 1981. Biological and environmental condition for the development of mycosel caused by *Beauveria and Meterhizium*. Insect Pathology, 6: 8 – 20.

7. VITA

RATHOD GOKUL PUNDLIK

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of
MASTER OF SCIENCE (AGRICULTURE)
in
AGRICULTURAL ENTOMOLOGY
2012

TITLE OF THESIS : STUDY ON STORABILITY OF *Metarhizium anisopliae* METSCHNIKOFF (SOROKIN)
MAJOR FIELD : Agricultural Entomology

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EDUCATIONAL

- Passed S.S.C. in first class with distinction (75.46%) from Highschool Marwali Tal. Naingaon, Dist. Nanded during 2003.
- Passed H.S.C. in first class (68.17%) from Post Basic Jr.College, Marwali Tal. Naigaon, Dist. Nanded during 2005.
- Received Bachelor of Science (Agri.) degree in first class (CGPA 7.78) from College of Agriculture, Latur, M.A.U.,Parbhani. during 2009.
- Selected for ICAR (16th rank) Merit scholarship for M.Sc. Agri. Entomology during 2009 and joined at M.P.K.V., Rahuri, Dist. Ahmednagar (M. S.) India.

EXTRA CURRICULAR ACTVITIES

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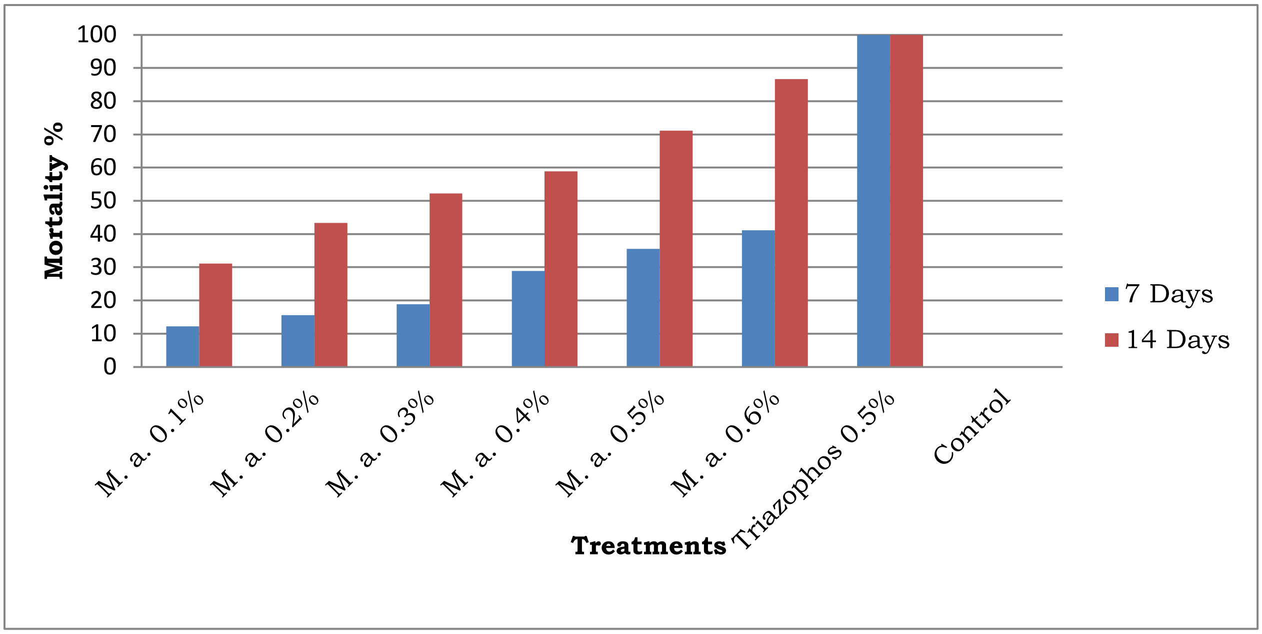


Fig. 5 Bioefficacy of *M. anisopliae* 3% AS against *S.litura* before storage 1 month under refrigerator condition.

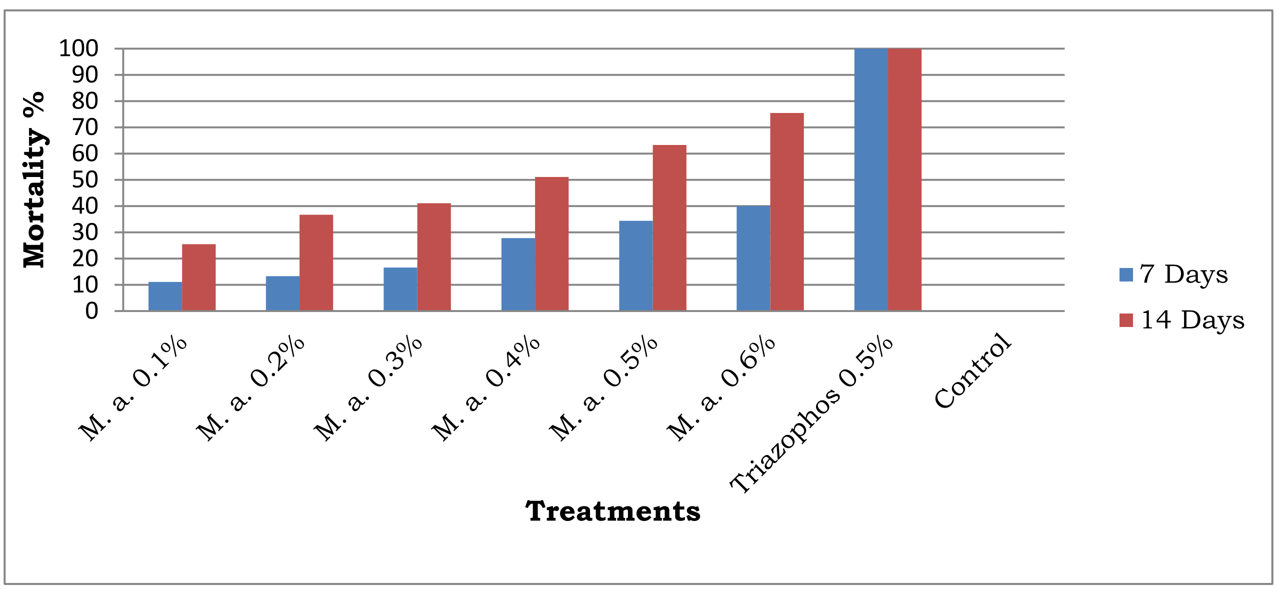


Fig. 6 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* before storage 1 month under refrigerator condition.

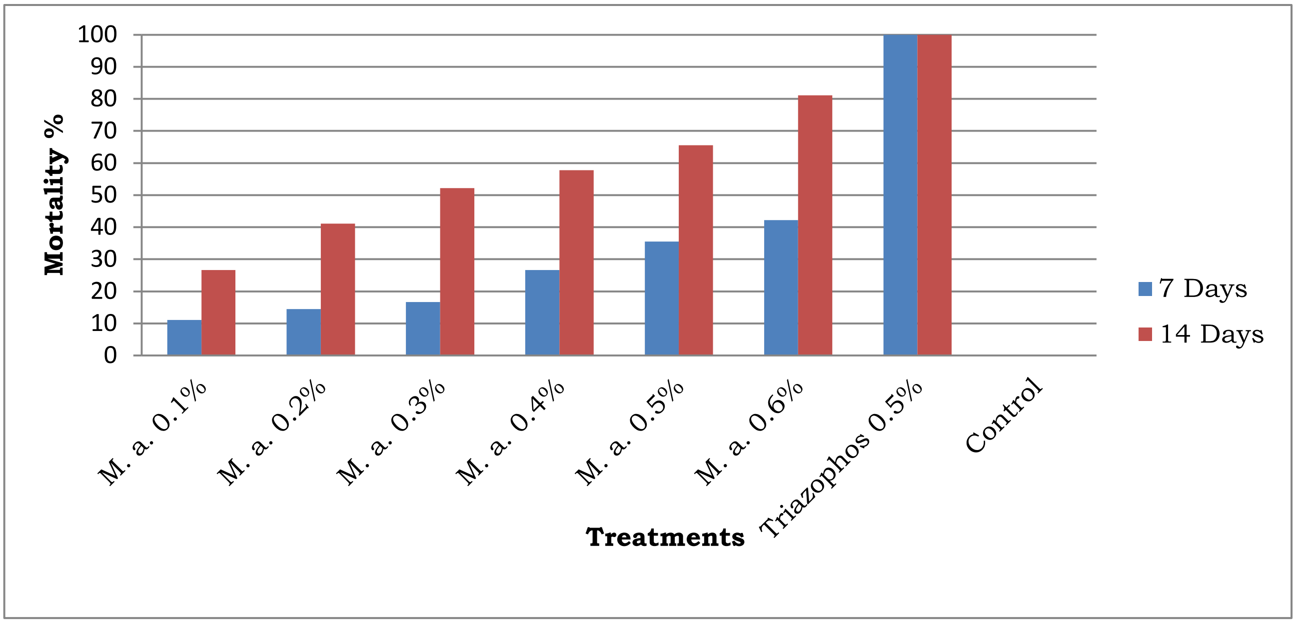


Fig. 7 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 1 month under aambient condition.

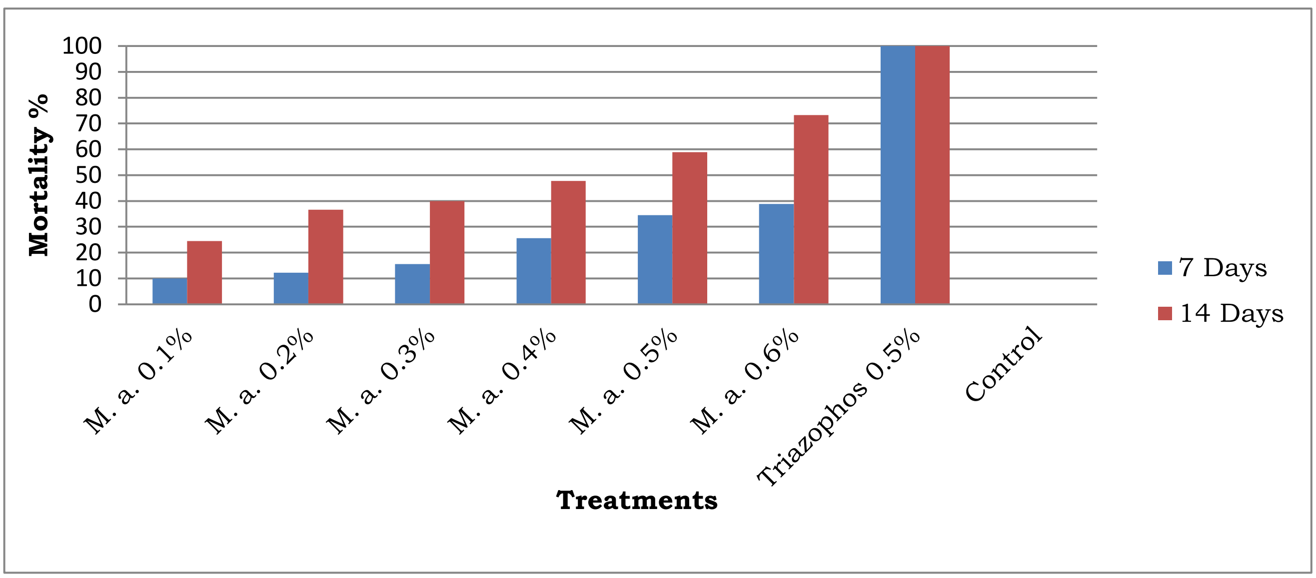


Fig. 8 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 1 month under aambient condition.

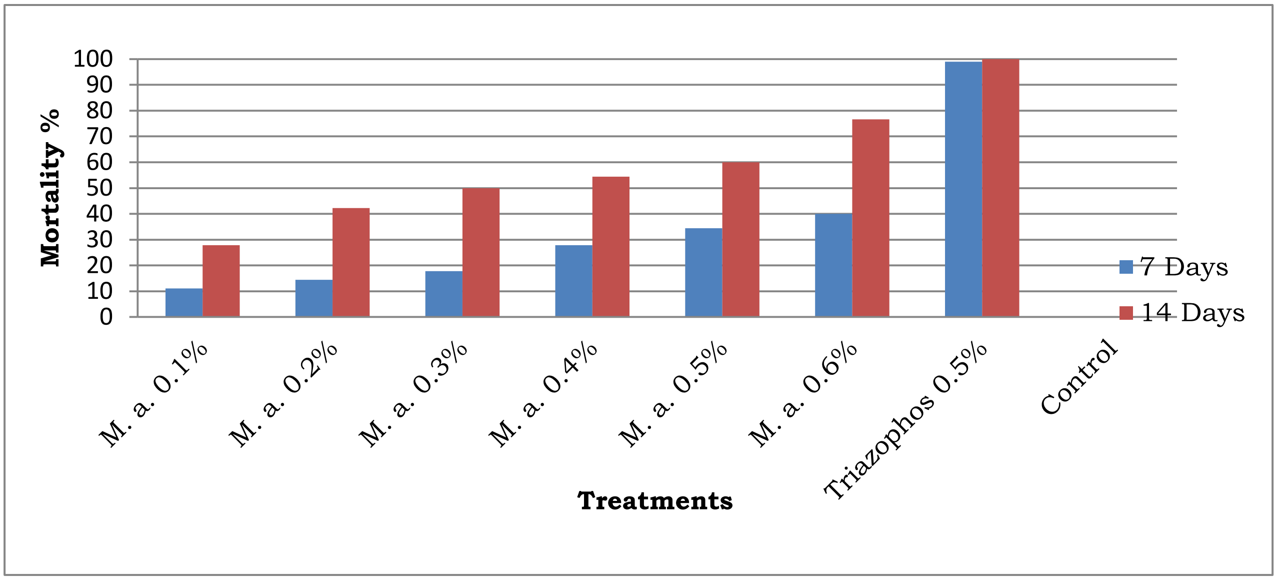


Fig. 9 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 6 month under refrigerator condition.

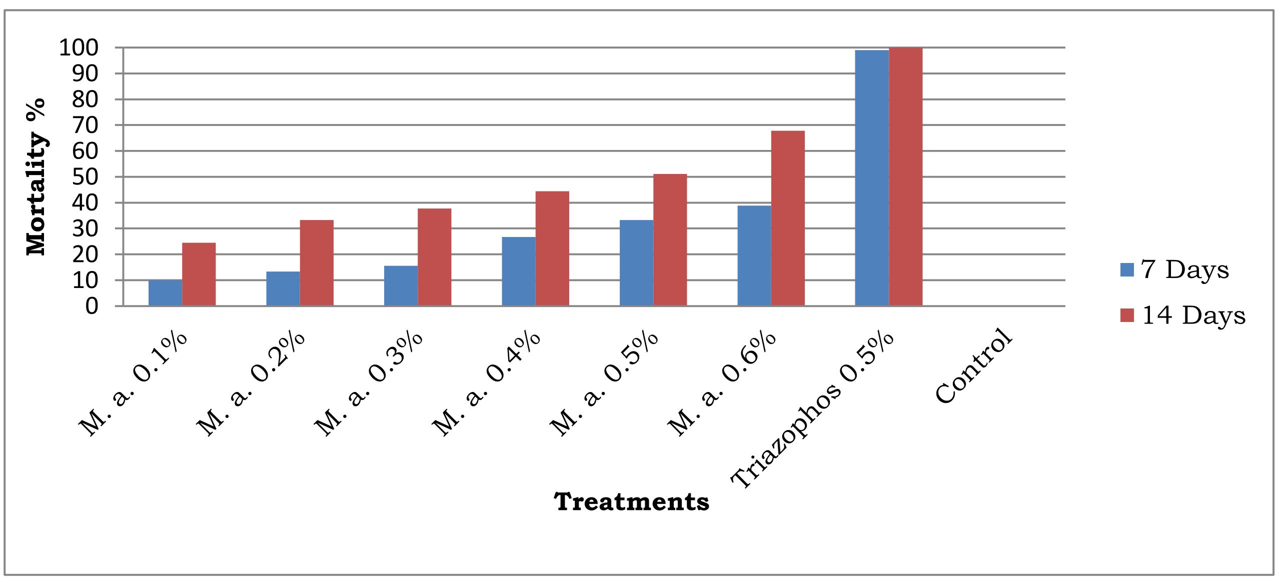


Fig.10 Bioefficacy of *M.anisopliae* 1.15% WP agains *S.litura* after 6 month under refrigerator condition.

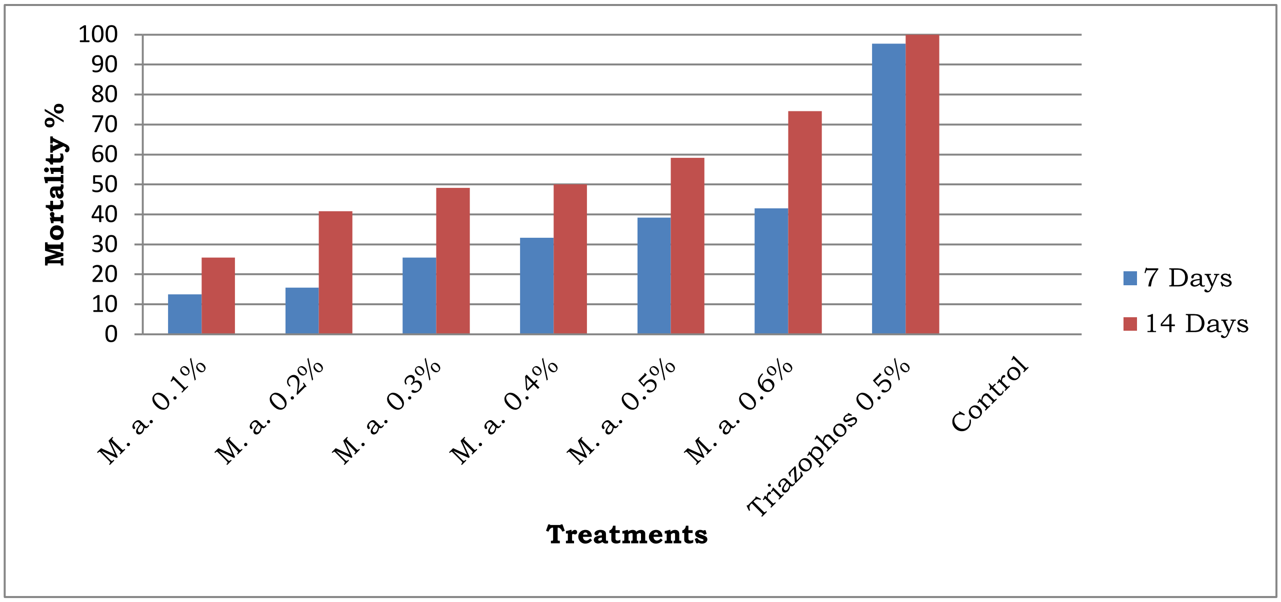


Fig.11 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 6 month under aambient condition.

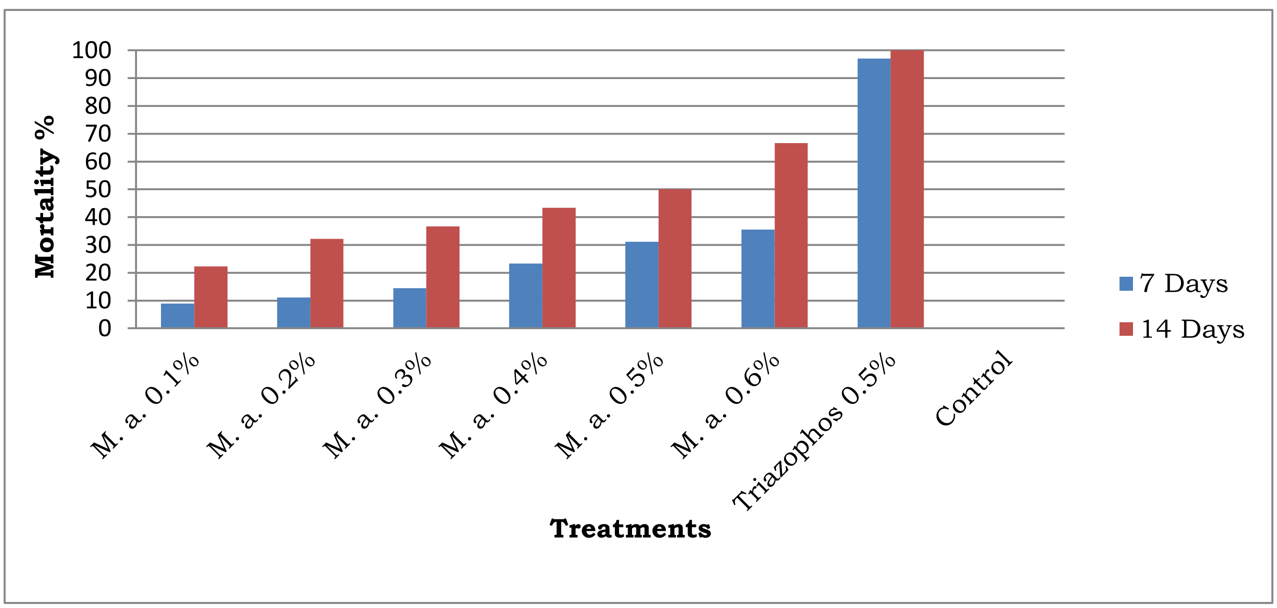


Fig.12 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 6 month under aambient condition.

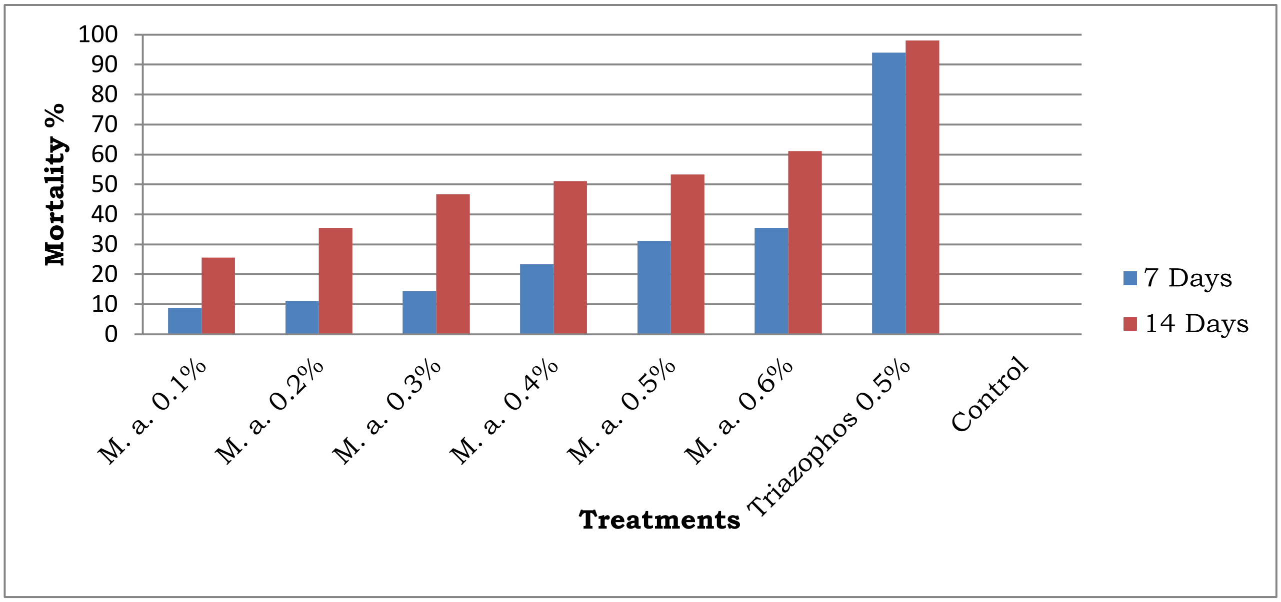


Fig.13 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 12 month under refrigerator condition.

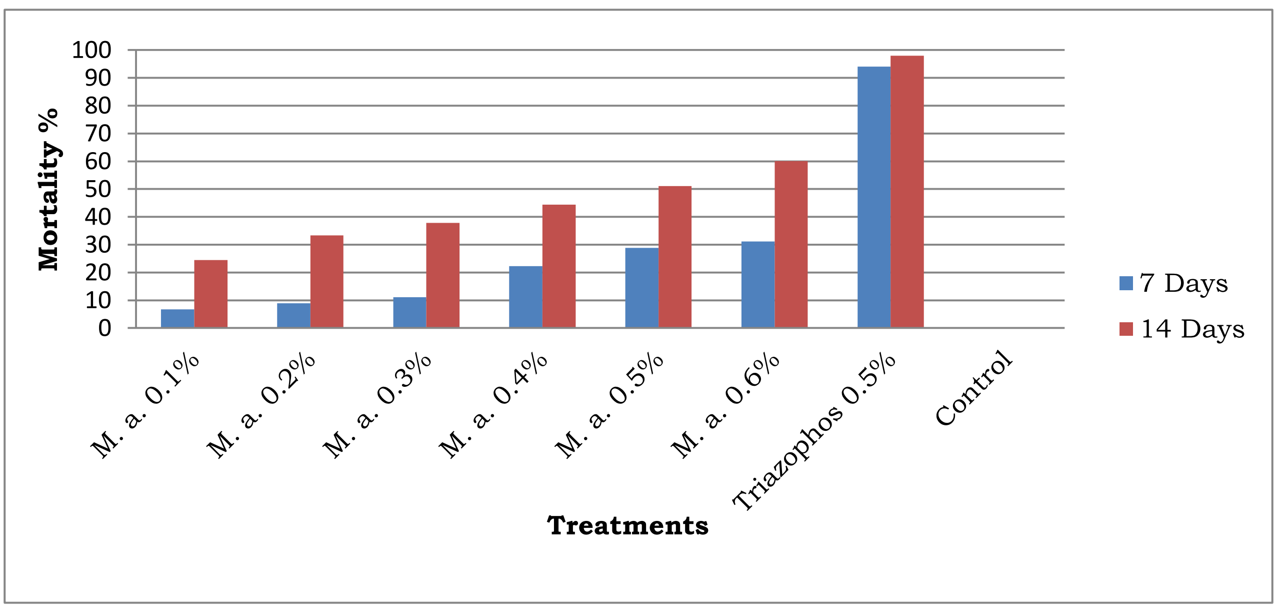


Fig.14 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 12 month under refrigerator condition.

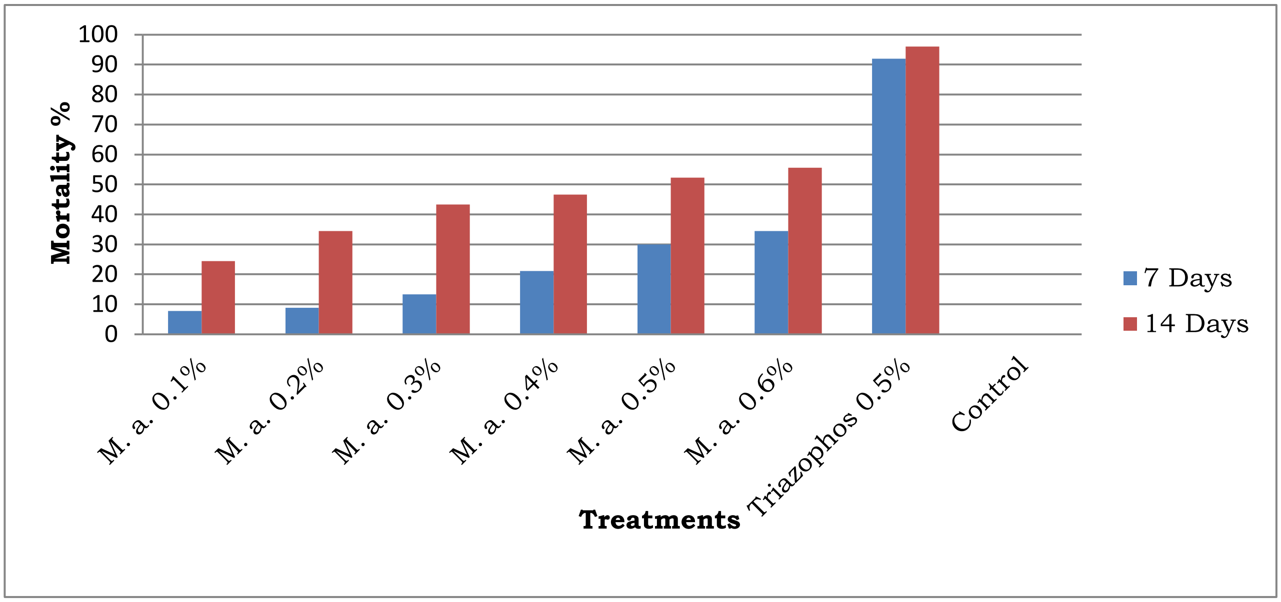


Fig.15 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 12 month under aambient condition.

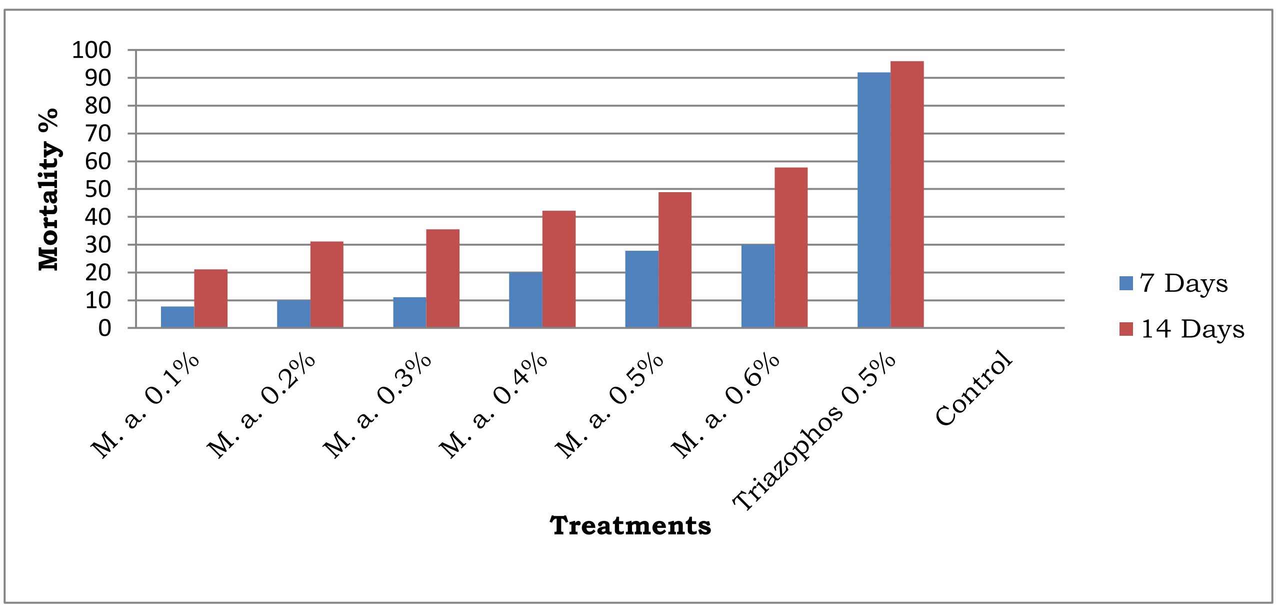


Fig.16 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 12 month under aambient condition.

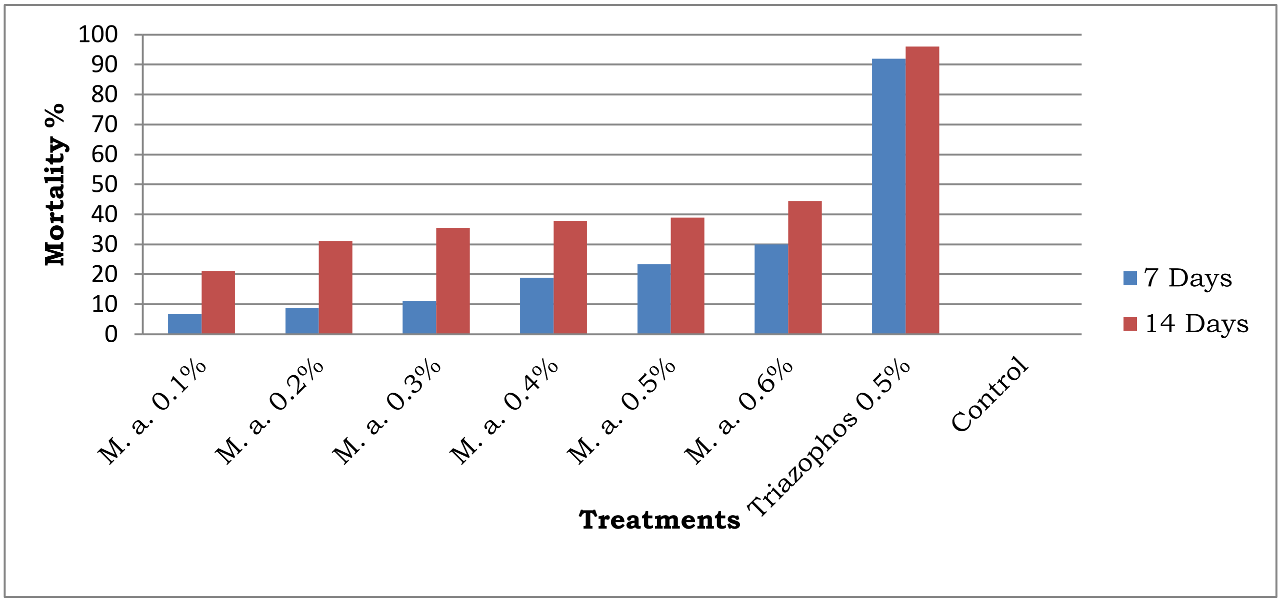


Fig.17 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 18 month under refrigerator condition.

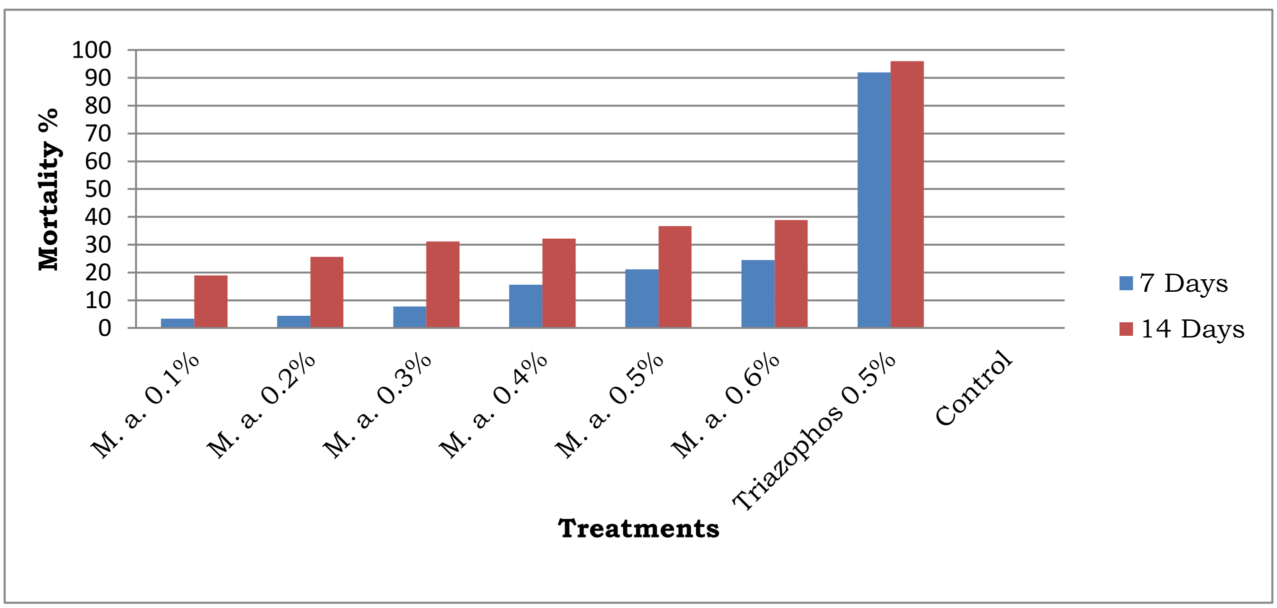


Fig.18 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 18 month under refrigerator condition

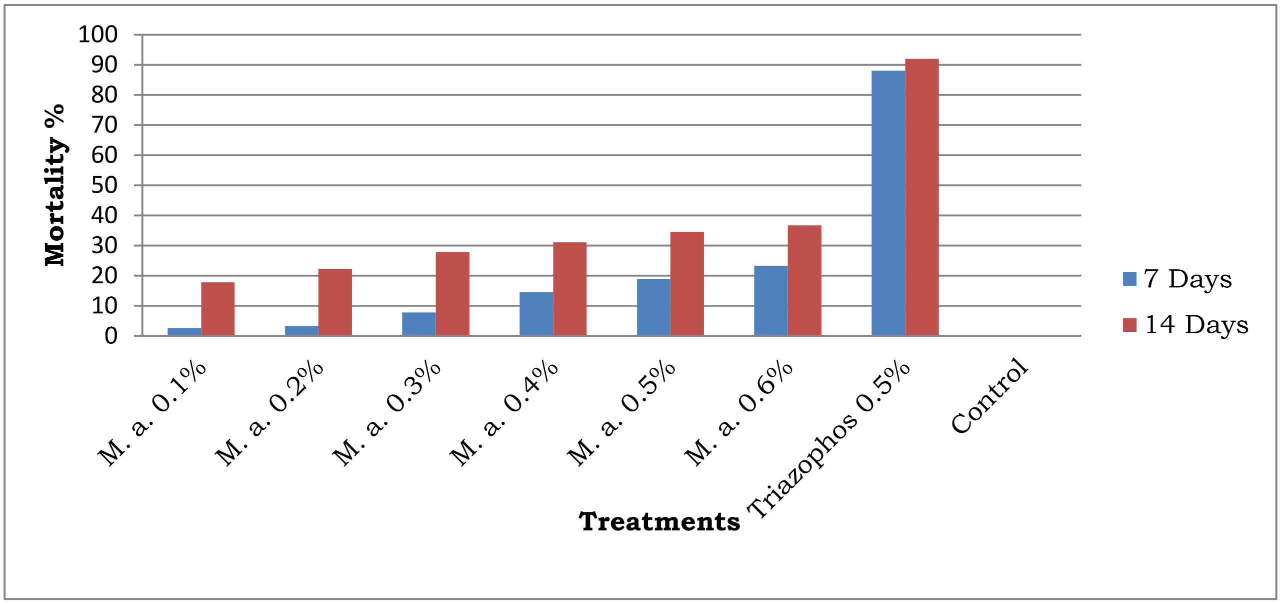


Fig.19 Bioefficacy of *M.anisopliae* 3% AS against *S.litura* after 18 month under aambient condition.

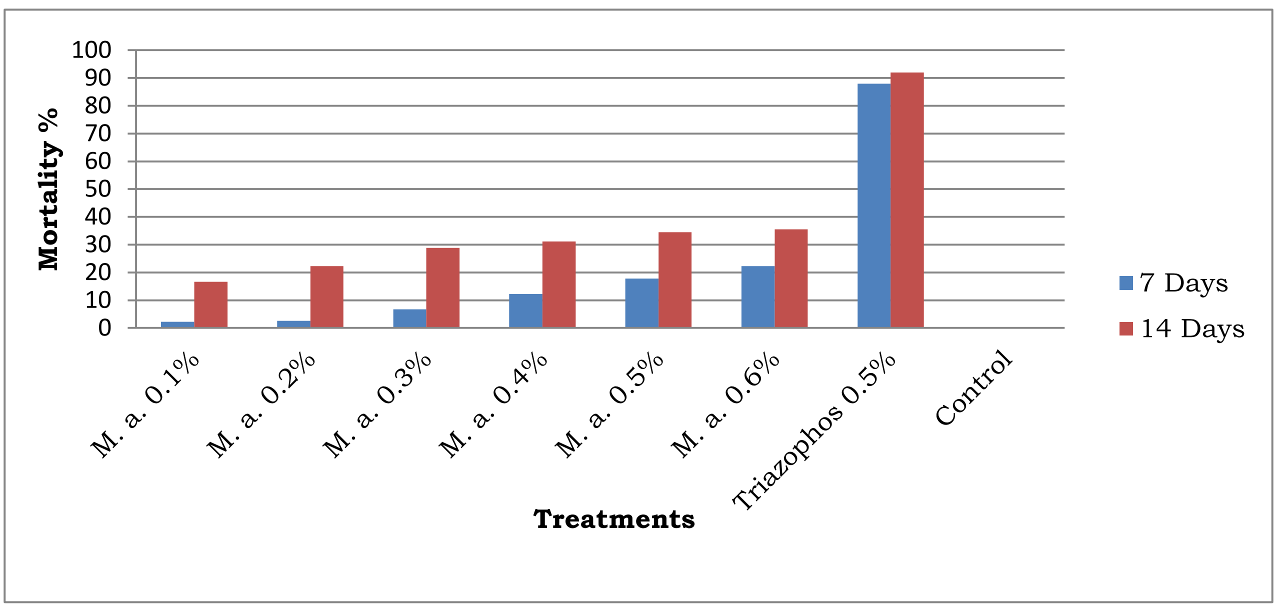


Fig.20 Bioefficacy of *M.anisopliae* 1.15% WP against *S.litura* after 18 month under aambient condition.

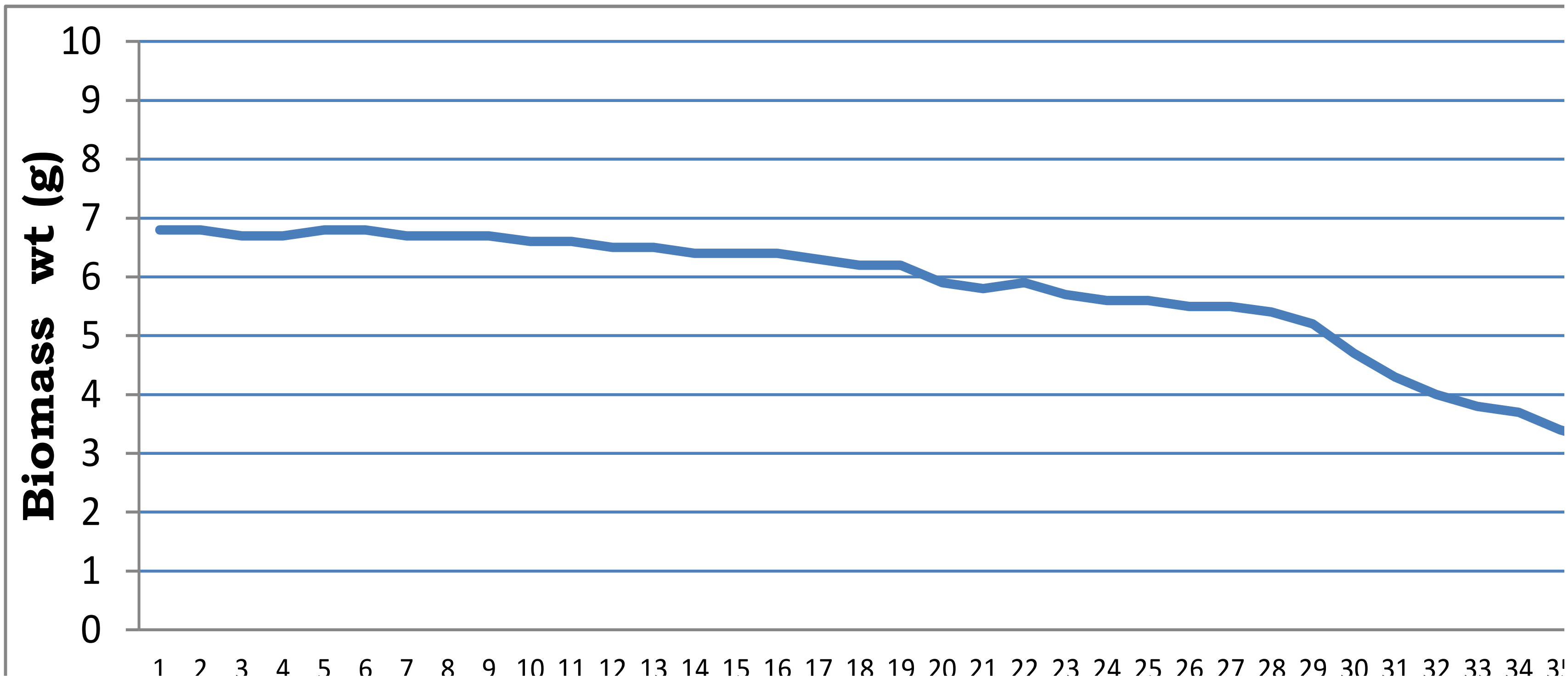


Fig. 3 Effect of storability on biomass production of *M. anisopliae* 3% AS stored under refrigerator condition.

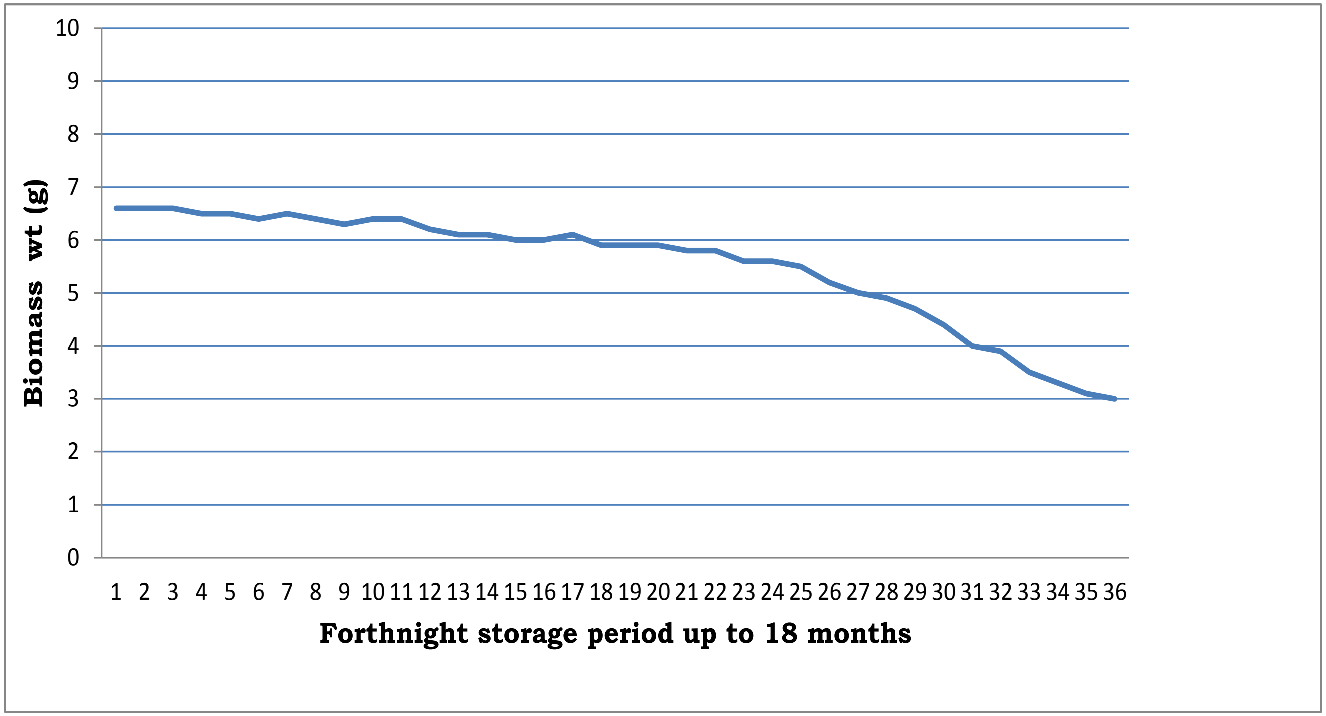


Fig. 4 Effect of storability on biomass production of *M. anisopliae* 3% AS stored under ambient condition.

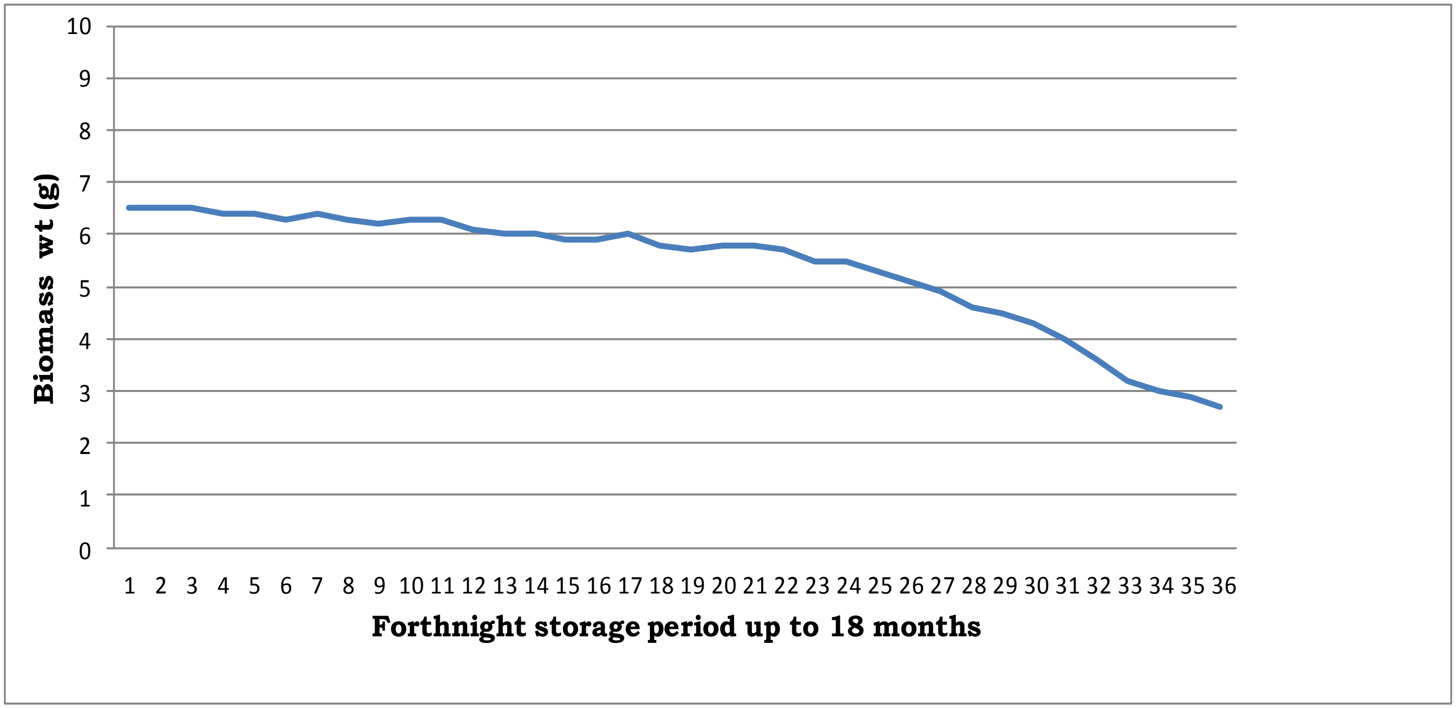


Fig. 5 Effect of storability on biomass production of *M. anisopliae* 1.15% WP stored under refrigerator condition.

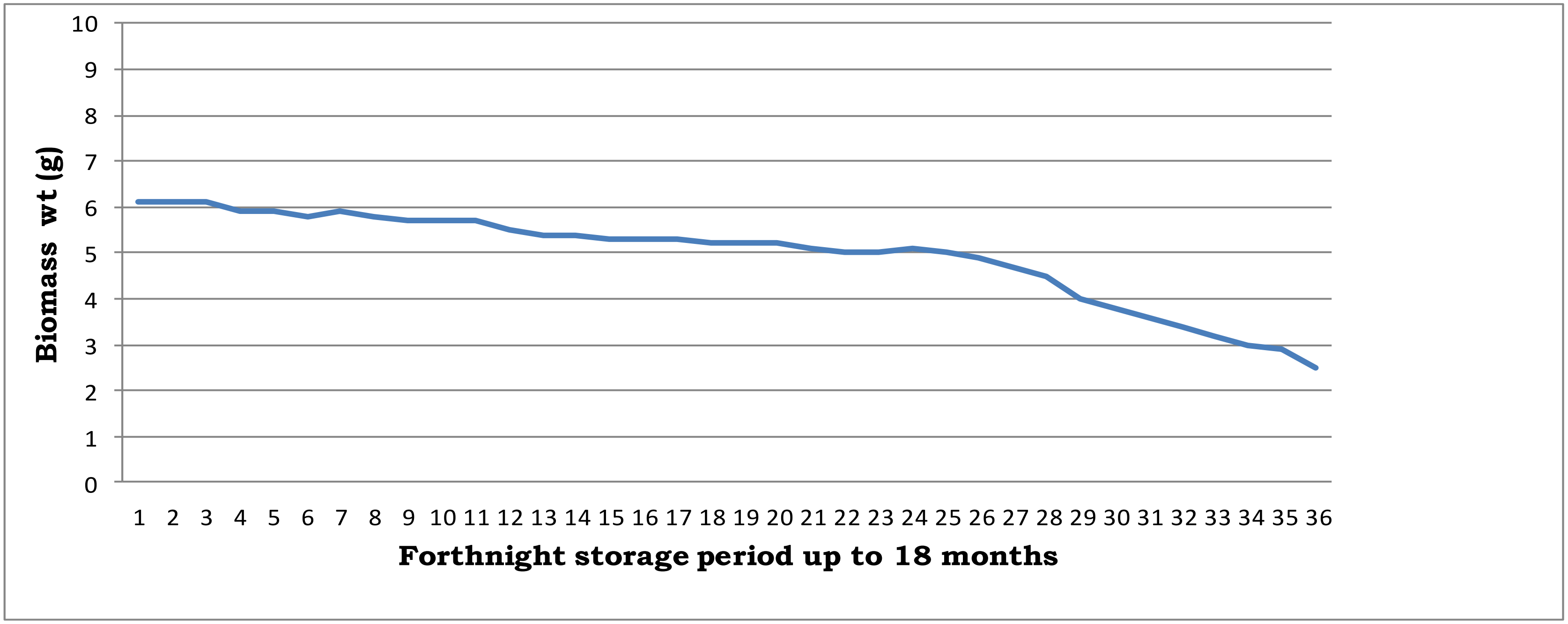
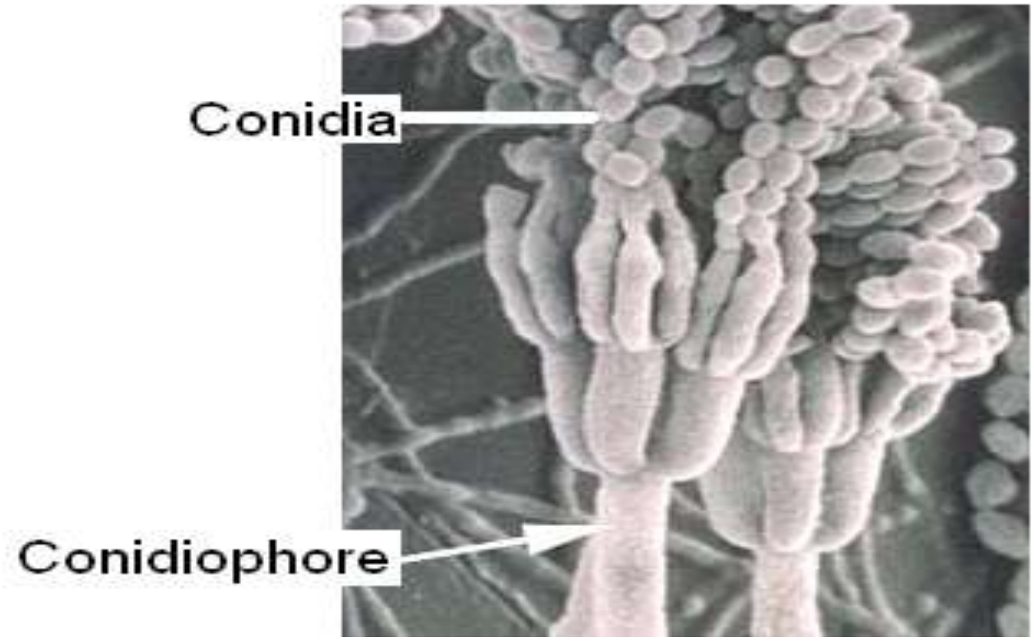
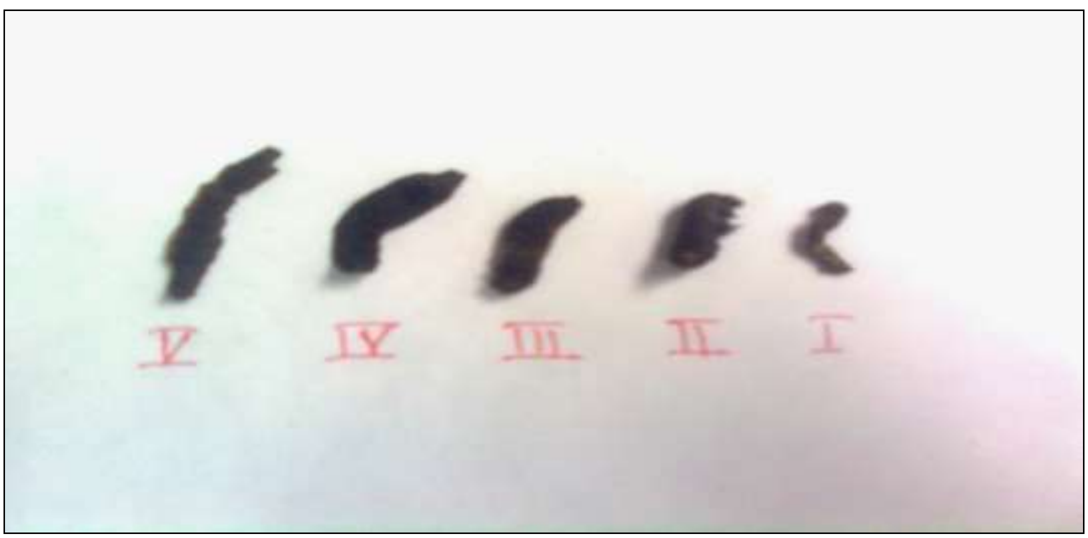


Fig. 6 Effect of storability on biomass production of *M. anisopliae* 1.15% WP stored under ambient condition.



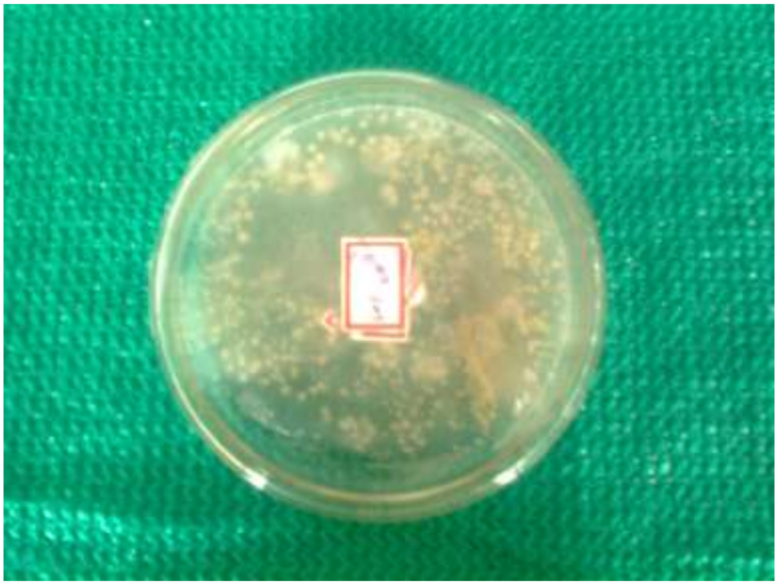
A) ***M.anisopliae*** **– Conidia and Phialides.**



B) S.litura larvae infected with ***M.anisopliae***

Fig. 1 ***M.anisopliae*** **structure and infected larvae**

a) Cfu at 10^8 after 24 hour inoculation



b) Cfu at 10^8 after 48 hour inoculation

fig 2. *M.anisopliae* cfu