DEVELOPMENT OF WP FORMULATION OF *Nomuraea rileyi* (FARLOW) SAMSON AND *Metarhizium anisopliae* (METSCH.) SOROK.

By

SANJAY DHONDU PATIL

[Reg. No. 09/42]

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

DOCTOR OF PHILOSOPHY (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 thereof

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. *(S.D.PATIL)*

Dated: - 31/05/2012

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Shri. SANJAY DHONDU PATIL, under my guidance and supervision and no part of the thesis has been submitted for any other degree, diploma or publication in any other form. The assistance and help received during the course of this investigation has been duly acknowledged.

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Dr. J.R. Kadam, Professor and Ex.Head, Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri and that no part of the thesis has been submitted for any other degree, diploma or publication.

Place: MPKV, Rahuri
Dated: 31/05/2012

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LIST OF ABBREVIATIONS

Abbreviation		Description
%	:	Per cent
/	:	Per
+	:	Plus
@	:	at the rate of
>	:	More than
^{0}C	:	Degree Centigrade (Celsius)
a.i.	:	Active ingredient
AS	:	Aqua suspension
Av	:	Average
BA	:	Boric Acid
Bt	:	*Bacillus thuringiensis*
BAI	:	Bioactive Ingredient
CD	:	Critical Difference
cfu	:	Colony forming Unit
cm	:	Centimeter
CMC	:	Carboxymethyl Cellulose
Cm^2	:	Square Centimeter
C.R.D.	:	Completely Randomized Design
Conc.	:	Concentration
CNO	:	Coconut Oil
DAS	:	Days After Sowing
DAT	:	Days after treatment
e.g.	:	Example
EC	:	Emulsifiable Concentrate
et al.	:	and others
etc.	:	etceteras
Even.	:	Evening
Fig.	:	Figure

Form.	:	Formulation
FYM	:	Farm Yard Manure
G	:	Granule
GH	:	Ghee
GLY	:	Glycerol
GRO	:	Groundnut Oil
g	:	Gram
ha.	:	Hectare
hrs or h	:	Hours
i.e.	:	id est (Latin : for instance)
Kg	:	Kilogramme
L	:	Liquid
μm	:	Micro meter
μ	:	Micron
LC_{50}	:	Lethal concentration
l	:	Liter(S)
LD_{50}	:	Lethal dose
LT_{50}	:	Lethal time
m	:	Meter
M.a	:	*Metarhizium anisopliae*
Max.	:	Maximum
Min.	:	Minimum
Morn.	:	Morning
m^2	:	Square Meter
mg	:	Milligram
Min	:	Minute
ml	:	Milliliter (s)
mm	:	Millimeter
mm^2	:	Millimeter Square
ME	:	Malt Extract
MUO	:	Mustard Oil
N.r.	:	*Nomuraea rileyi*

No.	:	Number
nm	:	Nanometer
pp.	:	Pertaining pages
PDY	:	Potato dextrose broth with yeast extract
PD	:	Potato dextrose broth
PG	:	Potato Glucose Broth
PP	:	Potato Peptone Broth
PM	:	Potato Maltose Broth
q	:	Quintal
R.H.	:	Relative humidity
rpm	:	Revolutions per minute
SE	:	Standard Error
SDY	:	Sabouraud's dextrose broth with yeast extract
SMY	:	Sabouraud's maltose broth with yeast extract
SFO	:	Sunflower Oil
SBO	:	Soybean Oil
SC	:	Soluble concentrate
Sp	:	Species
Sr.	:	Serial
t	:	Tonnes
Tr.No.	:	Treatment Number
Temp.	:	Temperature
TW	:	Tween 80
TX	:	Triton-X-100
UV	:	Ultra violet
viz.	:	Videlicet (Namely)
WP	:	Wettable powder
WUVC	:	Without UVC
YEG	:	Yeast Extract Glucose Broth

ABSTRACT

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2012

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

The investigations on "DEVELOPMENT OF WP FORMULATION OF *Nomuraea rileyi* (FARLOW) SAMSON AND *Metarhizium anisopliae* (METSCH.) SOROK." were carried out during 2009-2012 at Biocontrol Research Laboratory, Department of Entomology, M.P.K.V. Rahuri, to develop potential WP formulations of *N.rileyi* and *M.anisopliae*. The results revealed that out of nine test media, Sabouraud's dextrose broth with yeast extract (SDYE) emerged as best medium for mass production of *N.rileyi* as well as *M.anisopliae* which yielded highest biomass of 6.10g and 7.20g/40ml medium with viability of $8.33x10^8$ and $12.33x10^8$ cfu/ml, respectively. The biomass productivity and virulence of both the fungi increased with the increase in concentration of the inoculums and the standardized optimum concentration of

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bioactive ingredient was 30% fungal culture at 10 DAI (v/v) for the formulations considering the biomass, viability and bioefficacy.

On the basis of performance of the adjuvants comprising chemicals, edible oils and other edible substrates for production of biomass of *N.rileyi* and *M.anisopliae* GLY 2.0%, BA 2.0%, CMC 0.5%, TW 0.5%, SFO 1.0%, GH 0.5%, GNO 0.5%, SBO 0.5%, MUO 0.5% and CNO 1.0% and HO 1% were emerged as most promising adjuvants producing 8.83 to 8.97 g and 6.50 to 8.37 g biomass of *N.rileyi* and *M.anisopliae*, respectively. On exposure of *N.rileyi* and *M.anisopliae* test formulations containing adjuvants to UVC rays for 10 to 50 min. and 2 to 5 hrs the sunflower oil, groundnut oil, glycerol, carboxymethyl cellulose, boric acid, honey and milk performed as UVC protectants for both fungi even after the exposure of 5 hrs.

The growth, development, viability and bioefficacy of an entomopathogenic fungus, the highly promising advance stage *N.rileyi* formulations were *N.r.*+HO 1%, *N.r*+SFO 1%, *N.r.*+GH 0.5%, *N.r.*+TW 0.5%+GH 0.5%, *N.r.*+GLY 2%+SFO 1%, *N.r.*+GLY 2%+GH 0.5%, *N.r.*+SFO 1%+GH 0.5%, *N.r.*+TW 0.5%+GLY 2%+SFO 1%+CMC 0.5%, *N.r.*+TW 0.5%+GLY 2%+HO 1% and *N.r.*+TW 0.5%+GLY 2%+CMC 0.5%. Similarly, the high performance formulations of *M.anisopliae* were *M.a.*+TW 0.5% +CMC 0.5%, *M.a.*+SFO 1.0%+CMC 0.5%, *M.a.*+SFO 1.0%+HO 1.0%, *M.a.*+GNO 0.5%+BA 2.0%, *M.a.*+GNO 0.5%+CMC 0.5%, *M.a.*+GNO 0.5%+GH 0.5% and *M.a.*+GH 0.5%+HO 1.0%. These formulations possessed UV rays protecting ability.

On the basis of wet fungal mat weight/volume of both fungi the standardized concentration of bioactive ingredient was 5 per cent. The two best *N.rileyi* 5% WP formulations coded as A ($N_{30}S_{1/1}$) and B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) while, those of *M.anisopliae* 5% WP formulations coded as A1($M_{30}S_{1/1}C_{1/}2$) and B1($M_{30}S_{1/1}H_{1/1)}$ were emerged as the

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best potential formulations. The LC_{50} values of *N.rileyi* formulation A($N_{30}S_{1/1}$) for II and III instar larvae of *S.litura* were 0.0116% and 0.0157% and those for formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0120% and 0.0176% BAI, respectively. Similarly, The LC_{50} values of *M.anisopliae* 5% WP formulation A1($M_{30}S_{1/1}C_{1/2}$) were 0.0174% and 0.0180% and those of formulation B1($M_{30}S_{1/1}H_{1/1}$) were 0.0149% and 0.0163% for II and III instar larvae of *S.litura*, respectively.

Considering biomass production and viability (cfu/g) the shelf life of *N.rileyi* 5% WP formulation A, B and control (without adjuvants) was 10, 10 and 7 months, respectively. *M.anisopliae* 5% WP formulation A1, B1 and control could be stored upto 10, 10 and 6 months, respectively.

The *N.rileyi* 5% WP formulations A 0.025 % and 0.03 %, B 0.03 % were highly promising (1.67 to 1.73 larvae/m row) and were at par with quinolphos 0.05% (1.53 larvae/m row) for the lethal effect against *S.litura* on soybean. Although quinolphos 0.05% recorded maximum yield of 28.66 q/ha, it was at par with formulation B 0.03% (27.47q/ha) and 0.025% (27.17q/ha). Similarly, besides the considerable bioefficacy of *N.rileyi* 5% WP formulation B 0.03% recorded highest yield of 27.47 q/ha and it was at par with B 0.025% (27.17q/ha), A 0.03% (26.93q/ha), A 0.025% (26.67q/ha), B 0.02% (26.40q/ha) and A 0.02% (26.13q/ha). The *M.anisopliae* 5% WP formulations 0.03% B1 and A1 were highly effective in controlling the *S.litura* larvae on soybean. These were at par with formulation B1 0.025%. The *M.anisopliae* formulation A1 0.03% recorded highest yield of 26.67 q/ha and it was at par with formulation B1 0.03%, A1 0.025%, B1 0.025% and A1 0.025%. The both WP formulations of *N.rileyi* and *M.anisopliae* did not caused any phytotoxicity to soybean plants at 0.01 % to 0.08 % concentrations.

1.INTRODUCTION

Insecticide resistance in insects and other deleterious effects of chemicals on the environment and human safety have provided a strong impetus for the development of microbial pesticides to use in IPM (Puri *et al.*, 2005). A diverse assemblage of entomopathogens comprising viruses, bacteria and fungi is currently being exploited as microbial control agents for crop pests. The mycopathogens have distinct advantages over others because of their wide microbial options, host range and amenability for easy mass production (Manjula and Krishnamurthy, 2005). They are associated with pests in diverse habitats. Further, these are epizootics in nature effective against both chewing and sucking pests and their mode of action is through contact while ingestion is must for other microbes. Thus, entomopathogenic fungi have wider scope in the biological control programmes in different crop ecosystems. The fungi are frequent opportunistic parasites of arthropods worldwide (Shah and Pell, 2003).

Many chemical insecticides are recommended to control the insect pests. Growing awareness about the failure of chemical pesticides on one hand and health hazards on the other hand have compelled the agricultural entomologists to develop sustainable pest management strategies which are economically viable and eco-friendly. In this direction biological control, especially the microbial control is desirable to advocate as the first line of attack. Microbial control is the biological suppression of insect pests employing microbial world. It has the advantage of analogous control like chemicals, higher host specificity, virulence, safety to natural enemies, shelf life, ease in production, farmers and producer's friendly, eco-friendly and compatibility with other methods. Thus "Biological control" is getting impetus recently and became an inevitable component of IPM in many crops for the development of sustainable cropping system leading to produce safe food. There is ample scope for microbial control of the crop pests. Several micro-organisms are currently under consideration as control agents of pests, because of their high host- specificity, non-persistence and non-

toxicity to environment, unique mode of action and appreciable shelf life. So the insect viruses, bacteria, nematodes and fungi are emerging as potential bioagents (Pandey and Kanujia, 2005).

At the end of 2001, there were approximately 195 registered biopesticides and 780 formulated products for the control of insects (38.10 %), bacteria (37.00 %), nematodes (15.7 %), fungi (4.7 %), viruses (2.85 %) and protozoa (2.14 %) (Anonymous, 2003). Among the pathogens used in microbial control, entomopathogenic fungi have played an important role in the history of insect pathology and microbial control of insects.More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (Hajek and Lager, 1994). The entomopathogenic fungi causing diseases to the insects are practically more significant as they are epizootic in nature. Also they have the advantage of ease of production and contact action which allow direct penetration of the host cuticle without ingestion.

Nomuraea rileyi (Farlow) Samson Moniliales, Moniliaceae is a fungus of cosmopolitan nature. *N.rileyi* infects mainly Lepidoptera, particularly economical important and polyphagous noctuid insect pests. *N.rileyi* is an entomopathogen causing natural mortality in as many as 51 Lepidopteran insects throughout the world (Lingappa and Patil 2002). *N.rileyi* frequently cause epizootics in nature, is one promising because of its wide spread occurrence and relative abundance due to its wide host range which included many catterpiller pests. The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes such as chitinase, proteases and lipases. Progress of research on *N.rileyi* in India is slow though the results of the few studies have revealed that *N.rileyi* as a potential mycoinsecticide (Vimladevi *et al.*, 2002).

The green muscardine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, Moniliales, Moniliaceae is another potential entomopathogenic candidates for biological control. Metschnikoff (1879) was first to isolate fungus *M.anisopliae* from the larvae of grain weevil and also first to demonstrate entomopathogenic nature of the fungus against chrysomelid, curculonid and scarabaeid beetles. *M.anisopliae* capable of infecting more

than 100 different insect pests belonging to a variety of insect orders *viz.*, Orthoptera (Grasshopper and Cockroaches), Homoptera (Spittle bug, *Nilaparvata lugens*) and Lepidoptera (*Helicoverpa armigera, S.litura*) (McCoy *et al.*,1988). Gopalakrishnan and Narayanan (1987), reported 80-100% mortality of *H.armigera* by *M.anisopliae*. It also used for the control of *Earias insuana* (Aly and Rashad, 1997), cabbage semilooper (Wikramatileki *et al.*, 2000), *Maruca vitrata* (Ekesi *et al.*,2002) diamond back moth (Silva *et al.*,2003). This fungus is also used for control of sucking pests of important field crops. Virulence of *M.anisopliae* against mustard aphid (Pandey and Kanujia, 2004), *Aphis gossypii* and *Myzus persicae* (Loureiro and Moino, 2006) and *Maconellicoccus hirsutus* (Ujjan and Saleem Shahzad, 2007) has been documented.

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 peg penetrates 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, also 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.

A considerable number of mycoinsecticides have reached the market place and millions of hectares are treated annually with entomopathogenic fungi worldwide (Faria and Wraight, 2007). The lepidopterous pests cause serious havock to most of the crops. The leaf eating caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera) is one of the polyphagous and cosmopolitan pests. The sporadic pest of past recently assumed serious form on soybean, cotton, potato, okra, cabbage, cauliflower, castor, groundnut, lucerne, sweet potato, beat root, grapes and polyhouse crops. The defoliator

caused maximum economic damage in 2009-2010 to these crops in Maharashtra (Anonymous, 2011).

Some pesticide and other beneficial organisms have been very effective in the laboratory only to fail at some stage in the field, even after development of product for marketing. Common causes of this demise are poor stability of the product during storage prior to application, too little active material reaching to the target and rapid degradation of the active material. Formulation plays a vital role in helping to solve these problems. Formulated organisms are suspended in a suitable carrier which is supplemented by additives to maximize survival in storage, optimize the application for the target and protect pest pathogen after application. Formulation technology must be considered at all stages from production of an entomopathogens to its eventual action on the target pests. The solution for foregoing problem is developing suitable formulations.

Efficiency of entomopathogens in the field depends upon virulence towards target pest, coverage and persistence on target site. However, major constraints for successful use of such bio-agents are their difficulties in use of pure cultures, survival on crop after application, loosing virulence by ultra violet (UV) rays, short shelf life, and dependability on the prevailing environmental conditions are the problems reported by Kaur *et al.* (1999).

The formulation of the fungi still awaits a serious efforts in formulation technology. Exploring formulation of *N.rileyi* and *M.anisopliae* as a tool in the pest management of Lepidopteran pests is one of requisite mandate.The foregoing problem can largely be overcome by developing suitable formulations. The performance and shelf life can be improved by adding suitable adjuvants subsequently leading to growth, development and viability of the fungus that may act as nutrient, adhesive, UV protectants, wetting agents etc. Presently crude suspensions of the fungi with short shelf life of around one to two months for liquid and 5 to 6 months for WP are marketed. For developing wettable powder formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before the formulation. There is a need to develop a viable and potential WP

formulation of *N.rileyi* and *M.anisopliae* for biological pest management. Hence, the present investigations were planned and executed with following objectives:

i. Find out suitable medium for mass production of *N.rileyi* and *M.anisopliae*.

ii. Development of WP formulations of *N.rileyi* and *M.anisopliae* using adjuvants.

iii. Studies on bioefficacy of the developed formulation against Lepidopteran pest.

iv. Shelf life assessment of WP formulations.

v. Evaluation of phytotoxicity of the formulations.

vi. Field evaluation of the formulations against *S.litura* on soybean.

2. REVIEW OF LITERATURE

Nomuraea rileyi (Farlow) Samson and *Metarhizium anisopliae* (Metschnikoff) Sorokin green muscardine fungus belongling to subdivision Deuteromycetes, class Hyphomycetes, order Moniliales, family Moniliaceae are of the disease producing fungus reported to be effective against many insect pests for their management. Development of microbial insecticide formulations should contribute to pathogen stability, efficacy and acceptance by the end users. The performance and shelf life can be improved by adding suitable ingredients that may acts as nutrient adhesive, UV protectants, wettable agent sticker, surfactants and sunscreen.

The literature pertaining to formulation and efficacy of *N.rileyi* and *M.anisopliae* with respect to objective to research are reviewed and presented in the following headings.

Entomopathogenic fungi :

2.1 Occurrence and distribution of *N.rileyi* and *M.anisopliae*

World

***N.rileyi*:**

The name of *Spicaria rileyi* (Farlow) Charles was changed to *Nomuraea rileyi* (Farlow) Samson (Kish *et al.*, 1974). Higher infection of *N.rileyi* in *Heliothis sp*. Larvae collected from a closed canopy of cotton (Delta pine 215) than collected from an open canopy variety (Louisiana Okraleaf) was observed by Burleigh (1975).

The fungus was found to be commom pathogen affecting the population of *Plathypena includense* Walker, *Helicoverpa sp.* and *Anticarsia gemmatalis* Hubner, in soybean ecosystem in North Carolina (Carner *et al.,* 1975). Seasonal Incidence of *Nomuraea* (*Spicaria*) *rileyi* associated with noctuid pests of soybean in Columbia was recorded by Ignoffo *et.al.* (1975b).

In South Carolina *N.rileyi* occurred frequently enough to reduce the population of soybean caterpillar (Ignoffo *et. al.*, 1976a). Initiation and peak

incidence was advanced by atleast 12 days by heavy application of *N.rileyi* (1.1 – 2.2x10^{13}conidia/acre) (Ignoffo *et. al.*, 1976).

Garcia and Ignoffo (1977) reported that the dislodging of conidia was initiated when the wind speed was 2.7 km per hr, suggesting it as the threshold speed. As the wind speed increased upto 5.9 km per hr, the number of conidia dislodged increased. Nearly 90 per cent of conidia were dislodged within 3 minutes.

Wind as an important means of dislodging and dispersal of conidia. Dispersal of conidia from early season killed larvae in soybean ecosystem by wind resulted in the late season epizootic (Ignoffo *et al.,* 1977).Widespread occurrence of the fungus *N.rileyi* on *Anticarsia gemmatalis* Hubner was observed by Panizzi *et al.* (1977).

The entomopathogenic fungi *N.rileyi* was described for the first time by Farlow in 1883 as *Botrytis rileyi* later it was transferred to *Spicaria* by Charles in 1936. However, the taxonomy of the fungus was not stable and it did not have a valid name till 1966 (Ignoffo, 1981).

Santos (1982) reported that the peak incidence occurred after the damage by defoliating caterpillars in untreated plots. The prevalence of *N.rileyi* on *A. gemmatalis* on soybean in Brazil between February to mid April ranged from 16.6 to 88.5 per cent and were lowest in area of heavy application of insecticide.

Epizootics of *N.rileyi* in the 2nd generation of green clove worm, *P.scabra* was dependent on density of 1st generation. If the density during 1st generation was more, an early epizootic will be established in the succeeding generation (Thorvilson and Pedigo, 1984).

N.rileyi causing mortality of both *Thysanoplusia orichalcea* (Fubricius) and *Chrysodensis eriosoma* (Doubleday) on soybean was observed for the first time in New Zealand (Hill *et al.,* 1987).

M.anisopliae:

Mechnikov demonstrated in Russia for the first time the potential of microorganisms for pest control when using the enthomopathogenic fungus *M.anisopliae*, isolated from larvae of the bread beetle *Anisoplia austriaca*. (Ismailov *et al.*, 2002).

Fungi were isolated from soil by means of *G.mellonella* larvae as baits. Three entomopathogenic fungal species: *Beauveria bassiana*, *M.anisopliae* and *P.fumosoroseus* were isolated. *M.anisopliae* occurred only once in the soil from wheat field. The frequency of the occurrence of entomogenous fungal species in the soil under different managements, measured by the percentage of infected *G. mellonella* larvae, was 77.3% in woodland, 27.5% in wheat field, 16.5% in lucerne field and 9.3% in grassland (Tkaczuk *et al.*, 2003).

Soil samples were taken from forest stands, conventionally and organic treated fields, vineyards, orchard, hedgerows and fallow in 1998 and 1999 from Austria. Entomopathogenic fungi were isolated from a major part of the soil samples (84%). The two most common species of fungi were *B.bassiana* (74%) and *M.anisopliae* (29%) (Hozzank *et al.*, 2003).

Entomopathogenic fungi have been isolated from 81.25% of the soil samples. Incubation temperatures were 10, 15, 20, 25 and 30°C. The most abundant species were *B.bassiana* (89.7% of samples) and *M.anisopliae* (43.5%). Neither the percentage of organic matter nor the pH has any relationship with the occurrence of *B.bassiana* and *M.anisopliae* in the soil. (Maranhao *et al.*, 2003).

M.anisopliae was the dominant species isolated from all soil samples. The highest mortality of *G. mellonella* larvae caused by this species was observed in samples from arable soil. (Sapieha Waszkiewicz *et al.,* 2003).

India

N.rileyi:

In India the natural incidence of the fungus *N.rileyi* was reported for the first time on *Diacrisia oblique* by Singh and Gangarde (1975). In Maharashtra its occurrence was recorded for the first time on *S.exigua* on black gram and bajra. Similarly, it was recorded on *Spodoptera litura* on tobacco in and around Poona during *kharif*, 1977 (Phadke *et al.,* 1978).

M.anisopliae var. minor and *N.rileyi* are reported infecting mature larvae of the noctuid *Heliothis armigera* (*H.armigera*) for the first time in India. In the first case, *H.armigera* was collected from tomato and in the second from field beans both in Karnataka (Gopalakrishnan and Narayanan, 1988). Higher rates of fungal infection in *H.armigera* were found on *C.cajan* (37%) compared to *P.vulgaris* (28.2%) and tomato (20.5%) (Gopalakrishnan and Narayanan, 1989).

Under natural condition, occurrence of *Nomuraea sp*. has been reported from Akola district of Vidarbha in Maharashtra on *H.armigera* infecting the groundnut crop of *kharif* season (Men *et al.*, 1990) (Rajak *et al.,* 1991).

Epizootic of entomofungal pathogen *N.rileyi* on Lepidopteran pests of groundnut, castor and niger was recorded in Rajendranagar and Narkhed of Andhra Pradesh by Vimladevi *et al.,* (1996). Outbreak of the same fungus was observed on *S.litura* in soybean during October, 1996 by Ambethgar and Loganathan (1998) at Udamalpet regions of Tamilnadu.

N.rileyi was recorded infecting 11% of the larval population of *S.litura* on groundnut at Patancheru, Andhra Pradesh, in September 1999 (Venkatesan *et al.*, 2000).An outbreak of tobacco caterpillar, *S.litura*, was observed in soybean during the last week of August 2000 in Western Maharashtra, India. Natural epizootic of the entomopathogenic fungi,

N.rileyi, was observed in the larval populations of *S. litura* and this fungus controlled the pest by 80-90% (Nakat *et al.*, 2001).

The incidence of *N.rileyi* on *H.armigera* in cotton and red gram was noticed from forty-third to forty-ninth and forty-third standard weeks and attained its peak in the forty-eighth and forty-seventh standard weeks during 1996 and 1997, respectively in Dharwad, Karnataka. (Kulkarni and Lingappa, 2002).

N.rileyi on *S.litura* in groundnut was observed between thirty-second to fortieth standard week (SW) with peak activity in the thirty-seventh standard week (10-16 September) during 1996 and 1997 and in the thirty-fourth (20-26 August) during 1998. (Kulkarni and Lingappa, 2002).

In 2001, the entomopathogenic fungus *N.rileyi* was observed in populations of the soybean green semilooper, *C.acuta*, in the humid Southeastern Plain Zone of Rajasthan, India. The fungus caused 100% mortality of the larvae of *C.acuta*. (Gupta, 2003).

The occurrence of *N.rileyi* on *H.armigera* and *S.litura* on cotton, chilli, tomatoes, red gram, black gram and groundnuts was determined in Bapatla, Andhra Pradesh, India from October to March of 1998-99. Cotton and groundnut recorded 100% mycosed larvae of *H.armigera* and *S. litura* due to infection by *N.rileyi*. The incidence of *N.rileyi* showed positive association with morning relative humidity. *H.armigera* recorded higher infection compared to *S. litura* in groundnuts. However, higher infection was observed in *S.litura* compared to *H.armigera* in black gram (Manjula *et al.*, 2003).

The first appearance of *N.rileyi* was recorded naturally in the 30th standard week, which continued up to the 39th standard week and peaked between the 33rd and 37th standard weeks in Karnataka. (Patil *et al.*, 2003).

Epizootics caused by *Beauveria bassiana* and *N.rileyi* have been observed on boll worms and *S.litura* in south Indian fields during winter since the last 15 years. During the *N.rileyi* induced natural epizootics, some boll worms were found surviving without infection. (Devi *et al.*, 2003).

During a survey in August 2003 (*kharif*) in various villages in Akola and Washim districts of Maharashtra severe infestation by *N.rileyi* was observed in *Diachrysia orichalcea* on soybean, and in *H.armigera* and *S.litura* on green gram. Lower incidence of *N.rileyi* was evident in *Spilosoma obliqua* on soybean. (Ingle *et al.*, 2004).

Disease incidence on *S.litura* and *H.armigera* was more in Bailhongal than at Dharwad mainly because of close canopy crops such as soybean, groundnut and green gram, grown in large areas, which provided a favourable niche for the development of the pathogen. (Rachappa and Lingappa, 2007).

M.anisopliae:

In a survey in potato fields in Himachal Pradesh in 1973-74, large numbers of white grubs were found dying from infection by *M.anisopliae.* This is the first report of *M.anisopliae* infecting these soil-inhabiting potato pests in India (Singh, 1978). Studies were carried out on the occurrence and natural enemies of cutworms infesting potato in Karnataka, India. *Agrotis ipsilon* (Hfn.) was a serious pest of potato in Patna and Jullundur *M.anisopliae* infesting *A.ipsilon* was found in the Simla Hills (Singh, 1982).

The percentage incidence of infection of larvae of *S.litura* with *M.anisopliae* on groundnuts at a place 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 2nd fortnight of June, was high in mid-August and fell to nil in November (Siddaramaiha, *et al.*, 1986).

M.anisopliae var. minor and *N.rileyi* are reported infecting mature larvae of the noctuid, *H.armigera* for the first time in India. In the first case, *H.armigera* was collected from tomato and in the second from field beans both in Karnataka. (Gopalakrishnan and Narayanan, 1988).

M.anisopliae var. anisopliae was found on the larvae of the sugarcane internode borer *Chilo sacchariphagus* indicus under field conditions in Coimbatore, Tamil Nadu. The number of fungal spores produced per larvae was higher on fourth instar than on third instar larvae. This is the first report of *M.anisopliae* on sugarcane internode borer (Easwaramoorthy *et al.*, 2001).

During field surveys in India, *M.anisopliae* was isolated from the larvae of the teak defoliator, *H.puera*. The fungus exhibited pathogenicity to all larval instars of the teak defoliator in the laboratory (Shamila *et al.*, 2003).

Green muscardine, *M.anisopliae* was noticed in Belgaum, Raichur, Koppal and Dharwad on *Plutella xylostella* on cabbage, *Approerima modicella* on groundnut, *Nilaparavata lugens* on paddy and *Oryctes rhinoceros* in FYM pit, respectively (Rachappa *et al.*, 2007).

2.2 Isolation of entomopathogenic fungi

***N.rileyi* :**

Natural occurrence of entomopathogenic fungi was observed in soils from different surrounding of Darmstadt. A total of 100 soil samples were examined using larvae of *G.mellonella*, and *Tenebrio molitor*. *M.anisopliae*, *B.bassiana*, *P.fumosoroseus* and *P.furinosus* were isolated (Kleespies and Zimmermann, 1989).

There were three fungal species *viz. Aspergillus parasiticus, B.bassiana* and *M.anisopliae var. anisopliae,* Smith *et al.* (1998). Isolated fungus *B.bassiana* sp. was obtained from *Sitophilus seamis* and *Tribolium* sp.

***M.anisopliae* :**

The larvae of *G.mellonella* had been used successfully as bait for insect-parasitic nematodes in soil, could also be used to detect entomopathogenic fungi. Species such as *B.bassiana*, *M.anisopliae* and *P.fumosoroseus* were detected in many soil samples using larvae of the pyralid (Zimmermann, 1986).

Carrion *et al*. (1996) isolated the entomopathogenic fungi from bodies of dead female of leaf cutting ants *Atta maxicana*. Three fungal species were found *viz*., *Aspergillus parasiticus*, *B.bassiana* and *M.anisopliae* var. *anisopliae*.

Deshpande *et al*. (2001) reported that entomopathogenic fungi as mycoinsecticides are useful against Lepidopteran pest in pulses. Under the biopesticide programme of Indo-Swiss Collaboration in Biotechnology (ISCB), NCL, Pune, around 56 different fungal strains have been isolated from the soil by *Galleria* bait method (GBM), plating on selective medium from the infected *Spodoptera* and *Heliothis* larvae.

Hassani *et al*. (2001) isolated more than 40 strains of entomopathogenic hyphomycetes belonging to *M.anisopliae*, *N.rileyi* and *B.bassiana* sp. either from soil samples using bait method or a selective medium or from infected *Spodoptera lituralis* larvae from Wagholi near Pune, India.

The occurrence of entomogenous fungi was determined by means of the insect bait method, using larvae of *G.mellonella*. The insect bait was put in plastic petri plates filled with soil and placed at 17, 20 and 25^{0}C temperatures. The first observation of larval mortality was carried out after 7 days and the next ones every 4 days. Three species of entomogenous dominating fungi were identified on dead insect bait: *M.anisopliae, B.bassiana and P.fumosoroseus*. *M.anisopliae* was the dominating species in the soil from buckwheat cultivation. (Sapieha Waszkiewicz, *et al.*, 2006).

2.3 Classification and Morphology

***N.rileyi* :**

According to Kish *et al.*, (1974) conidiophores of *N.rileyi* bear whorl of branches, each of which bears two or three phialids and thereby dense clusters and formed around the conidiophores.

N.rileyi vegetative hyphae are smooth walled, septate, hyaline to vary slightly pigmented, 2-3 µ diameter. Conidiophores are erect arise in a tight

stand, forming dense compacted clusters of phialids. All these structures are hyaline or pale greenish, phialids are short and rounded, cylindrical to almost globes, with very swollen base tapering abruptly to narrow neck which is very short or even almost lacking. Conidia appears dusty pale leek green and are aseptate, in chains, smooth, hyaline, elliptical to sometime cylindrical 3-4 (4.5) x 2-2.5 (3) µ (Onions, 1974).

Morphological characters of *Nomuraea* as conidiophores with conidiogenous cells in dense, individually distinct whorls, conidiogenous cells are short, blocky, with no distinct neck, conidia, one-celled, in short, divergent chairs and conidial mass light (gray-green) (Samson, 1974).

Entomopathogen *N.rileyi* is a dimorphic hyphomycetes having invasive hyphal stage and yeast like vegetative stage as per Pendland and Boucias (1997). They also reported that conidial germ tube produce hyphal bodies (yeast like cells) in complex mycological medium and only mycelium produces on less complex medium.

Vimaladevi (2000) investigated the morphology of fungus *N.rileyi* and noted that initial growth is by yeast like hyphal bodies produces cream coloured sticky growth with sucet musty odour. The colour of colonies progresses from white to green to malachite green.

M.anisopliae:

Rombach *et al.* (1987) gave the morphological characters of *Metarhizium* as conidioiphores in low mounds, covered by conidia, branched closely or loosely grouped forming a sporulating layer, sporogenous cells borne singly, in pair or in whorls; conidia apical produced in basipetal chains compacted into columns, long- ovoid to cylindrical with rounded ends, celled, olive green in mass.

Multinucleate hyphae were observed on complete medium and binucleate hyphae on potato dextrose agar. Conidia were usually uninucleate and without significant differences in shape and size on either

medium. Better growth of colonies was obtained on complete medium (Ribeiro *et al.*, 1992).

The characteristic conidia form was observed in the strains of *M.anisopliae* var. anisopliae. The growth and colour of colony were different among strains. The conidial mean size was 6.0 mm long (5.2-7.1 mm) and 2.2 mm wide (2.0-2.6 mm). The conidial mean germination varied significantly among the strains, with a 24.0 to 92.3% range and a mean of 49.3% for Scarabaeidae and 83.8% for Curculionidae (Guerrero *et al.*, 1999).

A strain of *Metarhizium sp*. was isolated from the infected *P.brevitarsis* seulensis larvae in Korea. Under the scanning electron microscope, the isolate, *Metarhizium sp*. KMA-1, showed distinct formation of conidia on the palisade-like mass which were comprised of elongate chains and this shape is a typical feature of *Metarhizium* species (Choi *et al.*, 2003).

2.4 Persistivity

***N.rileyi* :**

Conidia survived for 12 months when stored in a glass vial with a porous cloth bag containing silica gel compared to six months in the absence of silica gel (Bell, 1975). Soil is the natural reservoir of conidia that start annual epizootics of *N.rileyi* (Ignoffo *et al.*, 1977).

M.anisopliae No. 32 and *N.rileyi* No. 5 were substantially degraded after 6 months' incubation (70-80% dry weight loss). In contrast, the activity of *M.anisopliae* No. 51 remained at the initial level after 21 months. The persistence of conidiospores depended on the fungal strain (Fargues and Robert, 1985).

Fargues and Robert (1985) reported that conidia of *N.rileyi* on cadavers of *A.gemmatalis* stored in jelly capsules in glass container with silica at -18°C were viable and pathogenic upto to six years of storage (Silva and Loach, 1987). The persistence of conidia applied to soil in a groundnut field along with crushed sorghum substrates was high even at two weeks after application (Vimaladevi, 1995).

***M.anisopliae* :**

Inocula of *M.anisopliae* No. 32, *N.rileyi* No. 5, *P.fumosoroseus* No. 39 and *B.bassiana* No. 18 were substantially degraded after 6 months incubation (70-80% dry weight loss). In contrast, the activity of *M.anisopliae* No. 51 remained at the initial level after 21 months (Fargues and Robert, 1985).

The persistence of *M.anisopliae*, released as a conidial suspension at low (10^9conidia/m^2) and high levels ($6x10^{11}$conidia/m^2), was investigated in lucerne fields located at Snubbekorsgaard, Denmark. In control plots, the natural level of *M.anisopliae* varied between 0 and $3x10^2$cfu per g of surface soil. In treated plots, the level of *M.anisopliae* decreased between 100- and 1000-times during 84 weeks. Investigation of the vertical distribution of *M.anisopliae* revealed no significant differences between the surface and deep-layer soils in control plots. Similarly, in plots treated with a low dose of *M.anisopliae*, the level of cfu was 10^2-10^3 per g of soil for both the surface and the underlying layer (Vestergaard *et al.*, 2000).

Studies were carried at South Andamans, Andaman and Nicobar Islands, India to study the prevalence of *M.anisopliae* on *O.rhinoceros* larvae. The prevalence ranged from 1.70 to 7.80%. Its maximum prevalence was in July, and the least was in March (Prasad *et al.*, 2002).

The spores of *M.anisopliae* are persistent on the soil surface over one week without significant density reduction. In contrast, the persistence of spores on leaves decreased rapidly under controlled as well as under field conditions. Within 24 hours most of the spores on the oilseed rape leaves disappeared or lost viability (Pilz *et al.,* 2005).

2.5 Mode of action

N.rileyi:

N.rileyi germinated and penetrated the cuticle within 6 hrs of treatment. Within 24 hrs, penetration hyphae had reached the cuticular epidermis and,

via a budding process, initiated the hyphal body stage in the haemocoel. The hyphal bodies, suspended in haemolymph, multiplied and spread throughout the host larvae. By 6-7 days after treatment, the majority of larvae were mummified. Within 12 hrs of death numerous conidiophores emerged, producing a confluent mycelial mat over the entire cuticular surface. Numerous hydrophobic conidia were formed on phialides present on the aerial conidiophores (Boucies and Pendland, 1982). El-Sayeed *et al.*, (1993) suggested that both protease and chitinases enzyme may be involved in penetration of the larval integument by germinating conidia of *N.rileyi*.

The mature conidia of *N.rileyi* germinate on the larval integument of *Bombyx mori* within 24 hrs and penetrate the cuticle within 36 hrs after inoculation at 24.0 ^{0}C temperature and 80% relative humidity. The penetrating hyphae multiply by budding and septa formation in the hemocoel, and the larva succumbs to the infection 6–7 days post-treatment. The hyphal bodies elongate and become interwoven with other hyphae forming a mycelial complex across different tissues. The ramification of hyphae along the epidermal tissue results in larval mummification in 7–8 days. Numerous conidiophores emerge, producing a confluent white fungal mat over the entire surface of the host larva by 9–10 days. Pale green conidia develop, making the larval body green. Life cycle of the fungus on *B. mori* is completed in 10–11 days (Vineet Kumar *et al.*, 1997).

The infected larvae of *H.armigera* showed shrunk skin with smooth texture and most of the larvae died in characteristics. *N.rileyi* infected *H.armigera* larval body became slightly bent, tough and mummified by fifth day (Manjula *et al.*, 2003).

Seven concentrations of *N.rileyi* (spores/ml), i.e. $1x10^{8}$ to $1x10^{2}$ suspensions, were used against 30 third instar larvae of *H.armigera* in plastic vials. *N.rileyi* with the infected larvae showed shrunk skin with smooth texture and most of the larvae died in a characteristic way (Snegapriya and Manjula, 2008).

***M.anisopliae* :**

Hystolysis of cuticle by fungal enzymes secreted by *M.anisopliae* play a major role in cuticle penetration. Within the larvae, toxins produced by the fungus incite a progressive degeneration of the tissues, with no appreciable swelling or shrinkage of the cells. A rapid excretion of fluids by the Malpighian tubules, and, to a more limited extent, by the midgut epithelial cells is indicated, which enhances desiccation and mummification (Zacharuk *et al.*, 1973).

Gopalakrishnan and Narayanan (1988) reported that the infected caterpillars of *H.armigera* with *M.anisopliae* were sluggish and ceased to feed on the third day after inoculation. The body became slightly bent, tough and mummified on the fifth day. Initial growth of the fungus was noticed on the seventh day and on the whole body was covered with turf of pure white mycelia growth with green spores covering the entire body of caterpillar.

The properties and mode of action of a biological preparation (BIO 1020) containing the fungus *M.anisopliae* consists of insoluble mycelium granules on which the infectious spores are formed after mixing with soil. Fungal spores remain inactive in the soil and germinate on the cuticle of host insects. The spores penetrate the insect cuticle and the hosts die following systemic infection (Stenzel, 1992).

Entomopathogenic fungus *M.anisopliae* conidia adhered to and germinated readily on the surfaces of live aphids and at sites under the elytra of live beetles. On dorsal elytra and the ventral thorax of live beetles poor adhesion and germination was attributed to fungistasis because no such behaviour was noted on dead beetles. The cuticle also influenced appressorium morphology and the mode of pathogenesis. *M.anisopliae* also penetrated the cuticle directly, i.e. without producing appressoria. Sites under the elytra of live beetles were more vulnerable to infection than sites on the dorsal elytra or ventral thorax resulting in higher mortalities and lower $LT_{50.}$ (Butt *et al.*,1995).

The effect of infection by *M.anisopliae* on the haemocyte number and soluble protein in the haemolymph of the 3rd-4th instar larvae of *D. punctatus* was investigated. It was found that, from 1 to 4 days after infection, the total haemocyte counts and the concentration of haemolymph soluble protein were significantly higher in infected larvae than non-infected ones. The results revealed that *M.anisopliae* affected the metabolism of the host's proteins, and that the peroxidase activities exhibited a decreasing tendency (Song Zhang *et al.*, 2002).

2.6 Toxin production

***N.rileyi* :**

Coudron *et al.*, (1984) reported that the chitinase and exochitinase activity were found low levels in conidia and germinating conidia of the 3 entomopathogenic fungi *B.bassiana*, *M.anisopliae* and *N.rileyi*. A rapid increase in activity occurred during mycelial growth, with maximum activity near the time of sporulation.

The entomogenous fungus *N.rileyi* produces excessive amounts of exocellular slime material that appears to be mainly composed of polysaccharides. The slime comprised exclusively D-glucans, and the polysaccharide have probable role in host infection (Latge *et al.*, 1988).

An insecticidal toxin, a new polypeptide was extracted from mycelia of the entomogenous fungus, *N.rileyi* (a sclerosis pathogen in the silkworm *Bombyx mori*), using an organic reagent (Ye *et al.*, 1992). Onofre *et al.*, (2002) reported that the entomopathogenic fungus *N.rileyi* produced a peptide active against *A.gemmatalis* 3rd instar larvae.

***M.anisopliae* :**

Many entomogenous fungi produce insecticidal toxins in submerged culture and these compounds are source of new toxophores. The most extensively studied toxins are the cyclic depsipeptides from *M.anisopliae*, termed destruxins. *B.bassiana* also produces the cyclic peptides termed beauvericin, beauverolides and bassianolide (Gillespie and Claydon, 1989).

Five strains of *M.anisopliae* from widely divergent isolation sources were cultured in vitro on media containing gelatin, glucose plus nitrate, or purified cuticle from larvae of *G.mellonella.* The highest levels of proteinases and endochitinase were produced in cuticle-grown cultures. Three of 5 strains produced exceptionally high levels of chymoelastase (47 000-98 000 IU/mg protein) on cuticle. The highest levels of N-acetyl glucosaminidase were produced in gelatin-grown cultures. Most strains produced esterase under all growth conditions. The source of insect cuticle did not strongly influence the production of enzymes (Gupta *et al.,* 1991).

Destruxins are hexacyclodepsipeptidic mycotoxins produced by *M.anisopliae*. The insecticidal and cytotoxic effects of 13 natural and hemisynthetic destruxins against larvae of *G.mellonella* were studied. Brominated destruxin was relatively active, displaying particular modalities of cytotoxic effects which reflected a certain originality of its mode of action (Dumas *et al.*, 1994).

Destruxins (DTXs) are cyclic peptide toxins secreted by the entomopathogenic fungus *M.anisopliae* var. anisopliae. The effects of DTX E, the most active compound of this family on haemocytes, the immunocompetent insect cells, and on the dynamics and efficacy of the multicellular defence of insect hosts have been investigated. Ultrastructural alterations have been observed in circulating plasmatocytes and granular haemocytes, and in attached haemocytes of *G.mellonella* larvae treated with a toxic dose of DTX E (LC_{50}) (Vey *et al.*, 2002).

The insecticidal activity of cyclodepsipeptide destruxin on the larval stages of *S.litura* showed an ascending trend in LD_{50} values with increasing age. The value for 12-day-old larvae in the combined application assay was as low as 0.045 µg/gbody weight of crude destruxin from M-19 strain when compared with the corresponding values of 0.17 µg/g body weight in the ingestion assay and 0.237 µg /g body weight in the topical application assay. On the other hand, values were higher in the treatments with crude destruxin from the low-virulence M-10 strain of *M.anisopliae* showing the

least quantities of A and E components of destruxin. (Sowjanya Sree *et al.*, 2008).

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 hrs of treatment with the mycotoxin at a dosage of 0.147 micro g/g 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.

2.7 Formulation of Entomopathogenic fungi

Medium for mass production

N.rileyi:

Sabouraud's Maltose Agar supplemented with one per cent yeast extract (SMAY) was routinely used to multiply *N.rileyi* in culture tubes and Petri dishes (Bell, 1975a).

Polished rice grains were evaluated as culture media for the entomogenous fungus *N.rileyi*. The use of 3 different proportions of liquid and solid did not give significant differences in sporulation. Boiling the rice grains for 1 min before sterilization resulted in more spores being produced. The spores produced on culture media maintained their pathogenicity to larvae of the noctuid *A.gemmatalis* (Silva and Loach, 1987).

M.flavoviride var. *minus* grew well on all the media tested, except when sorbitol was the carbon source. The growth of *H.strigosa* was high on all the media tested and highest on media containing dextrose. Yeast extract was necessary for the mycelial growth of the fungi. All the fungi tested grew well in media with pH 5-8. Sporulation of *M.flavoviride* var. minus was as high on media containing 1-2% yeast extract and on all the carbon sources used. The best carbon source for sporulation of *N.rileyi* was dextrose (Im *et al.*, 1988).

Carrot-malt-agar, oats-malt-agar, tomato-malt-agar, potato- dextrose-agar and Sabouraud-maltose-agar plus yeast extract media were evaluated for 2 isolates of the entomogenous fungus *N.rileyi*. The carrot-malt-agar and oats-malt-agar media were effective for sporulation of *N.rileyi*. A pH of about 7 enhanced the growth of both isolates (Balardin and Loch, 1989).

Maximum of $1.44x10^9$conidia per gram of crushed sorghum was obtained after 8-9 days at 25°C (Vimaladevi, 1994).

The entomogenous fungus *N.rileyi* was grown in solid media made of ground pupae of *B.mori*. The largest production of conidia was obtained on SMAY ($1x10^{12}$conidia/litre of medium). The growth medium containing silkworm pupae and potato (FCBA) was the most economical. The virulence of the fungus on SMAY was greater than on the other growth media (Neves and Alves, 1995).

Kulkarni (1999) has reported $13.45x10^8$ and $13.15x10^8$ conidia per g of crushed sorghum and rice grains, respectively followed by maize and bajra after 10 days.

Carrot emerged as the cheapest source of substrate for mass production of *N.rileyi* to harvest 6.0 g spores/kg at the cost of Rs.1.00/g of spores. Rice was next best with same amount of spore but it did not have comparative advantage in cost (Rs. 2.67/g) (Gopalakrishnan and Mohan, 2000).

Crushed sorghum and rice grains with 1% yeast extract proved as the most favourable food media for the faster as well as higher conidial production over other food grains. LC_{50} value of the fungus multiplied on sorghum to the *S. litura* larvae was lowest ($5.425x10^6$conidia/l) (Kulkarni and Lingappa, 2002a).

Mycelial growth was obtained in SMY containing PP or MP, while conidiation was observed in SMAY containing these. The highest wet weight (22.2 mg) was obtained in medium containing MP, while the lowest in the one with PP. Mycelia and conidia were observed in liquid and solid media

containing BP. The conidia produced on agar and stationary liquid cultures were pathogenic to third instars of *S.litura* (Kumar *et al.*, 2002).

The excellent growth of the fungus *N.rileyi* was obtained with Sabouraud's dextrose agar with yeast (60.17 mm) followed by Saboraud's maltose agar medium with yeast (58.83 mm). Potato dextrose agar and carrot dextrose agar media recorded comparatively lesser growth of 59.50 mm and 53.83 mm, respectively (Manjula and Krishnamurthy, 2005).

Locally available grains, namely sorghum, rice and bajra were used as substrates for mass production of *N.rileyi*. Sporulation was relatively high on sorghum and rice grains ($2.9x10^9$ and $2.4x10^9$ spores per gram, respectively) compared to bajra ($1.2x10^9$ spores per gram) (Lalitha *et al.*, 2008).

Potato maltose agar was significantly superior over all other media for maximum biomass, mycelial growth and conidial production of *N.rileyi*. Among the three isolates studied maximum radial growth, biomass and spore production were observed in PDBC isolates both in solid as well as liquid media (Sonai Rajan and Muthukrishnan, 2008).

Among the six nitrogen sources tested, the maximum radial growth, biomass and spore production of *N.rileyi* was observed in potato peptone agar medium both in solid as well as in liquid. Similar trend of growth and development was observed with Directorate of Oil Seeds Research and local isolates of *N.rileyi* (Sonai Rajan and Muthukrishnan, 2009).

Among the grains, rice supported the maximum ($5.53x10^7$spore/g) spore production of *N.rileyi* followed by refuse raw bananas ($4.2x10^7$spores/g) and sorghum ($4.01x10^7$spores/g) on the 11^{th} day after inoculation of spore suspension; whereas, wheat ($3.55x10^7$spores/g) and refuse potato chips ($3.1x10^7$spores/g) supported less spore load than other substrates on the 11^{th} day and on the 15^{th} day after inoculation of spore suspension respectively (Mamta Thakre *et al.*, 2011).

***M.anisopliae* :**

The entomogenous fungi, *M.anisopliae* was cultured in liquid culture media containing various commercial peptone sources to determine the effect of these on growth and sporulation. Tryptone, Casitone and yeast extract were effective for mycelial growth and sporulation of *M.anisopliae*, and yeast extract was the most effective in the production of spores. (Barnes *et al.*, 1975).Bean broth was the best of 4 liquid culture media tested for producing spores of the entomopathogenic fungus *Metarhizium anisopliae* (Bastos *et al.*,1987).

Czapeck's Dox medium was the most suitable, supporting the highest radial growth, sporulation and biomass production *of M.anisopliae*. The optimum temperature for growth was 25°C. Among various cereal grains tested as growth media, rice was the most suitable substrate, and among waste materials tested, puffed rice waste gave the best results (Patel *et al.*, 1990).

The liquid fermentation of *M.anisopliae* for dry mycelium production was investigated. Several culture media based on sucrose, dextrose, yeast-water and yeast extract were tested in agitation cultures (150 r.p.m.). The best medium composition contained sucrose (4%) and yeast extract (1%). Dry mass and pH varied according to the initial concn of conidia. The highest dry mass was obtained at 10^8conidia/ml per flask. The most significant change in pH was detected at high concn of conidia (>10^7 conidia/ml). In the fermenter, a dry mass yield of 9.05 g/litre was observed at time 72 hrs of culture, with a 0.13 g/litre per hrs productivity rate and 69% level of sugar consumption. (Magalhaes *et al.*, 1994).

M.anisopliae was cultured in liquid culture media containing various amino acids, vitamins, KNO3 and (NH4)2SO4 to determine their effect on growth and sporulation. The results showed that various amino acids and vitamins had significant effects on growth and sporulation of submerged conidia (Song Zhange *et al.*, 2000).

Sabouraud's dextrose agar+yeast (SDA+Y) medium was superior, resulting in the highest radial growth (4.07 cm) of *M.anisopliae* followed by Emerson YPSS medium (4.01cm), at 10 days after inoculation (DAI). The highest spore count ($9.43x10^6$spores/ml) of fungal suspension was observed in Barner's medium, followed by Emerson YPSS medium ($8.29x10^6$) and SDA+Y medium ($7.16x10^6$), at 10 DAI (Kulat *et al.*, 2002).

M.anisopliae produced the maximum sporulation (3.50x10/ml) on molasses yeast broth, while Sabouraud's medium was the best for sporulation in Ma1, M2 and M3 isolates. Richards broth medium supported excellent growth for all the eight isolates but proved poor for sporulation. Among the agar media, molasses yeast, and Sabouraud's media were suitable for sporulation in all the three entomopathogens. All the grain media supported good sporulation, however, bajra, sorghum and maize grains for *M. anisopliae* (Shashi Sharma *et al.*, 2002).

Wadyalkar *et al.* (2003) conducted an experiment on mass multiplication and formulation (10^8, 10^7, 10^6, 10^5 and 10^4 concentration) of *M.anisopliae* in different media and different temperature (20, 25, 27 and 30^0C). Among several media tested (Potato dextrose agar, Sabourad›s dextrose agar + yeast, yeast phosphate soluble starch agar, (zapek's medium and Barners medium), yeast phosphate soluble starch agar the best for all temperatures for germination, growth and sporulation of *M.anisopliae.*

Sabouraud's Dextrose with yeast extract and sorghum-based medium were the best among synthetic and grain-based culture media gave significantly higher biomass and conidial counts of entomogenous fungi *M.anisopliae* (Purwar and Sachan, 2006).

Maximum sporulation was observed on crushed bajra + 1% yeast extract ($22.77x10^8$ spores/g of substrate) and rice bran + 10% molasses ($33.24x10^4$ spores/g of substrate). In general, sporulation increased with increasing incubation time up to 20 days. Also, addition of molasses increased the sporulation of the entomopathogen (Bharati *et al.*, 2007).

M.anisopliae conidial sporulation on wheat, barley, sorghum, rice or bajra grains was studied. The fungus sporulated on all substrates at 21 days after inoculation. Conidial sporulation reached $2.17x10^8$ g on wheat, $1.68x10^8$ g on barley, $1.78x10^8$ g on bajra, $4.22x10^8$ g on rice, and $3.97x10^8$ g on sorghum. Although conidial production was greatest on rice grains, difficulties in the extraction of conidia from this substrate were observed (Mahesh kumar *et al.*, 2007).

Mass production of *M.anisopliae* was carried out in the laboratory by using liquid media. The spore count, radial growth, sporulation and biomass were maximum when *M.anisopliae* were cultured both czapeck dox agar and potato dextrose agar media. Hence, these two media can be used for successful mass production of fungus, *M.anisopliae* (Soundarapandian and Chandra, 2007).

Screening locally available substrates for mass multiplication of *M.anisopliae* such as, broken rice grains, broken maize, broken jowar ,broken wheat, and broken ragi grains, showed that, broken rice followed by broken jowar served as the most productive media for conidial production of the fungus, with a yield of $3.45x10^8$ and $3.2x10^8$ spores per ml, respectively (Babu *et al.*, 2008).

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 III instar larvae of *S. litura* with LC_{50} value of 1.72×10^6 conidia/ml 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.

M.anisopliae was cultured on seed media: white rice, unhusked rice grain, rice bran chaff, broken-milled rice, maize and sorghum. The result

showed that white rice was the best media which the fungi could produce many spore, i.e. 1.41x105 spores/ml (Suasa-ard *et al.*, 2008).

Various liquid growth media *viz* sabouraud dextrose, sabouraud sucrose, sabouraud maltose supplemented with yeast extract, potato dextrose and coconut water. Among the media, the conidia harvested from sabouraud maltose yeast extract broth (SMYB), potato dextrose broth and coconut water were found to be pathogenic to all the larval instars of *C.partellus*. However, blastospores derived from none of the media did cause mortality (Namasivayam and Kumar, 2009).

Conidia of the entomopathogenic fungi *M.anisopliae* (SBT # 27 and SBT # 28) were produced under standard condition and then examined for influences on vitro conidial germination speed and virulence to an insect host, *H.armigera.* Conidia were most virulent (based on mortality at 6 d) and had the fastest germination rates when produced on SMB (Sabouraud maltose broth) and the media pH is 6.0 - 6.2. The second instar larvae were exposed to the fungus and the concentration is of 1x 10^7 and 1 x10^8 conidia/ml were tested against host insects at 28^0C, SBT # 27 isolates showing 98 - 100% mortality in 8 days against *H.armigera* and SBT # 28 showing 90 - 92% in 8 days. Among the two isolates SBT # 27 is superior in terms of high percent kill as well as 100% germination of conidia within 48 hours. However, SBT # 27 isolate showing greater pathogenicity against insect pest (Vijayavani *et al.*, 2010)

Formulation procedures

N.rileyi:

Fermentation process is the standard method employed for the production of microorganisms in general and entomopathogens in specific. Conidia of the *N.rileyi* could be readily produced on surface of solid or semi solid media under aerated conditions (Bell, 1975; El Sayeed *et al.*, 1991; Lingappa and Patil, 2002). Submerged fermentation with liquid media was

tried by many workers. Blastospores obtained from submerged fermentation were not infective to larvae of *H. zea* (Bell, 1975).

M.anisopliae:

M.anisopliae is used as potential entomopathogen. The culture medium for its multiplication is rice grains mixed with water; it is placed in 1-litre bottle capped with aluminium foil. A hypodermic syringe is used to inoculate the medium through the foil, the hole so made being sealed to avoid unwanted contamination. Two weeks after inoculation, the fungus and the medium are removed from the bottle, drained in a sieve and placed in plastic sacks that are stored at a low temperature until the fungus is required for use. It has recently been found that the fungus can be cultured satisfactorily in the sacks themselves (provided these are of polypropylene) and the sacks and their contents autoclaved thus simplifying the procedure (Aquino *et al.*, 1977).

A simple and inexpensive method for culturing entomopathogenic fungi using pans, bran, Cellophane and autoclave bags is described. With this method, large quantities of fungus can be obtained free from substrate contamination. Conidial yields of 10^{10}/g dry biomass were obtained for *Tolypocladium cylindrosporum*, *Verticillium lecanii* and *Beauveria bassiana*, 10^9 for *Metarhizium anisopliae*, and 10^8 for *Culicinomyces clavosporus* (Goettel, 1984).

Molasses yeast broth was selected as a synthetic medium for mass production for pathogens which produced $8x10^8$ and $1x10^9$conidia/ml in the slurry of *M.anisopliae* and *B.bassiana*, respectively. Amongst grain media, crushed maize grain for *M.anisopliae* and whole cowpea grain for *Beauveria spp*. were employed for mass multiplication using 2 kg high density polypropylene bags each containing 100 g of grains moistened with 60 ml of distilled water. *M.anisopliae* and *B.bassiana* at their respective temperature yielded a grain spore dust of $2x10^9$, and $1.5x10^9$conidia/g dry grain weight. Fungal slurry and dried grain spore mass, after blending in electric mixer for 30 s was incorporated in sterilized talcum powder (carrier) in the proportion

of 1:2 to 1:5 depending on density of spores in order to achieve 4-5x10^8conidia/g in formulation (Shashi Sharma *et al.*, 1998).

Effect of adjuvants on potency of formulations

Oils as adjuvants

N.rileyi:

The coconut oil formulation of *B.bassiana* was proved to be effective against cocoa weevil adults *Pantorhytes plutus* (Oberth) by recording lower LD_{50} of 1.18x10^3 conidia per ml, when inoculated on the mouth parts as compared to water + 0.01% Tween–80 which showed LD_{50} of 4.29x10^4 conidia per ml at the end of 5th day (Prior *et al.*, 1988).

Vegetable oils at 0.5 per cent concentrations (v/v) were emulsified in 0.02 per cent Tween 80 solution containing 2x10^{10}conidia/100 ml of spray solution. These preparations were applied after one hour to *S.litura* larvae on castor leaves and allowed to feed for 48 h. Cumulative larval mortality nine days after treatment due to fungus indicated the similarity between treatments with vegetable oils (Safflower, groundnut, sunflower, rapeseed, mustard, sesame, cotton seed and coconut oil) indicating the safety of these vegetable oils to the fungus (Vimaladevi and Prasad, 1996).

Out of two formulations like conidia of *B.bassiana* in mineral oil suspension, conidia in water suspension, the aqueous formulation was efficient like as the oil formulation in cotton ecosystem against cotton boll weevil (Silva and Silva, 2001). *B.bassiana* in corn oil formulation @ 1×10^8conidia per ml against larvae of *Diabrotica speciosa* (Germal) (Coleoptera: Chrysomelidae) showed 65 per cent mortality (Consolo *et al.*, 2003).

The groundnut oil formulation of *B.bassiana* has recorded 100 per cent mortality of adults of *Bemisia tabaci* Gen. (Manjula *et al.*, 2003a), which was followed by coconut oil (97.8%), sunflower (85.6%) and castor oil formulation (64.4%) at 72 h after inoculation.

Twelve different oil based formulations of *N.rileyi* were evaluated for conidial germination on the day of formulation and two weeks after formulation at two environmental conditions of 4^0C and 30^0C. Most of the oil formulations are resulted in >62 per cent germination after two weeks of storage at 4^0C, where in case of 30^0C only peanut oil and castor oil are found to have germination upto 30 per cent remaining all oils were recorded the germination ranging from 0 to 18 per cent (Wiwat, 2004).

Formulations of *N.rileyi* with sunflower oil (2%) and Tween 80 (0.02%) resulted in maximum cumulative mortality of *S.litura* (95.00%) followed by talc based wettable powder (83.10%) and unformulated crude formulation (77.00%) under laboratory conditions (Nagaraja, 2005).

Among nine vegetable oils and seven WP formulations of *N.rileyi* studied conidia of *N.rileyi*. The viability of conidia after one year of storage was 22.21 per cent in refrigerated condition, while it was only 15.64 per cent at ambient room temperature. Rice flour, talc and sorghum flour emerged as the best among carrier materials evaluated, while skimmed milk powder and gram flour appeared to be non-suitable (Ramegowda, 2005).

Oil based formulations recorded lower LC_{50} and LT_{50} values compared to WP formulations of eight WP formulations crude WP had registered lowest LC_{50} value of $80.09x10^3$conidia per ml followed by talc based WP and rice flour. Among oil formulations, safflower oil ($1.42x10^4$conidia/ml) recorded lowest LC_{50} values followed by groundnut and sunflower oils. Among the WP formulations crude formulation recorded 82 per cent mortality followed by talc and rice flour. Among oil formulations groundnut oil registered highest of 96 per cent followed by sunflower and safflower oils (Ramegowda, 2005).

The infectivity of *N.rileyi* in oil, wettable powder or crude formulation ($2x10^8$conidia/ml) to third-instar larvae of *S.litura* and *H.armigera* was evaluated under laboratory conditions. The level of mortality of the larvae of *S.litura* and *H.armigera* increased as the number of days after treatment increased. At 10 DAS, the highest rates of mortality of *S.litura* and

H.armigera were obtained with the oil formulation (95.0 and 93.20%, respectively), followed by the wettable formulation (83.1 and 87.2%) and crude formulation (77.0% for each) (Nagaraja *et al.*, 2006).

***M.anisopliae* :**

Ramoska (1984) reported that *M.anisopliae and B.bassiana conidia* in oil have provided much evidence of infection at low humidities and penetration at intersegmental membrane of chinch bug (*Blissus leucopterus*).

A study of the effect of neem oil on conidia germination and mycelium sporulation of the entomopathogenic fungus *M.anisopliae*, which is being evaluated for the possible control of the rice pests *Nilaparvata lugens* and *Scotinophara coarctata*, showed that the highest concentration used (95% in a liquid medium with 1% saccharose [sucrose] and 1% yeast extract) completely inhibited both processes. The effects of the lower concentrations (5 and 50%) were not so severe, but even at 5% neem oil had a significant inhibitory effect. It is recommended that if neem oil is used in a control programme with *M.anisopliae*, the two should be applied separately (Aguda *et al.*, 1986).

Prior *et al.* (1988) reported that laboratory studies primarily with grasshopper have shown that oil formulation of aerial conidia of *M.anisopliae* are more efficacious than aqueous formulation under various temperature and moisture conditions. Combination of oil and powdered formulations stimulated mycelia growth.

The corn starch formulation of *M.anisopliae* and *B.bassiana* stored for 13 weeks at 4^0C has recorded 100 per cent mortality of *Diabrotica undecimpunctata* Howardi Barber, which is followed by corn starch oil formulation which recorded 100 and 93 per cent mortality in *M.anisopliae* and *B. bassiana*, respectively. Whereas, the fungal formulations stored at 22^0C has showed mortality up to 92 per cent in corn starch oil formulation by *M.anisopliae* and 84 per cent by *B.bassiana* (Pereira and Roberts, 1991).

The laboratory assay on desert locust (*Schistocerca gregaria*) (Forsk) formulation of *M.flavoviridiae* Gamsand and Rozsypal, conidia in cotton seed oil showed superior performance by recording lowest LD_{50} value of 8900 conidia per insect at 5 days, where it is $>10^6$ conidia per insect for water based suspension and this was especially pronounced at low humidity *i.e.,* at 35 per cent (Bateman *et al.*, 1993).

The entomopathogenic fungus *B.bassiana* is potential fungus for pest management. The sunflower and groundnut oil accelerate the spore germination as well as growth. Some combinations showed additive effect. Conidial germination rate decreased with increase in concentration of oil formulation. The mycelia weight increased with increase in concentration with oil. Oil formulation promotes conidial germination as well as mycelia growth. The water component will help in germination and oil will help in retension of humidity as well as adhesion. Formulations of fungal pathogen in oils increased there efficacy and speed of kill compared to that with water based formulation (Kaur *et al.*, 1999).

Kaaya and Hassan (2000) reported that *B.bassiana* and *M.anisopliae* oil formulation (10^9conidia/ml) induced 100 per cent mortality in larvae of *Rhipicephalus appendiarlatus* Neum and *Amblyomma variegates* (F).

Alves *et al.* (2001) evaluated the effects of different formulations the viability of *M.anisopliae* conidia. The viability of conidia mixed with eight emulsifiable adjuvant oils (EAO), seven wetter/spreaders, three vegetable oils and four mineral oils was evaluated 24 hrs and 48 hrs after spreading over SDA medium surface. The effects of different formulations on medium term storage (40 weeks) of *M.anisopliae* were evaluated at 10^0C and 27^0C. Five EAO, one vegetable oil, a mixture of mineral oils and pure dry conidia were tested. The oil formulation did not cause any negative effect on conidial germination.

M.anisopliae isolate FI-1248 was the most virulent isolate in both water and oil suspensions with LC_{50} values of $6.4x10^5$ and

$3.4x10^4$conidia/ml, respectively. *M.anisopliae* isolate FI-0985 was found to be the least virulent (Cannard *et al.*, 2002).

The efficacy of *M.anisopliae* against *Trialeurodes vaporariorum* and *Bemisia tabaci* without additives was about 50%. At 1/20 of their recommended dosages, all compounds tested significantly increased the efficacy of *M.anisopliae* for the control of *T.vaporariorum*, with the formulated sunflower oil Biola giving the highest synergistic effect, reaching nearly 100% control. Not only was the level of control increased but also the speed of action was improved, resulting in a higher reliability of control (Malsam *et al.*, 2002).

The conidia of the entomopathogenic fungus *M.anisopliae* were formulated as (water-in-oil formulation) with a coconut/soybean oil provided the most stable emulsion layer (93%) and lowest viscosity (27 cps). The fungal conidia remained viable in the formulation for 30.8 months with a 50% reduction (half-life) in conidial viability after 4.6 months at 20±1 °C. While dry non-formulated conidia became non-viable after 2 months, with a half-life of 0.5 month. Under laboratory $5x10^6$conidia/ml killed (66.70 to 100%) nymphs of the tobacco whitefly, *Bemisia tabaci*, within 3 days of treatment, with cadavers mycosis within 4-5 days under laboratory conditions. The mortality was 30.0% to 92.2% on eggplants in field (Batt, 2003).

A lipase from a Brazilian strain of *M.anisopliae* was investigated. The effect of different lipid sources added to basal medium was verified to improve enzyme production. Biomass was highest for sunflower oil (21.88 mg/ml), however lipase specific activity was highest for olive oil (108.38 U/mg). In the presence of surfactants, the highest lipase activity occurred when SDS and Tween 80 were added after 50 hrs *M.anisopliae* growth. SDS (4.54+or-0.46 U/ml) and Tween 80 (4.15+or-1.13 U/ml) were the best surfactants. The best temperature for lipase production was 32 ^{0}C. This is the first report investigating lipase production by an entomopathogenic fungus (Silva *et al.*, 2005).

Four isolates in each of *B.bassiana*, *M.anisopliae* and *V.lecanii* were tested for their pathogenicity to the sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner in oil emulsion formulations under field conditions at Arabhavi, Karnataka. Mycosis was observed with six isolates *viz., B.bassiana*, Bb4 (10%), Bb5a (19.8%), Bb6 (8.3%) and *M. anisopliae*, Ma2 (4.7%), Ma3 (16.2%) and Ma4 (42.3%). Pathogenicity was confirmed by re-isolation of respective fungal isolates from the mycosed aphids. None of the *V. lecanii* isolates showed mycosis on *C.lanigera*. In the laboratory bioassay studies, *M.anisopliae* (Ma4) and *B.bassiana* (Bb5a) were found pathogenic to the predator of sugarcane woolly aphid, *Dipha aphidivora* causing 29.3 and 10.4 per cent mycosis. *M.anisopliae* (Ma4) isolate was also found pathogenic to another predator of SWA, *Micromus sp*. causing 29.14 per cent mycosis (Nirmala *et al.*, 2007).

The oil formulations were more effective than the water formulations. The most potent treatment at 15 ^{0}C was the oil suspension of isolate of *M.anisopliae* IMI098376 (LC_{50}=4.47x10^4 conidia/ml) with an LT_{50} at the lowest concentration (1.4x10^4 conidia/ml) of 13.3 weeks and earliest mortality at highest and lowest concentration occurring in 3 and 4 weeks, respectively (Makaka, 2008).

Nutrients as adjuvants

***N.rileyi* :**

Casein at a concentration of 0.04% and trehalose at 0.28% promoted the growth of the fungus, *N.rileyi,* however, the ascorbic acid levels tested had no significant effect on mycelial development. In vivo utilisation of available complex proteins, trehalose and other nutrients may govern the parasitic nature of the pathogen (Sutton *et al.*, 1981).

The growth of *M.anisopliae* and *N.rileyi* fungi in 100 ml of basal salts containing 1% (w/v) alkanes or N-acetylglucosamine was assessed. After a lag period of 2-24 days, both fungi grew in media containing n-alkanes (C >8) as the sole carbon source, although their ability to use n-alkanes varied.

N.rileyi could exploit a wider selection of n-alkanes than *M.anisopliae* (Leger *et al.*, 1998).

Glucose was found to be the best carbon source and maize steep powder was found to be the best nitrogenous source for *N.rileyi*. The maximum point of cell production when the medium contained 3.2% glucose and 0.5% maize steep powder and dry weight of cell was 12.1g/l (Lin Ming Shen *et al.*, 2003).

Wiwat (2004) reported that the sugars like maltose (control), D-glucose, lactose, sucrose were evaluated for conidial germination and conidial production of *N.rileyi*. Among these, all the four sugar sources influenced the conidial germination uniformly upto 95 per cent. Where in case of conidial production lactose is found superior with 1.80×10^9conidia per ml over other sugars which given conidial production ranging from 1.1 to 1.3×10^9conidia per ml.

***M.anisopliae* :**

Pre-gelatinized corn starch was more suitable than sodium alginate for the formulations with mycelia of *B.bassiana* and *M.anisopliae* (Marques *et al.*,1999).

Maltose and peptone were the best carbon and nitrogen sources for the production of destruxins from *M.anisopliae*. With the addition of 0.1% (w/v) beta-alanine to the basal medium, the yields of cyclodepsipeptides DA and DB were 7.2 and 279 mg/litre, respectively, which was 2-fold higher than that of the control experiment (Liu Binglan, *et al.*, 2000).

Molybdenum was the most important while zinc was stimulatory. The production of submerged conidia was highest upon treatment with boron + copper + iron + manganese + molybdenum + zinc. Vitamin B6+VH and compound vitamin B were more effective for producing submerged conidia of *M.anisopliae* than other treatments (Song Zhange and Song, 2001).

Iskandarov *et al.*(2006) reported the effects of nutrient medium composition and temperature on the germination of conidia of the fungi

B.bassiana and *M.anisopliae*. It was demonstrated that the presence of carbohydrates to germinate where as the germination of *B.bassiana* spores was inhibited. The optimum temperature for spore germination was 20-35^{o}C in both fungal species.

Three substrates; rice, barley and sorghum at variable pH, moisture content and yeast extract concentrations. These three factors were found to be important, affecting *M.anisopliae* spore production. Moisture content of 75.68% for sorghum, 73.21% for barley and 22.34% for rice produced optimal results. Maximal conidial yield was recorded for rice at a pH of 7.01; at 7.06 for sorghum and at 6.76 for barley (Prakash, *et al.*, 2008).

Chemicals as adjuvants

N.rileyi:

B.bassiana conidia dispersed better in oil (Mycotech 9209) than in 0.05 aqueous Tween-80, while substantial clumping occurred in 5 per cent emulsion of the oil in water, even after homogenization (Inglis *et al.*, 1993).

Drying of conidia along with the substrate over silica gel or calcium chloride failed to cause any mortality. Whereas, drying conidia in an air stream in a laminar flow chamber resulted in significant mortality comparable to freshly harvested conidia without drying (63-87% mortality). The conidia formulated with sunflower oil (90%) and Triton-X-100 (83.9% mortality) or Tween-80 (65.7% mortality) performed equivalent to unformulated conidia (70.2% mortality) against *S.litura* (Vimaladevi *et al.*, 2002).

The laboratory evaluation of *N.rileyi* formulations *viz.,* oil formulation (sunflower oil + Tween-80 (0.02%), wettable powder (talc) and crude formulation at 2 x10^{8} conidia per ml concentration against third instar larvae of *S.litura*. At the end of 10^{th} day the cumulative mortality was 95.00 per cent in oil formulation followed by wettable powder and crude formulation by recording 83.10 and 77.00 per cent, respectively (Nagaraja, 2005).

***M.anisopliae* :**

Tryptophan and alanine were most effective for growth and sporulation of *B.bassiana*, although glutamine and potassium nitrate also produced large numbers of regularly shaped spores. Tryptophan, glutamic acid and histidine were all well-utilised for both growth and sporulation of *M.anisopliae*. Nitrogen sources containing sulfur were poorly utilised for sporulation by *M.anisopliae*. Both fungi attained nearly the same growth maximum on asparagine medium (Campbell *et al.*, 1978).

B.bassiana grew best on melezitose but sporulated best on sucrose, trehalose and D-glucose. *M.anisopliae* grew best on D-mannose but sporulated best on i-inositol and glycerol (Campbell *et al.*, 1983).

The time required to cause 100% mortality in first-instar larvae of *Holotrichia consanguinea* by *M.anisopliae* and *B.brongniartii* was shortest at 25°C. Adding carboxymethyl cellulose to the *Metarhizium* formulation reduced the time to 100% mortality by more than a week (Shashi Sharma *et al.*, 1998).

Culture media influenced the germination of conidia, appressorial development and mycelial growth of *M.anisopliae*. Although the addition of KCl to Sabouraud dextrose agar (SDAM) significantly reduced germination of the conidia of three isolates in *vitro* and lowered germination and appressorial development on the cuticles of *Myzus persicae*, conidia grown on SDAM and minimal medium (MM) were more aggressive than conidia derived from SDA or yeast extract agar (Ibrahim *et al.*,2002).

In maize oil, the viability of conidia was > 90% for the first month, but was < 85% in the following months. The viability of conidia in neem oil and glycerin, in both types of flask, was < 85% from the beginning, with 0% in the first month of evaluation (Rodriguez Colorado *et al., 2*002).

Others

N.rileyi:

Zhang *et al.* (1992) tested the wettable powder formulation of *B. bassiana* @ 50x10^9spores per g against *Ostrinia furnacalii* Gn. under laboratory conditions the results showed 95 per cent mortality.

Two formulations of *B.bassiana* isolate IMI 330194, oil palm kernel cake based formulations of conidia (OPKC) and conidial powder (CP) against adult banana weevils showed that both formulations resulted in the same level of weevil mortality of 75.5 per cent compared with only 1 per cent mortality in the control (Godonou *et al.*, 2000).

Orozco Santo *et al.* (2000) reported that commercial formulation *i.e.,* wettable powder of *B.bassiana,* "mycotrol" increased the mortality of whitefly nymphs and adults when compared to control.

Ramarethinam *et al.* (2000) evaluated commercial wettable powder formulation of "Priority" of *P.fumosoresus* (wize) Brown and Smith against red spider mite, *Oligonychus coffea* in tea under laboratory conditions. The mortality of *O. coffeae* ranged from 58.2 to 64.83 per cent on 10th day after spraying and 75.68 to 95.68 on the 15th day after spraying.

Sood *et al.* (2001) evaluated commercial wettable powder formulation of *B. bassiana* (Daman) against third instar larvae of *P. xylostella* @ 2 x10^7 cfu per ml showed it was more effective in bringing mortality.

2.8 Pathogenicity and *Invitro* evaluation of formulation

2.8.1 *Spodoptera* sp.

***N.rileyi* :**

A dense aqueous spore suspension of *N.rileyi* caused 100 per cent mortality in seven days old larvae of *S.litura* (Rao and Phadke, 1977). Mortality of *S.litura* caused by crude formulation of *N.rileyi* at the concentration 1.2×10^8spores per ml ranged from 80 to 100 per cent within 5

to 11 days (Anonymous,1989). Trial on crude form of *N.rileyi* at 1.2×10^8conidia per ml against the third instar larvae of *S.litura* caused 95 per cent mortality in 6 days after treatment in cabbage (Anonymous, 1990).

The susceptibility of third instar *S. frugifera* larvae to fungal infection were similar whether larvae were reared on *Brassica sativa* (Linn.) cotton, faba beans or aubergins. There was no significant difference in either LD_{50} (0.3×10^3– 0.7×10^3) conidia per cm^2 or LT_{50} (4.0–4.3 days) at 3×10^4conidia per cm^2 (Fargues and Maniania, 1992)

Mortality of larvae were ranged from 50.7 to 76.6 per cent between 1 to 4th instar and 32.1 per cent in fifth instar larvae of *Spodopter exigua* (Hub.) when treated with *N.rileyi* (Goh *et al.*, 1992). Strain No. 3 of *N. rileyi* killed 100% of 1st- to 5th-instar larvae and 77% of 6th-instar larvae. Fungi were considered to have potential as biological control agents (Lezama *et al.*, 1993).

N.rileyi was cultured on crushed sorghum together with a 1% yeast extract. A maximum of $1.4x10^9$conidia/g was yielded after 8-9 days at 25°C. The efficacy of the fungus in the control of *S.litura* on *Ricinis communis* var. VP.1 was then evaluated in the laboratory, net house and in the field. In the laboratory, application of 10^8, 10^9, 10^{10} and 10^{11} spores/litre of spray solution to 1st-instar larvae resulted in 100% mortality within 5 days. LD_{50} values at 6-8 days after application for 2nd-instar larvae were 2.23×10^{11}, 2.89×10^{10} and 2.20×10^{10}, resp. The LD_{50} for 3rd-instar larvae was 2.89×10^{10} at 8 days. In the net house studies, initial mortality was observed at 7-8 days, with an LD_{50} of $2.2x10^{10}$spores/litre. Larval mortality was initially observed in the field at 9 days after spraying with *N.rileyi* conidia. Even the lowest dose of $2x10^{11}$spores/litre resulted in a significant cumulative larval mortality. (Vimladevi, 1994).

Soybean plants coated uniformly with spore suspension containing 10^7 spores per ml were fed by larvae of *S.litura* under caged condition. Mortality started after three days and at eight days it was 59 per cent (Ambethgar and Loganathan, 1998).

Two strains of *N.rileyi*, isolated from *S.depravata* (F889) and *A.gemmatalis* (F815) in Japan were inoculated into the larvae of *S.litura*. During the time from infection to host death, larvae were reared on artificial diet. Five concentrations of conidia suspension were tested (10^7, 10^6, 10^5, 10^4, 10^3 conidia/ml). The results showed LC_{50} values were 5.38 (F889) and 5.71 (F815). Mortalities at a concentration of $1x10^7$ conidia/ml were 89.5 (F889) and 84.2% (F815) (Zhang *et al.*, 1999).

Early instars of *S.litura* are highly susceptible to *N.rileyi* with a mortality of 96.25 per cent by spraying the aqueous spore suspension of the fungus ($1.26x10^6$conidia/ml). As the larval stage advanced, the mortality also decreased significantly. Lowest mortality of 20 per cent was recorded in fifth instar larvae. The LT_{50} values for 1^{st} to 5^{th} instars were 130.71, 137.77, 148.04, 235.55 and 263.10 hrs, respectively (Patil, 2000).

The virulence of *B.bassiana*, *M.anisopliae* and *N.rileyi* isolates against the second instar of *S.litura* was investigated 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 x 10^5 conidia/ml, respectively (Dayakar and Kanujia, 2001).

Pathogenicity of *N.rileyi* to important lepidopterous pests in laboratory indicated that the cumulative mortality of larvae increased with the increase in concentration and exposure period. Treatments comprised: $1.2x10^8$, $1.2x10^7$, $1.2x10^6$, $1.2x10^5$ and $1.2x10^4$ *N.rileyi* conidia/litre on *Spodoptera litura*, *Mythimna separata*, *Earias vittella*, *Achaea janata*, *Cydia ptychora* and *Galleria mellonella* larvae. The fungus was pathogenic to all six test insects with different degrees of pathogenicity. At the highest concentration ($1.2x10^8$ conidia/litre), it caused 86.50% mortality of *S. litura*. (Kulkarni and Lingappa, 2002b).

The entomopathogenic fungus *N.rileyi* in sunflower oil formulation along with Triton-X-100 was showed 83.9 per cent mortality of 7 to 8 days

old *S. litura* larvae, whereas, sunflower oil + Tween-80 (0.02%) has recorded only 65.7 per cent mortality under laboratory conditions. The fungus in oil in water emulsion spray has recorded 65.9 per cent mortality in post-rainy season groundnut and 62.8 per cent mortality in rainy season castor against *S.litura* (Vimaladevi *et al.*, 2002).

Conidial suspensions (10^4-10^{10} conidia/ml) of the entomopathogenic fungi *B.bassiana*, *M.anisopliae* and *N.rileyi* were topically applied to second-fourth instar larvae of *S.litura* infesting tobacco to determine the pathogenicity of the fungi to the pest. The median lethal concentrations (LC_{50}) of *B.basiana*, *M.anisopliae* and *N.rileyi* against the second instar larvae were 6.43, 13.97, 11.33x10^5conidia/ml, whereas the median lethal time (LT_{50}) was 4.34, 5.33 and 4.85 days, respectively. The LC_{50} values for the third and fourth instar larvae of the three fungi ranged from 14.85 to 71.69x10^5conidia/ml, whereas the LT_{50} values recorded for the third and fourth instar larvae ranged from 5.13 to 6.45 days. The results of the bioassays indicated that the susceptibility of the pest decreased with age of the larvae in terms of both LC_{50} and LD_{50} (Dayakar and Kanujia, 2003).

In the laboratory, *N.rileyi* at 2x10^6spores/ml was highly virulent, resulting in approximately 97.5, 93.33, 80.0 and 100.0% mortality of *D.orichalcea*, *S.litura*, *Spilosoma obliqua* and *H.armigera*, respectively (Ingle *et al.*, 2004).

Wiwat (2004) evaluated the pathogenicity of *N.rileyi* against *S.litura* under laboratory condition. Among the WP formulations evaluated *N.rileyi* conidia along with bentonite and sucrose powder (1:7:7), aluminium silicate (1:18), bentonite soil (1:7:7) and bentonite (1:18) recorded lower LC_{50} values of 168, 311, 416 and 586 conidia per larva, where fresh conidia recorded LC_{50} of 797 conidia per larva.

The first instar larvae of *S.litura* were found more susceptible to *N.rileyi* compared to fifth instar larvae which recorded no death for aqueous spore suspension with 1×10^9spores per ml of *N.rileyi*, where 91.2 per cent

mortality recorded in first instar larvae. As the concentration decreases the mortality was also found decreased (Manjula and Krishnamurthy, 2005a).

The laboratory evaluation of *N.rileyi* formulations *viz.,* oil formulation (sunflower oil+Tween-80 (0.02%), wettable powder (talc) and crude formulation at 2×10^8conidia per ml concentration against third instar larvae of *S.litura*. At the end of 10^{th} day, the cumulative mortality was 95.00 per cent in oil formulation followed by wettable powder and crude formulation by recording 83.10 and 77.00 per cent, respectively (Nagaraja, 2005).

Oil based formulations recorded lower LC_{50} and LT_{50} values compared to WP formulations (Ramegowda, 2005) of eight WP formulations crude WP had registered lowest LC_{50} value of 80.09×10^3conidia per ml followed by talc based WP and rice flour. Among oil formulations, safflower oil (1.42×10^4conidia/ml) recorded lowest LC_{50} values followed by groundnut and sunflower oils. The cumulative per cent mortality after nine days was relatively higher with oil formulations compared to wettable powder formulations. Among the WP formulations crude formulation recorded 82 per cent mortality followed by talc and rice flour. Among oil formulations groundnut oil registered highest of 96 per cent followed by sunflower oil and safflower oil (Ramegowda, 2005).

Sonai Rajan and Muthukrishnan (2009) carried out pathogenicity assay of some isolate of entomopathogenic fungus, *N.rileyi* against different instars of *S.litura* (F) under in *vitro* conditions. Bioassays on *S.litura* with *N.rileyi* isolates revealed that, PDBC isolate was most virulent against *S.litura* and also had lower LC_{50} and LT_{50} values than DOR and local isolates of *N.rileyi.*

Fungi, *N.rileyi* was highly compatible with oils and caused mortalities near 100% in all oil treatments; the lowest LT_{50} values were 4.7 days in mineral oil. The second set included additional fungal strains and oil formulations (mineral, canola, sunflower, olive and peanut oils) tested against larvae of *S.exigua, S.frugiperda, H.zea* and *H.virescens*. The highest

activity was that of *N.rileyi* in oil against *Spodoptera* spp., with LT_{50} values of 2.5 days. (Paulina Vega-Aquino *et al.*, 2010).

M.anisopliae:

The virulence of *B.bassiana*, *M.anisopliae* and *N.rileyi* isolates against the second instar of *S.litura* was investigated 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 x 10^5 conidia/ml, respectively (Dayakar and Kanujia, 2001).

The number of days taken for mummification to occur in treated 2^{nd} instar larvae was shorter than that obtained with the 4^{th} instar larvae. In general, the mortality of *S.littoralis* larvae increased with increasing dosage of the pathogen. The LT_{50} values recorded for *M.anisopliae* against the 2^{nd} instar larvae of *S.littoralis* were 8.11, 8.74, 8.81 and 9.06 days at concentrations of $5.7x10^8$, $5.7x10^7$, $5.7x10^6$ and $5.7x10^5$conidia/ml, respectively (Bekheit and Abbas, 2002).

Dayakar and Kanaujia (2003) reported that the conidial suspensions (10^4x10^{10} conidia/ml) of the entomopathogenic fungi. *B.bassiana, M.anisopliae* and *N.rileyi* were topically applied to second-fourth instar larvae of *S.litura* infecting tobacco to determine the pathogenicity of fungi to the pest. The median lethal concentrations (LC_{50}) of *B.bassiana, M.anisopliae*, and *N.rileyi* against second instar were 6.43, 13.97, $11.33x10^5$conidia/ml.

Pandey (2003) investigated pathogenicity of *M.anisopliae* in eggs and pupae of the lepidopteran pests, *S.litura*, *Spilosoma obliqua [Spilarctia obliqua]* and *H.armigera*. An aqueous suspension at $5×10^6$ and $5×10^7$conidia/ml sprayed on eggs and pupae of the insects. *M.anisopliae* at the lower rate resulted in egg mortality values of 32.3-40%. *M.anisopliae* caused mean egg mortalities of 49.5, 44.0 and 38.1%, and pupal mortalities of 41.8, 40.8 and 22.5%, on *S. litura*, *Spilosoma obliqua* and *H.armigera*, respectively.

Pandey and Kanaujia (2003) studied the pathogenicity of two isolates of *M.anisopliae*, cultured on SDA medium supplemented with and without larval extract, using *S. litura* as test insect. The results indicated that the virulence of both isolates increased when cultures were grown on SDA medium supplemented with the larval extract. The LC_{50} values of Pantnagar isolate and MUCL 8237, grown on SDA medium supplemented with larval extract, were 1.08 $\times 10^6$,1.42 $\times 10^6$, 1.73$\times 10^6$ conidia/ml and 6.42$\times 10^6$, 2.65 $\times 10^6$, 7.58$\times 10^6$ conidia/ml against II,III and IV instar larvae, respectively, showing higher virulence of the former in terms of LC_{50} and LT_{50} values against *S.litura*.

Pandey and Kanujia (2004) conducted experiment on pathogenicity test of entomopathogenic fungi *viz.*, *B.bassiana*, *M.anisopliae* against *S.litura*, at 20^0 and 30^0C temperature. The LC_{50} values of *B.bassiana* at 20 and 30^0C were 4.2 and 15.1x10^6 conidia/ml, 6.2 and 24.3x10^6 conidia/ml and 8.5 and 25.8x10^6 conidia/ml against second, third and forth instar larvae, respectively.

Purwar and Sachan (2005) studied the differential toxicity of two isolates of entomogenous fungi viz. *B.bassiana* and *M.anisopliae* against tobacco caterpillar, *S.litura* and *Spilarctia obliqua* and indicated that the activity decreased with advancement of age of larvae. On the basis of LC_{50} and LT_{50} values, Pantnagar isolates of both fungi had more potency to kill *S.litura* and *S.oblique* than IMTECH, Chandigarh Strain.

Hu *et al.* (2007) reported bioactivities of destruxins (dtx), depsipeptides isolated from *M.anisopliae* against *S.litura*. For contact toxicities dtx-E was more effective than dtx-A and dtx-B. The LC_{50} values of dtx-A, B and E were 197.98, 292.00 and 113.99/mg at 48h after treatment, while the LT_{50} were 42.65, 59.45 and 23.68 h at 300 mg/l.

Amer *et al.* (2008) observed that the conidiophores of *M.anisopliae* and *M.flavoviridae* have the most effective isolates. *M.anisopliae* gave the highest

mortality (60 and 55%) to the II and IV instar larvae of *S.litura* with lethal time (LT_{50}) 7 and 10 days, respectively.

Pandey Renu and Hasan Wajid (2009) studied efficacy of entomogenous fungus *M.anisopliae* in controlling *S.litura* by bioassay test. The LC_{50} of *M.anisopliae* isolate MTCC 4101 were 4.44×10^4, 4.45×10^6 and 1.62×10^8conidia/ml for 4-5, 10-11 and 15-16 days old larvae of *S.litura* respectively, while in case of MTCC 4103 isolate the corresponding figures were 1.95×10^5, 6.71×10^6 and 6.9×10^8conidia/ml, respectively. The LT_{50} values that were calculated at highest concentration of the test fungus were 101.16, 116.51 and 149.75 h for 4-5, 10-11 and 15-16 days old larvae of *S.litura* in MTCC 4101 isolate, while 99.27, 119.53 and 201.03 h for MTCC 4103 isolate respectively. MTCC 4101 was comparatively more virulent than MTCC 4103.

The pupae of *S.litura*, (Lepidoptera: Noctuidae), a polyphagous pest affecting common crops in Indian subcontinent, were treated with different concentrations of conidia. Suspensions (10^8/ml) of conidia harvested from Sabouraud dextrose agar yeast extract (SDAY) plates resulted in the highest mortality (85.8%) with *M.anisopliae* (Rajesh Anand *et al.*, 2009).

Bioassays were conducted with MUCL 38502 strain of *B.basiana* and MUCL 8237 of *M.anisopliae* cultured on SDA and SDA supplemented with host insect larval extract against third instar larvae of *S.litura.* However, in both the fungi the biomass, linear growth, conidial count and viability of the conidia were increased with the supplementation of larval extract. The LC_{50} and LT_{50} values of MUCL 38502 grown on SDA medium and medium supplemented with larval extract were 14.85 and 9.65 $x10^5$ conidia/ml and 123.02 and 113.95 h at $5x\ 10^7$ conidia/ ml respectively. The LC_{50} and LT_{50} values recorded with MUCL 8237 were 45.23 and 21.32 $x10^5$ conidia ml-1 and 138.72 and 130.93 h at $5x10^7$ conidia/ml.The virulence of both the fungal isolates increased when grown on SDA medium supplemented with larval extract (Dayakar and Subbarao, 2011).

2.8.2 Other lepidopteran pest

***N.rileyi* :**

Gopalakrishna and Narayanan (1988) reported cent per cent mortality of third instar larvae of *H.armigera* due to infection of *N.rileyi* spores in aqueous suspension in five to eight days.

In laboratory tests, spraying with a spore suspension of *N.rileyi* at $8x10^6$/ml gave 60-77% mortality of 1st- to 2nd-instar larvae after 106-120 h, and 73.3% mortality of 4th-instar larvae after 154 h. The LC_{50} of *N.rileyi* was $3.27x10^6$/ml spore suspension for 1st-instar larvae, $3.12x10^6$ for 2nd-instar larvae, and $5.02x10^6$ for 4th-instar larvae. At the same spore concentration, the LT_{50} of older larvae was greater than that of younger larvae. In field trials, infection of *H. armigera* larvae was 22.6%, 6 days after spraying a spore suspension of *N.rileyi* (Lu YongYue *et al.*, 1998).

The entomopathogenic fungus *N.rileyi* caused 90.5-100% mortality of 4th-instar larvae of *H.armigera* when applied at 10^7conidia/ml to maize silks, and leaves of soyabean, tomato and chrysanthemum. The LT_{50} was 5.9-6.7 days. The 5th-instar larvae showed a mortality of 94.6% on soil with 20% water content, and 41.7% on soil with 10% water content, when the soil surface was sprayed with 10^8 conidia/ml suspension (Tang and Hou,1998).

Five isolates of *M.anisopliae* and one isolate of *N.rileyi* were bioassayed against larvae of *Plutella xylostella*. Larvae were treated by exposing cabbage leaf discs previously immersed in conidial suspensions, and were then incubated at 25°C and observed daily. The *M.anisopliae* isolate FI1248, was the most virulent against *P. xylostella*. The LC_{50} was estimated as 2.03×10^4 conidia/ml and the LT_{50} was 4.97 days at 10^7 conidia/ml. Isolate FI63 (*N. rileyi*) showed virulence to *P. xylostella* in both tests, but cadavers did not sporulate (Ma, 2000).

Gundannavar (2001) reported that the aqueous extract of *N.rileyi* found pathogenic to all stages (I-V instars) of *H. armigera* and recorded LC_{50}

values, 1.07×10^6, 1.64×10^7, 1.86×10^8 and 3.42×10^9 conidia/ml for I, II, III and IV instar larvae, respectively.

Cumulative mortality of larvae of *H.armigera* increased with increase in concentration of spores of *N.rileyi* and exposure period (Gundannavar, 2001). Mortality of all larval instars (I-V instars) of *H.armigera* due to *N. rileyi* in aqueous spore suspension at the highest concentration (10^8 conidial/ml) was marginal on fifth day after treatment. Cumulative mortality steadily increased to reach maximum (47.5–100%) within 10 days. The pathogen was found more virulent against early instars compared to later ones.

Three isolates each of *B.bassiana* (B.b-4, B.b-5 and B.b-7), *M.anisopliae* (M.a-2, M.a-3 and M.a-4), *Verticillium lecanii* (V.l-1, V.l-2 and V.l-3) and *N.rileyi* (N.r-1, N.r-3 and N/r-4) were tested against *H.armigera* and *S.litura* under laboratory conditions. *N.rileyi* isolates showed the maximum mean mortality for *H.armigera* (54.44%) and *S. litura* (76.66%). Among the 3 *N.rileyi* isolates, N.r-3 caused the highest mortality for both insects (Ramanujam *et al.*, 2003).

The spore suspension of *N.rileyi* @ 1×10^9 spores per ml was sprayed against different instars of larvae of *H.armigera*, where second instar larvae were found more susceptible as they showed 95.0 per cent mortality rather than first instar larvae which showed 81.2 per cent mortality, while fourth instar larvae recorded least of 36.2 per cent mortality. The mortality was found decreasing as the concentration decreased (Manjula and Krishnamurthy, 2005a).

Nagaraja (2005) reported that the oil formulation of *N.rileyi* @ 2×10^8 spores per ml exerted considerable pathogenicity of 26.2 per cent mortality to the third instar larvae of *H.armigera* at 3 DAT. The cumulative mortality steadily increased to reach 93.20 per cent on the 10th day after treatment.

Gundannavar *et al.* (2008) studied the dose mortality response between different instars of *H.armigera* and *N.rileyi* indicated that fungus

performed better at its higher concentration (10^8conidia/ml) compared to lower concentration *viz*., 10^7,10^5,10^3 and 10^2conidia/ml. Early instars were more susceptible to fungus.

M.anisopliae:

Larvae of *G.mellonella* were dipped in conidial suspensions of *M.anisopliae* prepared from isolates collected in Recife (Brazil) and Yaritagua (Venezuela), mortality reached 14 and 97%, respectively, after 10 days (Valdes, 1974).

Gopalakrishnan and Narayanan (1987) conducted preliminary pathogenicity test by spraying spore suspension of *M.anisopliae* at 1.8x10^9spore/ml against *H.armigera* which revealed high susceptibility of *H.armigera* to this fungus recording 80-100% mortality of all the five instar tested.

In pathogenicity tests in the laboratory, *M.anisopliae* var. minor caused 100% mortality of 1st 4th instar larvae of *H.armigera* and 80% mortality of 5th-instar larvae. Exposure of 3rd-instar larvae to *Nomuraea rileyi* resulted in 100% mortality (Gopalakrishnan and Narayanan, 1988).

Saxena *et al.* (1990) evaluated virulence of *B.bassiana, M.anisopliae* and *N.rileyi*. Among these *B. bassiana* was the most virulent to *H.armigera* causing mortality between 65 to 100 per cent within four days after treatment. *M.anisopliae* and *N.rileyi* showed 50 to 70 per cent and 40 to 60 per cent mortality to the pest,respectively in seven days after the treatments.

Pandit and Samanta (1995) tested the efficacy of two entomogenous fungi *B.bassiana* and *M.anisopliae* against the larvae of *Spilosoma obliqua* (Walker) and reported 74-78 % and 75-91 % larval mortality of the pest, respectively.

Some responses were observed in the eggs and larvae of *E. insulana* under different concentrations of *M.anisopliae*. The fungus proved virulent against the two stages of the insect. Treatment of the eggs appeared more

susceptible to fungi than larvae. The treated eggs showed a highly reduced rate of hatchability and about 100% of the larvae from treated eggs failed to develop to adults and died. LC_{50} for eggs was $1.5x10^3$spores/ml, and for larvae, this was $3x10^3$spores/ml. *M.anisopliae* may be considered as a biological control agent against the *E.insulana* (Aly and Rashad, 1997).

In laboratory bioassays, *M.anisopliae* var. major of spore concentration of $40x10^8$ spores/ml caused 92 and 90% mortality of 3 to 5 day old and 5 to 8 day old cabbage semilooper larvae, respectively, within 7 days after treatment. Susceptibility of 10- to 12-day-old semilooper larvae was very low as the mortality caused by the spore concentration of 40 x 10^8spores/ml was only 8% (Wickramatileke *et al.*, 2000).

Aqueous spore suspension of *M.anisopliae* (at $1.2x10^7$spores/ml) of the fungus cultured on potato dextrose agar media was prepared and pathogenicity test was conducted by spraying suspension on 50 third instar larvae. The fungus proved pathogenic to *O.arenosella* and caused 80-90% mycosis of larvae after 4 days of treatment (Rachappa *et al.,* 2001).

Varhade (2001) studied the pathogenicity of *M.anisopliae* using different concentration ranging from $2.26x10^9$ to $2.26x10^6$ spore/ml of fungal suspension against 2nd instar larval of *H.armigera* and reported 95% larval mortality in 2.26x 10^9 spore/ml concentrations at 8th day after treatment.

Wadyalkar (2001) evaluated the pathogenicity of *M.anisopliae* using different concentrations from 10^4to10^8 spore/ml of fungal suspension against 2nd instar larval mortality in 10^8 spore/ml concentration at 8th day after treatment.

M.anisopliae were found to be highly pathogenic to eggs of *Maruca vitrata*, achieving 89-100% mortality (Ekesi *et al.*, 2002).

Wadyalkar *et al.*,(2003) conducted experiment of *M.anisopliae.* It is a potential microbial insecticide against *H.armigera* a potential threat to successful cultivation of many econoinically important crops. Pathogenicity

study conduced with 10^8 spores per ml concentration of fungal suspension revealed larval mortality of 100.00, 90.00, 76.67 and 56.67 per cent against I, II, III and IV instar larvae of *H.armigera*, respecitively.

All the isolates of *M.anisopliae* caused DBM larval mortality ranging from 70 to 96%. *M.anisopliae* was further evaluated at concentrations of 10^5, 10^6, 10^7 and 10^8conidia/ml against second instar DBM. These isolates at concentrations higher than 10^6conidia/ml caused DBM larval mortality ranging from 58 to 96%. The mean lethal time (LT_{50}) for DBM second instar were 0.7 and 5.8 days for *M.anisopliae* (Silva *et al.*, 2003).

Nahar *et al.* (2008) studied effect of repeated conidial sub-culturing of *M.anisopliae* on its virulence against *H.armigera* (Hubner).The LT_{50} observed against III instar larvae of *H.armigera* for the first sub-culture was 3.4 days; it increased to 4.5 and 5.6 days for the 20th and the 40th sub-cultures, respectively. The LT_{50} values after passage of the 40th sub-culture on *H. armigera* decreased to 4.4 and 3.7 days for the 40th (first in *vivo*) and the 40th (fifth in vivo) passages, respectively. Similarly, the LC_{50} of *M. anisopliae* towards III instar larvae of *H. armigera* increased from the first sub-culture (0.17×10^4) to (3.0×10^4) for the 40th conidial transfers on potato dextrose agar and again decreased to 0.74×10^4 and 0.23×10^4 in the 40th (first in vivo) and the 40th (fifth in *vivo*) passage, respectively.

2.8.3 Pests other than lepidopteran

M.anisopliae:

The highest *T.neocaledonicus* mortality in okra was recorded in the treatment consisting of $1.5x10^{14}$ spores/ha+0.03% dicofol (Hanchinal and Manjunatha, 2000).

Virulence of *M.anisopliae* against mustard aphid, *Lipaphis erysimi* was tested in the laboratory. Five days post inoculation the highest and lowest mortality was 98.33 and 76.66%, respectively, while 20% mortality was recorded in control (Pandey and Kanujia, 2004).

B.bassiana and *M.anisopliae* caused 100% mortality of aphids *Aphis gossypii* and *Myzus persicae* at the seventh day after inoculation, for both species (Loureiro and Moino, 2006).

M.anisopliae var. *acridum* were able to infect adults of *Maconellicoccus hirsutus* within 2 days after inoculation and showed 90% mortality by 8th day, whereas, 100% mortality of instars was observed by 4th day of inoculation when females were inoculated. *M.anisopliae* strain Ma1912 reduced the egg hatching of *M.hirsutus* by up to 60% (Ujjan and Saleem-Shahzad, 2007).

2.9 Evaluation of various mycoinsecticides formulations in field

Wettable powder formulation

***N.rileyi* :**

Ignoffo *et al.* (1977) evaluated entomopathogens *viz.,* bacterium (*Bacillius thuringiensis* Berliner), fungus (*N.rileyi*) and virus (*Baculovirus heliothis*) for control of *Heliothis zea* on soybean. Among different concentrations of *N.rileyi*, the treatment of 100 g per 0.4 ha was most effective and reduced the larval population upto 77 per cent.

Information on formulations of *N.rileyi* is scanty. Field applications of the fungus have generally been as foliar sprays of conidia, soil application of conidia along with substrate, dusting of dry formulations and field distribution of diseased cadavers. There is a solitary effort of field application with dust formulation of *N.rileyi.* Seven weekly applications of dust formulations with pyrophyllite (5.6 x 10^{13} conidia in 9.1 kg of pyrophyllite / 0.4 ha) gave significant control of *T. ni.*(Ignoffo, 1981).

B.bassiana was tested under field conditions to control *H.armigera* infesting chickpea for two crop seasons and was found very effective. At a spore concentration of 2.68×10^{7} spores per ml, the average pod damage was 6.8 per cent and yield of 2377 kg per ha. where untreated control recorded 16.3 per cent pod damage with an yield of 1344 kg per ha (Hem Saxena and Ahmad, 1997).

Murphy *et al.* (1998) reported that Botanigard a commercial wettable powder formulation of *B. bassiana* applied @ 0.5 and 1.0 lb per acre against sweet potato whitefly showed that treatment @ 0.5 lb per acre was sufficient to maintain aleyrodid populations below damaging levels.

The greater reduction (5.1 larvae/plant and 5.9 larvae/plant) of *P. xylostella* (L.) of 4th instar larval populations were obtained from wettable powder treatments of *B.bassiana* at higher rate ($5.0x10^{13}$ spores/ha) and from single application of emulsifiable suspension, respectively. Two applications of wettable powder at higher rate ($5.0 x10^{13}$ spores/ha) resulted in lower larval counts of 4.3 larvae per plant (Vandenberg *et al.*, 1998).

Ota *et al.* (1999) evaluated two formulations of *B.bassiana* as a emulsifiable suspension formulation (ES) and wettable powder against whitefly on tomato the results showed that *B.bassiana* both the formulations are found useful for the management of whiteflies attacking tomatoes.

Nankinga and Moore (2000) reported that application of *B.bassiana* maize flour formulated wettable powder at the rate of $2x10^6$ conidia per ha proved most effective in reducing the weevil population by 65.72 per cent within 8 weeks after a single application.

“Biopower”, talc based commercial formulation having *B.bassiana* as active ingredient was evaluated against diamond back moth, *P.xylostella* on cauliflower in greenhouse condition. All the dosage has shown a significantly higher efficacy. A mortality rate ranging between 47 to 92 per cent was recorded (Ramarethinam *et al.*, 2002).

Percent reduction of *H.armigera* larvae increased with longer exposure to the biopesticides. Pod damage decreased while crop yield increased with increasing rates of biopesticides. Among the biopesticides, NPV recorded the highest grain yield (8.25 q/ha), followed by *N.rileyi* (7.44 q/ha) and *M.anisopliae* (7.42 q/ha), while *M.anisopliae* recorded the lowest pod damage (18.06%), followed by *N.rileyi* (18.64%) and NPV (20.07%) (Kulkarni *et al.*, 2005).

All treatments were significantly superior to the untreated control which recorded the highest larval load of 3.02 larvae per plant. The percentage of pod damage increased with increasing larval population. All biorational treatments, which were statistically at par with each other, decreased the percentage of pod damage compared with the untreated control. Mean seed yield was highest (21.45 q/ha) in the recommended plant protection schedule, but it was at par with the other biorational treatments except GCK. *N.rileyi* at 1g/litre recorded the highest net return (Rs.27 936/ha) and benefit:cost ratio (4.69), followed by *M.anisopliae* at 1.0 g/litre and *N.rileyi* at 2.0g/litre. Hence, *N.rileyi* can be a good alternative for chickpea pod borer control when the crop is grown under irrigation conditions (Sreeniwas *et al.*, 2006).

M.anisopliae:

Dry mycelium, applied at rates equivalent to 700, 3500 and 7000 g/ha, and a suspension of conidia of the entomogenous fungus *M.anisopliae*, ($2.5x10^2$ conidia/ha), were evaluated for the control of *Nilaparvata lugens* on rice in the field gave significant control of the delphacid (Rombach *et al.*, 1986).

A 75 minute exposure to an inert dust containg 1 billion spores/g of *M. anisopliae* var. anisopliae caused 100% mortality of *A. atkinsoni* after 96 h (Vyas *et al.*, 1993).

M.anisopliae ($1x10^{14}$ or $5x10^{13}$), *B.bassiana* ($1x10^{14}$ or $5x10^{13}$) or chlorpyrifos (200 or 400g/ha) alone or in combination. *M.anisopliae* formulation applied at $5x10^{13}$conidia/ha along with chlorpyrifos 20 EC at 200 g a.i./ha was effective in controlling the grub population (56.5%) of white grub infesting potato exhibiting the maximum reduction in plant mortality (75-80%) and tuber damage (63.7%), which resulted in the highest tuber yield (155 q/ha) (Bhagat *et al.*,2003).

Field studies were conducted in Uttar Pradesh, India, during 2003-04 and 2004-05, to evaluate the efficacy of biological insecticides *B.bassiana*,

M.anisopliae, cow urine, cow dung and laboratory wash against the aphid, *Myzus persicae*, infesting potatoes. *B.bassiana* at 5 g/litre with water appeared to be comparatively more effective against the insect, followed by *M.anisopliae* (Shakti khajuria *et al.*, 2007).

Aqueous formulations

N.rileyi:

The field evaluation of entomopathogenic fungus *N.rileyi* was carried out on corn ear worm, *H.armigera* (Tang and Hou, 2002). The fungus caused 95 to 100 per cent mortality in fourth instar larvae, when applied at 10^7 conidia per ml. The fifth instar larvae showed a mortality of 94.6 per cent on soil with 20 per cent water content and 41.7 per cent on 10 per cent water content, when soil surface was sprayed with 10^8 conidia per ml suspension.

Spray of *N. rileyi* at both the doses, *viz.*, $1x10^8$ and $2x10^8$ conidia/l , were found to be effective in checking the larval population. The highest yield of 21.14 q per ha was obtained in *N. rileyi* spray at $2x10^8$ conidia/l and was on par with *N.rileyi* spray at $1x10^8$ conidia/l. The maximum cost-benefit ratio of 1:36.45 was obtained in *N.rileyi* spray at $1x10^8$ conidia/l followed by *N.rileyi* spray at $2x10^8$ conidia/l with a CB ratio of 1:29.44 (Shekharappa and Patil, 2008).

N.rileyi is a potential entomopathogenic fungus against Lepidopteran pests. It was formulated as wettable powder and oil based formulaltions to increase its efficiency in field by using different carrier materials and oils and these were evaluated in laboratory against two important noctuid pests, *H.armigera* and *S.litura*. Among the wettable formulations of *N.rileyi, viz.*, bentonite + glucose (7:1), talc + glucose (7:1), bentonite + sucrose (7:1) and talc + sucrose (7:1) recorded 87.0, 74.0, 72.0, 83.0 and 75.0 per cent mortality in *S.litura* and 79.0, 70.0, 66.0 and 88.0 per cent in *H.armigera*, respectively. The oil based formulations (tank mix) with pongamia oil, sunflower oil, seasame oil and ground nut oil recorded 74.0, 90.0, 83.0 and 87.0 per cent mortality in

S.litura and 73.0, 89.0, 87.0 and 87.0 per cent in *H.armigera*, respectively (Mallikarjuna, *et al.*, 2010).

M.anisopliae:

Okra plants inoculated with spider mites (*T. neocaledonicus*) were treated with *M. anisopliae* (0.75 x 10^8, 1.5 x 10^8 and 3 x 10^8 spores/ha) combined with dicofol (0.03 and 0.015%). Highest per cent mortality of spider mite and incidence of *M.anisopliae* were observed in 3 x 10^8 spores/ha+0.03% dicofol (Hanchinal and Manjunath, 2000).

In the field experiments where aqueous suspensions of *M.anisopliae* var. major dried powder of dead infected semilooper larvae were used, spore concentrations of 9.3x10^9, 8.4x10^{11} and 8.8x10^{13}spores/ml caused a cumulative mortality of 65% of 3 to 5 day old semilooper larvae. The considerably high mortality of cabbage semilooper larvae under field conditions indicates the suitability of *M.anisopliae* var. major as an effective biological control agent to be incorporated in cabbage semilooper management systems (Wickramatileke *et al.*, 2000).

Pathogenicity, entomopathogenic fungus *M.anisopliae* has been recorded for the first time on rice leaf folder, *Cnaphalocrocis medinalis*. Effective control of the pest was recorded under field conditions after application of spore suspension of *Metarhizium anisopliae* in gelatin (1%) at 1x10^8 spores/ml on the infested rice crop. Between 5 to 7 days after treatment, 60-70 per cent mortality was recorded (Padmaja and Kaur, 2001).

The application of *M.anisopliae* at a dilution rate of 1:20 gave a control efficiency of 77.48% against diamondback moth (*Plutella xylostella*) and 77.73% against common cabbage worm (*Pieris rapae*) on kohlrabi and 53.98% against tobacco budworm (*H.armigera*) on tomato, higher than those obtained by applying *M.anisopliae* at a dilution rate of 1:3000 (Zhao *et al.*,2001).

Per cent reduction of *H.armigera* larvae increased with longer exposure to the biopesticides. Pod damage decreased while crop yield increased with increasing rates of biopesticides. Among the biopesticides, NPV recorded the highest grain yield (8.25 q/ha), followed by *N.rileyi* (7.44 q/ha) and *M.anisopliae* (7.42 q/ha), while *M.anisopliae* recorded the lowest pod damage (18.06%), followed by *N.rileyi* (18.64%) and NPV (20.07%) (Kulkarni *et al,* 2005).

The lowest bollworm infestation in squares and flowers, green bolls, sheded materials, open bolls and loculi was recorded with endosulfan 0.07% followed by Bt 1000 ml/ha, *Ha*NPV 500 LE/ ha and *M.anisopliae* at 10 conidia/ml in that order. In these superior treatments, bollworm infestation in squares ranged from 15.35 to 17.86%, in green bolls from 14.82 to 17.72, in sheded material from 15.05 to 20.01, in open bolls from 17.85 to 21.34 and in loculi from 16.98 to 19.62%. The lowest percentage of bad seed cotton was recorded in endosulfan (13.96%) followed by *Ha*NPV 500 LE/ha (15.50%) and *M.anisopliae* at 10^{10} conidia/ml. However, in terms of seed cotton yield, *M.anisopliae* at 10^{10} conidia/ml proved to be the best treatment, with the highest seed cotton yield of 1185 kg/ha. The highest cost: benefit ratio of 1:14.31 was registered in the treatment of *M.anisopliae* at 10^{10} conidia ml followed by *B.bassiana* at 10^{10} conidia/ml (1:9.46) (Gadage *et al.*, 2009).

Chavan and Kadam (2010) studied liquid formulations A (VGTA50512) and B (VGTA502105) of the entomopathogenic fungus, *Verticillium lecanii* (Zimmermann) Viegas were significantly superior to untreated control recording 74.22 to 92.22 per cent mortality of grape mealy bug (*Maconellicoccus hirsutus* Green) at 14 days after treatment. *V.lecanii* at 1.00 per cent concentration of the liquid formulation gave the highest mortality of 90.50 per cent (Formulation A) and 92.22 (Formulation B).

Oil based formulations

***N.rileyi* :**

Vegetable oils synergize the pathogen. Higher efficacy of oil based formulation might be due to prevention of the desiccation of the conidia which helped in longer survival period and better penetration of peg into the integument. (Burges, 1998).

Field application of formulation containing conidia, sunflower oil and emulsifier Triton X-100 at a field concentration of $2x10^{11}$conidia/l @ 500l/ha resulted in 44.3 and 65.9 per cent mortality of *S. litura* on groundnut during rainy and post rainy seasons, respectively and 62.8 per cent mortality on castor during rainy season. (Vimaladevi *et al.*, 2002).

The effectiveness of oil based formulations of conidia of fungal isolate *N.rileyi* N812 was studied to evaluate potential to control *H.armigera* (Diesel: Sunflower oil 7:3) of *N.rileyi* conidia was found to be most effective in controlling *H. armigera*. The per cent efficacy was 61 per cent pod damage was 15.48 and yield was 12.62 per ha for *N.rileyi* treated plots (Nahar *et al.*, 2003).

The oil formulation of *M.anisopliae*, M 34412, *B. bassiana* B 3301 and *N.rileyi* N 3.12 are evaluated in field condition in pigeonpea against *H.armigera*. After two sprays, per cent efficacy from various treatments range from 55.51 to 70.93. The treatment with *M.anisopliae* M 3442 was found to be effective with maximum efficacy of 70.93 per cent, *N.rileyi* N 3.12 with 62.95 per cent. The per cent pod damage in *M.anisopliae* M 34412, *N. rileyi* 812 was 8.76 and 10.24, respectively (Nahar *et al.*, 2004).

Formulations like oil based (sunflower oil), wettable powder (talc) and crude formulation of *N.rileyi* were evaluated against *H. armigera* under chickpea ecosystem. The results clearly indicated that significantly higher per cent mycosis (26.56%) was recorded in oil formulation of *N.rileyi* @ 2×10^{11}conidia per ha which reflected on least pod damage (26.43%) which inturn resulted in getting higher pod yield (9.97 q/ha) as compared to other two formulations and control (Nagaraja, 2005).

Spray application of sunflower oil formulation with high volume sprayer in groundnut against *S. litura* resulted in higher mycosis (47.43%) and least leaflet damage (24.67%) and pod yield next only to chemical treatment. Similar results were obtained in chickpea ecosystem also. (Nagaraja, 2005).

According to Siddegowda *et al.* (2005), leaf area fed was least (44.69%) in sunflower oil based formulation compared to gingili oil, paraffin oil, palm oil and castor oil. The larval mortality was higher in sunflower oil formulation both in laboratory (89.90%) and cage (76.60%) studies.

Results showed that larval reduction increased in duration after spraying of oil formulation of *N.rileyi* under field condition. Two sprays of DC-Tron, soybean and sunflower oil formulation were found very effective in reducing larval population, pod damage and increase in grain yield of chickpea. However, endosulfan 35 EC 0.06 per cent treated plot recorded minimum pod damage and higher grain yield than the fungus formulation treatments. On the basis of ICBR, endosulfan ranked first having 1:21.14 ICBR followed by soybean oil (1:18.40), DC-Tron oil (1:16.57) and sunflower oil formulation (1:16.38) (Ingale, *et al.*, 2009)

Investigation was carried out on field evaluation of formulations of *N.rileyi* and spray equipments against *H.armigera* in chickpea. Among different formulations of *N.rileyi* and spray equipments, oil based (tank mix) formulation sprayed with high volume sprayer (42.96%) was found significantly superior in causing higher mycosis. However, high volume sprayer with RPP recorded significantly lowest per cent pod damage (22.83%) and significantly higher yield (11.31 q/ha) followed by high volume sprayer with oil based formulation (9.97 q/ha). High volume sprayer with RPP was also the most profitable with the highest net returns of Rs. 14,096 and B:C ratio of 3.5 followed by high volume sprayer with oil based formulation (net returns of Rs. 11,952 and B:C ratio of 2.9). High volume application of *N.rileyi* could be incorporated ss one of the components of managing *H.armigera* in chickpea (Nagaraj *et al.*, 2011).

M.anisopliae:

The oil formulation of *M.anisopliae*, M 34412, *B.bassiana* B 3301 and *N.rileyi* N 3.12 are evaluated in field condition in pigeonpea against *H.armigera*. After two sprays, per cent efficacy from various treatments range from 55.51 to 70.93. The treatment with *M.anisopliae* M 3442 was found to be effective with maximum efficacy of 70.93 per cent, *N.rileyi* N 3.12 with 62.95 per cent. The per cent pod damage in *M. anisopliae* M 34412, *N.rileyi* 812 was 8.76 and 10.24, respectively. The higher yield was obtained in treatment with *M.anisopliae* (M34412) (14.04 q/ ha) as compared to control (7.31q/ha) (Nahar *et al.*, 2004).

Oil formulations have been found to be more effective against target pests even under low RH and also possess a good shelf life (Rabindra and Ramanujam, 2007).

Aerial conidia of four isolates of *B.bassiana* (Bb734 and Bb2860) and *M.anisoplae* (Ma456 and Ma759) produced on rice were formulated with an emulsifiable oil and sprayed in block-randomized triple plots (6x8 m each) of two irrigated cotton fields (Trials 1 and 2) for control of summer populations of cotton spider mites, mainly *Tetranychus truncates* and *T. turkestani*. The spider mites were significantly controlled by all the fungal sprays despite some variation among the candidates. Pure sprays of Ma456 and Bb734 resulted in desirable control for 35 days in Trial 1 (sprayed twice at 15-day interval) (Shi WeiBing *et al.*, 2008).

Other formulations

N.rileyi:

The mortality of *S. litura* due to *N.rileyi* was 36.9 per cent in groundnut during 1991-92 at Bapatla in the coastal region of Andhra Pradesh (Sridhar and Devaprasad, 1996 & 1996a).

Four sprays of *N.rileyi* @ 1.2 x 10^{12} conidia/l reduced *H.armigera* larval load by 34.55 per cent after 14 days of spray. However, this was next to *Ha*NPV and *Bt* treatments (Kulkarni, 1999) in cotton.

Soil application of conidia along with crushed sorghum substrate exhibited greater promise for the control of *S.litura* on groundnut with higher mycosis and mortality (Vimaladevi, 1995 and Patil, 2000).

Treatment effect due to *N.rileyi* became visible at 7 days after spraying (DAS) and its superiority was more evident at 14 DAS. In soybean, the pathogen inflicted significantly higher reduction of *S.litura* larvae at higher concentration (1.2x10^{12}conidia/litre) at 14 DAS than S/NPV and *Bt* treatment and was at par in lowering percent leaflet damage. Pod and grain damage in soybean by lepidopteran pod borer at higher dose was on par with *Bt.* Grain yield did not vary between nuclear polyhedrosis virus (NPV) and *N.rileyi* treated plot but *Bt* treatment caused higher yield. Efficacy of *N.rileyi* increased with increase in dosage of fungus and all the doses of *N.rileyi* were significantly superior to untreated control. Chlorpyrifos was superior to all the microbial agents. In a comparison of the efficacy of *N.rileyi* between the crop ecosystems, soybean seemed to favour the fungal action far better (Kulkarni and Lingappa, 2002).

Among the treatments, NSKE+*N.rileyi*, *V.negundo*+*N.rileyi* and A.mexicana+*N.rileyi* were the most cost-effective, with B:C ratios of 12.28:1, 14.22:1 and 14.01:1, respectively. NSKE and *V.negundo* enhanced the bioefficacy of *N. rileyi* against *S.litura* in groundnut, resulting in high B: C ratio and yields comparable to that of monocrotophos, aside from being safer to environment (Patil *et al.*, 2003).

The field performance of *N.rileyi* at 2x10^{8} conidia/litre against soybean pests (*S.litura, H.armigera* and *Thysanoplusia orichalcea*) was compared with that of monocrotophos at 1 ml/litre and 1-cyhalothrin at 0.6 ml/litre in 2001-02 in Karnataka, India. Larval population was reduced by 28 and 62% in 10 days by *N.rileyi* after the first and second applications, respectively. Population reduction in pests in the chemical insecticide

treated soybeans was 42 and 65% after the first (monocrotophos) and second (1-cyhalothrin) sprays (Lingappa, *et al.*, 2002).

A field experiment was conducted in 1993 in Karnataka, India to investigate the efficacy of *N.rileyi* (at 1.6 and $3.2x10^8$spores/ml) on some pests of cabbage: *H.armigera*, *S.litura* and *Trichoplusia ni*. The lower rate was combines with endosulfan at 0.035%. Endosulfan was used at 0.07% for comparison. The maximum cabbage yield (35.9 t/ha) and insect control was observed with the fungi +endosulfan treatment (Gopalakrishnan *et al.*,2003).

The reduction of the larval population due to the spray of *N.rileyi* was compared with Neem Seed Kernel Extract (NSKE), insecticide (quinalphos 0.05%) spray. Insecticidal spray recorded significantly lower larval population (3.18, 2.53 and 0.82 larvae/m row at 40, 55 and 70 DAS, respectively) and leaflet damage in all the genotypes of groundnut. Significantly higher yield (16.89 q/ha) and lowest leaflet damage was recorded in quinalphos spray, whereas, *N.rileyi* and NSKE were next best in terms of yield (14.04 and 13.83 q/ha) with lesser leaflet damage (Navi *et al.*, 2006).

Chaudhari (2010) reported that the isolate *N.r.*kc showed lowest LC_{50} of 1.26 $x10^5$ and $1.60x10^6$ conidia /ml against second and third instar larvae of *S.litura* with lowest LT_{50} of 143.89 and 178.43 hr. respectively. Sorgum was found to be the most potential substrates to produce *N.rileyi* when fortified with 1% yeast extract. The *N.rileyi* formulated as liquid and wettable powder was better in shelf-life with the $3x10^6$and $5.50x10^6$CFU/ml after 180 days of storage at 27^0C. *N.r.*L-10 @ 4ml/l and *N.r.*T-S@ 4g/l was recorded high field pathogenicity, larval reduction, low leaf damage and high yield in soybean.

Entomopathogenic fungi *B.bassiana P.fumosoroseus V.lecani* were tested against groundnut pests, *Aphis craccivora* (Koch), *Aproarema modicella* (Deventer) and *S. litura* in field conditions. Among the tested fungi, *V.lecanii* suppressed 62% of *A.craccivora* population at 39 Days after Seedling Emergence (DASE). During the same period, *B. bassiana* reduced 72% of *S. litura* larval population (0.73 larvae). The infestation of *S. litura*

and *A. modicella* were greatly reduced after the treatment of *B. bassiana*; subsequently the yield (1721.31 kg/ha) and cost benefit ratio (1: 1.93) were increased (Sahayaraj and Namachivayam, 2011).

M.anisopliae:

Mortality rates (MR) of first, second and third *H.consanguinea* instar grubs, introduced into plastic cups with *M.anisopliae* inoculated soil, were determined. The highest MR (70%) for first instar grubs was recorded upon treatment with $1x10^{11}$spores for 16 days. Second instar grubs exhibited the highest MR (60%) when treated with 1×10^{11} and 5×10^{10} spores/ml soil for 30 days (Yadav *et al.*, 2000).

The fungus significantly reduced both the adult and larval populations of *F.occidentalis*, although the level of control of larval populations was much lower than for adults. Combined application of *M.anisopliae* and Methomyl, however, resulted in a significant reduction of both the larval and adult stages (Maniania *et al.*, 2002).

M.anisopliae was applied at the rate $1x10^{11}$conidia/ ml and dimethoate was applied at the recommended rate of 17.5 g a.i/ha for the control of onion thrips. In all the trials, thrips density and damage were significantly lower in the fungal and chemical insecticide treatments compared with the untreated control. In the third season trial *M.anisopliae* applied weekly recorded the highest yield (24 metric tones /ha (Maniania *et al.*, 2003)

Application of entomopathogenic fungi, *B.bassiana* and *M.anisopliae* (@ $5x10^{13}$ conidia/ha, each) indicated 43.02 and 47.16 per cent decrease in plant mortality in maize against white grub over control (Anjana Patial and Bhagat, 2005).

M.anisopliae when applied @ $5x10^{13}$ conidia/ ml in combination with imidacloprid 200SL at 48 g a.i./ha resulted in lowest plant mortality (2.28%) and grub population (1.12/pit) and highest yield of 6.79 q/ha (Badal Bhattacharyya *et al.*, 2008).

M.anisopliae and *B.thuringiensis* treatments similarly gave the highest cane yield (18.8 t/ha) while neem seed kernel extract gave the highest Brix reading (Deepthi *et al.*, 2008)

2.10 Shelf life assessment of formulations

N.rileyi:

Laboratory produced sclerotia (mummified cadavers of *H.virescens (F.*) and conidia of *N.rileyi* were held in field conditions for 281 days. Conidia were infectious after being held on the surface of the soil and in glass vial for 138 and 209 days, respectively (Spernkel and Brooks, 1975). Cadavers held on the surface of soil began sporulating after 47 days. The isolate of *N.rileyi* stored at 3°C in sterilized mineral oil showed no changes in viability or pathogenicity even after 6 months (Balardin and Loch, 1988).

Prior *et al.* (1988) studied the effect of storage conditions on survival of *B. bassiana* in coconut oil at room temperature (25^0C) and in refrigerated condition (2^0C). The conidia lost their viability in six days under room temperature, where conidia found to survived upto forty days under refrigerator conditions. Conidial suspension in oil was effective for field application because of its non-drying properties. The oil formulation *B. bassiana* exhibited the additional advantage of prolonged conidial survival.

The conidial production of treated mycelia stored 1.5 and 4.5 months at 4 ^{0}C was not significantly different for any procedure. For dry mycelium of *M.anisopliae*, Maltose and sucrose treated preparations produced more conidia than preparations sprayed with dextrose solution with water spray or not sprayed *B.bassiana* preparations dried soon (Periera and Roberts, 1991).

Dead larvae of the noctuid *A.gemmatalis* covered with spores of *Nomuraea rileyi* were collected in 6 counties in Brazil and maintained in jelly capsules in glass containers with silica at -18°C from 1984 to 1990. The isolates kept their viability and pathogenicity for up to 6 years of storage (Silva, *et al.*, 1993).

The virulence of 3 strains of *N.rileyi* was determined. Conidia were produced in rice, sorghum and soyabean and stored for 3 months at 4°C. The mortality of larvae of *A.gemmatalis* was reduced with stored conidia (Lopes-e-Lopes, *et al.*, 1995).

In case of *B.bassiana* higher conidial production occurred after storage at 22^{0}C.*B.bassiana* mycelium can be stored for longer periods of time than *M.anisopliae*. But, in both the fungi, corn starch formulation (500 ml mycelia paste + 25 g at gelatinized corn starch, later hardened by drying for 3-4 h) provided some protection for the material stored at room temperature which may be related to the presence of sugars in the formulations. The conidia of *B.bassiana* formulated as fat pellets, dispersible powder (with talc) and oil suspension (in shell sol) are evaluated for viability in two temperature conditions *i.e.,* at 4^{0}C and 25^{0}C stored for 45 days. The highest conidial viability after 45 days was recorded with the conidia in fat formulation (84.7% at 25^{0}C and 91.3% at 4^{0}C) as reported by Hidalgo *et al.* (1998).

The conidial viability of twenty four strains of entomopathogenic fungi (Hyphomycetes: *B.bassiana, M.anisopliae, N.rileyi, P.farinosus, P. fumosoroseus and P.lilacinus*) maintained in the culture collection and preserved by lyophilization and in liquid nitrogen, was assessed. Germination rates of 16- to 84-month-old cultures stored in liquid nitrogen decreased, on average, less than 13.3%. (Faria *et al.*, 1999).

Greater conidial viability and better survivability upto 11 months were observed in Ooty and upto 9 months in Coimbatore, where the average minimum and maximum temperature ranges between 5–25^{0}C and 19.23–34.7^{0}C, respectively, when compared to Chennai (upto 8 months) having an average minimum of 250C and maximum of 36^{0}C (Ramarethinam *et al.*, 2001).

Peanut oil, Tween-80 and Agral also gave high germination values after 24 h and 48 h (above 99%). Only soybean oil was significantly different from the other oils after 24 hrs, but not after 48 h. Viability of conidia in medium term storage (40 weeks) was better at 10^{0}C than 27^{0}C for all tested oil based

formulations and there were more significant differences between formulations stored at 27^{0}C than formulation stored at 10^{0}C. They remained viable above 97 per cent. Pure dry conidia and peanut oil were the only formulations that maintained conidial viability higher than 90 per cent in both temperatures after 40 weeks of storage (Alves *et al.*, 2001).

The conidial germination of *N.rileyi* N812 in different oil formulations was studied for 36 hours storage at room temperature. The fungus *N.rileyi* N812 conidial germination was >80 per cent in presence of sunflower oil, diesel: sunflower oil mixture (7:3), diesel: groundnut oil mixture (7:3) and Tween-80 (0.1%). The less per cent germination was seen in safflower oil and its combinations with diesel, groundnut oil which can be attributed to the high viscosity of oil which aggregated spores and reduced the germinations (Nahar *et al.*, 2004).

In case of WP formulations, it is aluminium silicate which was able to maintain 80 per cent viability upto 43 weeks at 4^{0}C, wherein it is less than 1 week in 30^{0}C. It is followed by bentonite and its combination with lactose, maltose, glucose and sucrose as proportion of 7:1 which were able to maintain >80 per cent viability upto 23 weeks at 40C wherein at 300C, they were found effective upto 1-3 weeks (Wiwat, 2004).

Among nine vegetable oils and seven WP formulations of *N.rileyi* studied, conidia of *N.rileyi* lost 99 per cent of their viability within a day of storage in vegetable oils (Ramegowda, 2005). The viability of conidia after one year of storage was 22.21 per cent in refrigerated condition, while it was only 15.64 per cent at ambient room temperature. Rice flour, talc and sorghum flour emerged as the best among carrier materials evaluated, while skimmed milk powder and gram flour appeared to be non-suitable.

M.anisopliae:

In Mississippi, conidiospores of *N.rileyi*, *M.anisopliae* and *B.bassiana* were stored outdoors for 12 months in continuous shade in culture tubes alone or with silica gel crystals (loose or in porous cloth bags), and the stored

spores were tested quarterly in the laboratory for pathogenicity to larvae of *Heliothis zea* (Boddie). Spores of *B.bassiana* were unable to survive any of the test conditions, but those of *N.rileyi* retained some activity for 12 months and *M.anisopliae* for 6 months when stored with silica gel in bags. For both species, some activity was retained for 6 months without silica gel (Bell, 1975).

Daust *et al.* (1983) studied effect of formulation on the viability of *Metarhizium* conidia. Among fourteen oils (12 are botanicals and two non-botanicals) are evaluated, conidial viability declined in all oils over a two months period at 19^0C and 26^0C. However, viabilities were considerably more at 4^0C, viability of conidia stored in all oils were considerably lower than those of dry conidia stored at 4^0C. The liquid vehicles like petroleum based oils; organic acids and water were even more detrimental to conidial survival than botanical oils. In contrast to liquid formulations, conidia stored at 10 % granules or 20 % dusts retained high viabilities over a 12 months period at 4^0C.

Spores of the entomopathogenic fungus *M.anisopliae* that had been stored dry in a freezer at -13°C were still 49.5% viable after 360 days. Spores stored in a refrigerator at 5°C were non-viable after 300 days, and those at room temperature at 120 days. Spores that had been stored in a freezer were more lethal to larvae of *G.mellonella* (Abreu *et al.*, 1987).

The viability of 64 formulations of the entomogenous fungus *M.anisopliae* under 2 types of storage conditions (at room temperature and refrigerated) and of pure conidia of the fungus and one formulation of the pure conidia plus silica gel under deep-frozen conditions was evaluated. The time for which the fungus could be stored increased by up to 33% at room temperature, and up to 52% in the refrigerator (at 2-3°C), depending on formulation, and formulations kept in the freezer were viable for up to 660 days (70% viability) (Alves *et al.*,1987).

Liquid cultures of *M.anisopliae* can be induced to form aggregates of hyphal cells under certain conditions. The cells remained viable for 8 months

at 4°C, but the shelf-life was increased to 18 months at this temperature when the granules were stored in a vacuum. The number of conidia formed was constant for about 6 months and biological activity was 60-100% (Wandersch *et al.*, 1990).

Blastospores of 3 strains of *M.anisopliae* were stored in 18 liquids at 4, 20 and 35°C for 18 weeks, 12 weeks resp. Viability was quantified by determination of their germination. Blastospores survived best at 4°C in 10% hydroxyethyl starch; for example, germination of *M.anisopliae* strain 97 still amounted to more than 80% after storage for 18 weeks. Other suitable liquids were deionized water, 25% Ringer's solution and 1% sodium alginate. The viability of blastospores stored at 20°C was considerably shorter than at 4°C. During storage for 12 weeks at 20°C the best protective liquids for *M.anisopliae* strain 97 were 25% Ringer's solution (43% germination), deionized water (23%) and 10% hydroxyethyl starch (23%). At 35°C, 45% of *M.anisopliae* strain 97 blastospores still germinated after storage for 7 days in 25% glycerol (Kleespies and Zimmerman, 1994).

Investigations on influence of carrier material and storage conditions on viability of *M.anisopliae* conidia were carried out in *in-vitro* conditions at Dharwad during from Jan. 2001 to Jan. 2002. Irrespective of the carrier material used, storage of conidia under refrigeration 4 ^{0}C, over a period of one year reduced viability to 62 per cent as against 84 per cent at ambient temperature. After one year of storage at ambient temperature, the colony forming units (39.6x10^{6}cfu/g) were almost 2.4 times lesser than storage under refrigeration (95x10^{6}cfu/g). Among different carrier materials used for wettable powder formulation, attapulgite and kaolinite retained viability of conidia significantly more (33.5 & 31.9% respectively after one year) followed by sorghum flour (27.9%) and talc (26.9%) (Rachappa *et al.*, 2007).

2.11 Compatibility with pesticides

N.rileyi:

Gardner *et al.*, (1979) reported that methyl parathion completely inhibited *N.rileyi* growth at all concentrations, while methomyl and carbaryl showed inhibition at 0.28 and 0.40 per cent concentration.

The organophosphate pesticides like dichlorvos, edifenphos, methyl parathion and dimethyl sulfoxide reduced the weight gain of mycelia significantly when compared to control under *in vitro* conditions (Ross and Brady, 1985). According to Terrible *et al.* (1991), diflubenzuron, carbaryl and λ – cyhalothrin had the lowest inhinitory effect on the fungus.

The experiment was conducted to evaluate the effect of endosulfan (175 g a.i), profenophos (110 g a.i.), trichlorfon (400 g a.i.) and permethrin (125 g a.i.) on sporulation under in vitro condition on SMYA medium. The insecticides permethrin and diflubezuron were found compatible, whereas trichlorofon, profenophos and endosulfan affected the sporulation of *N.rileyi* (Silva *et al.*, 1993).

Spraying of 0.02% Tween-80 served as the control. Results revealed that spraying of carbendazim after spraying of conidial suspension significantly inhibited the ability of *N.rileyi* to infect *S. litura* larvae (Kulkarni and Lingappa, 2001).

Sporulation on the surface of the agar slope was more than 75-100% of the area in all the methods of incorporation of neem seed kernel extract, which indicated that neem did not inhibit mycelial growth and sporulation of *N.rileyi*. Mancozeb completely inhibited *N.rileyi.* Carbendazim caused complete inhibition at all the 3 rates and was the inhibitoriest. Monocrotophos and endosulfan completely inhibited *N.rileyi* growth at X and 10X. Cypermethrin and fenvalerate exhibited 98.27 and 96.97% degree of inhibition, respectively (Vimladevi *et al.*, 2002).

M.anisopliae:

The effects of various insecticides on the mycelial growth, sporulation and conidial germination of *M.anisopliae* var. *anisopliae* isolate E9 were studied in the laboratory. Chlorpyrifos was the most toxic organphosphate insecticide to mycelial growth and sporulation at all concentrations. Temephos, malathion and leptophos were highly toxic to sporulation, while malathion was the most inhibitory to germination. The carbamates carbofuran, methomyl and oxamyl were moderately toxic to mycelial growth and sporulation, while oxamyl had an adverse effect on germination. The pyrethroids pyrethrins, permethrin and resmethrin and the insect growth regulators diflubenzuron and methoprene were not inhibitory to the various developmental stages of isolate E9. The chlorinated hydrocarbons (chlordane, lindane and toxaphene [camphechlor]) were more deleterious than all other insecticide groups tested. Among the fungicides, benomyl and maneb produced the greatest inhibition (Mohamed *et al.*, 1987).

Pari Pachamuthu *et al.* (1999) determined the compatibility of *M. anisopliae* strain ESC-1 with chloropyriphos. Insecticides did not affect conidial germination but adversely affect the growth and sporulation. The growth of *M. anisopliae* colonies on media amended 50 and 500 ppm of chlorpyriphos and treatment at 3, 6 and 9 day was significantly inhibited compared with the control.

Gupta *et al.* (2002) studied the compatibility of *M.anisopliae* and *B.bassiana* against 7 fungicides. Copper oxychloride was very well tolerated by *M.anisopliae* even at highest concentration of 2000 ppm. The growth inhibition at this concentration was only 11.1 per cent. This was closely followed by chlorothalonil exhibiting 53.4 per cent inhibition at 2000 ppm. Ridomil MZ, mancozeb and TMTD were tolerated at lower concentration showing 25.0, 33.3 and 50.00 per cent growth inhibition at 100 ppm, respectively. Bavistin and Benlate could not be tolerated even at 10 ppm.

2.12 Phytotoxicity

N.rileyi* and *M.anisopliae:

Mahajan (2003) reported that the entomopathogenic fungus, *V.lecanii* (both wettable powder and liquid formulation) with different concentrations did not show any phytotoxic effect on flowers and leaves of the hightech ornamental gerbera. But combination of *V.lecanii* 0.1 per cent with NSE 2 per cent and alone NSE 4 per cent caused severe phytotoxicity to the flowers and moderate one on the leaves. The flowers lost marketability due to NSE treatments

2.13 Congeniability

2.13.1 Light (Ultra violet)

N.rileyi:

Isolates of *N.rileyi* from cadavers of *A.gemmatalis* grew and sporulated on SMAY in both light and dark regimes indicating non influence of light on the growth and development. Optimum growth and sporulation occurred between 15 and 25°C and 80 and 100 per cent RH (Kish and Allen, 1978).

Viable spore half-life appeared to be dependent on sunlight intensity. Under sunny conditions the half-life was 3.6 hrs, but when plots were covered with a screen excluding direct sunlight it was 40 hrs. The pathogenic activity and viability of spores declined with time. The use of selective screens transmitting ultraviolet radiation (320-2500nm) or blocking wavelengths above 400 nm demonstrated the lethal effect of ultraviolet radiation on spores on leaves. An equation was formulated which predicted that in very sunny conditions a high concentration of *N.rileyi* ($3x10^6$ spores/cm2) could be reduced by 10 000 over 7 days, while viability decreased only 100 times during a cloudy period (Farguse, *et al.*,1988).

Entomogenous fungi with pigmented conidia varying from black to white (*Aspergillus niger*, black; mutant *A.n.* cinnamomeus, tan; *M.anisopliae*, dark green; *N.rileyi*, blue-green; mutant *N.rileyi*, yellow; and *Beauveria*

bassiana, white) were exposed to simulated sunlight (SUV) for 2, 4, 8, 16, 24 and 32 hrs. The black conidia of *A.niger* were significantly more stable (14.8 ± 2.7 hrs) when exposed to SUV than the lighter pigmented conidia of all the other isolates. The half-life of the other isolates ranged from 1.1 ± 0.2 h (yellow conidia of *N. rileyi*) to 2.0 ± 0.6 hrs (tan-coloured conidia of *A. n.* cinnamomeus). Also, dry conidia of *N. rileyi* were most stable when exposed to SUV (half-life of 2.4 ± 0.4 hrs) than wetted conidia (half-life of 1.6 ± 0.2 hrs). Because black-pigmented conidia were more tolerant to SUV, it may be possible to incorporate, by selection or genetic engineering, this phenotypic character into potential mycopesticides (Ignoffo and Garcia., 1992).

M.anisopliae:

Radiation in the ultraviolet (UV) region can kill or damage conidia, half lives of 100 and 360 minutes have been reported for *Metarhizium anisopliae* exposed to UV irradiation. (Zimmermann, 1982; Roberts and Campbell 1977). The most damaging ultraviolet wavelength is UV C which is largely filtered out by the atmosphere so conidia are, therefore, mainly exposed to UV A wavelengths ranging from (320-400 nm) and UV B(from 280-320 nm). The latter wavelengths are the most damaging for conidia of entomopathogen fungi. (Moore *et al.,* 1993).

Hunt *et al.* (1994) reported that chemical sunscreens were incorporated into oil formulations of conidia of two isolates of the entomopathogenic fungus *M.spp.* and exposed to simulated solar radiation after 2 hrs exposure several sunscreens gave protection as demonstrated by conidial germination after 24 hr. incubation than the unprotected control after 48 hrs incubation. During 5 hrs exposure, Eusolex 8021 failed to offer significant protection as demonstrated by conidal germination after 48 hrs incubation. Conidial damage was proportional to the duration of radiation received.

Conidial suspensions were prepared in water plus 10% emulsifiable oils; vegetable oil; mineral oils; and in water plus 0.05% Tween 80. Conidial

suspensions were exposed to 2, 4 and 6 hrs solar radiation from an 'Oriel' sunlight simulator. Unexposed plates of all formulations were the controls. After irradiation, the formulations were diluted and the resultant suspensions incubated on Sabouraud-Dextrose-Agar at 27 ± 0.5°C. Conidial viability was assessed after 24 and 48hrs incubation. Germination decreased for all treatments with increasing exposure time to solar radiation. Peanut [groundnut] oil, Shellsol plus Ondina, water plus Emoleo R, Codacide R, Natur'l oil R and Ashlade R significantly enhanced conidial tolerance ($P<0.05$) against UV light for up to 6 hrs of exposure compared with water plus Cropspray R, Cutinol R, Actipron R and Tween. Unexposed control plates of all formulations showed that germination of conidia was more advanced than in those plates subjected to simulated sunlight, thus confirming that UV exposure delays germination (Alves *et al.*, 1998).

Exposure to simulated solar radiation for a few hours can completely inactivate the conidia of the fungus. In the present study, we determined the effect of exposures to full-spectrum sunlight and to solar ultraviolet A radiation at 320-400 nm (UVA) on the conidial culturability and germination of three *M.anisopliae* strains. The strains showed wide variation in tolerance when exposed to full-spectrum sunlight as well as to UVA sunlight. Four-hour exposures to full-spectrum sunlight reduced the relative culturability by approximately 30% for strain ARSEF 324 and by 100% for strains ARSEF 23 and 2575. The relative UV sensitivity of the two more sensitive strains was different under solar UV from that under ultraviolet B radiation at 280-320 nm (UVB) in the laboratory (Braga *et al.*, 2001a).

The effects of irradiances of 920 and 1200 mW m-2 (weighted irradiance) on the conidia and germinants of the entomopathogenic *Hyphomycete M.anisopliae* were tested. The conidia were exposed to the two irradiances for 1, 2, 4, 6, 7 or 8hrs. Increased exposure decreased relative percent culturability. The inactivation provoked by the irradiance of 1200 mW m-2 was higher than for the 920 mW m-2, with a reduction in the 50%

lethal time (LT50) from 6 hrs and 40 minutes to 4 hrs and 26 minutes (Braga *et al.*, 2001a).

Carbon source greatly improved UV-B tolerance, but reduced conidial yield; while, on the other hand, preferred carbon sources improved conidial yield, but reduced UV-B tolerance (Rangel *et al.,* 2006)

The conidial tolerance of *M.anisopliae* var. *anisopliae* isolate ARSEF 2575 to UV-B irradiation is greatly influenced by growth-environment alterations. In this review, we report high variability in conidial UV-B tolerance in response to altered culture conditions. Conidia produced on insect cadavers [*Zophobas morio* (Coleoptera) or *G.mellonella* (Lepidoptera)] had low tolerance to UV-B radiation; and conidia produced on potato dextrose agar supplemented with yeast extract (PDAY) had medium UV-B tolerance; whereas conidia produced on a minimal medium without any carbon source (MM), on MM with a non-preferred carbon source such as lactose (=MML), on PDAY plus 1 M NaCl or KCl, or PDBY with high alkalinity had the highest UV-B tolerances (Rangel and Roberts, 2007).

The UV-A and UV-B wavelengths of solar radiation may seriously reduce viability of *M.anisopliae* conidia, a fungus used extensively in Brazil for insect pest control. Conidia formulated with oil emulsion had higher survival after 3 h of UV exposure, but the virulence of the conidia previously exposed to 2 h of UV radiation were similar when oil formulated or not (Francisco *et al.*, 2008).

2.13.2 Temperature

N.rileyi:

The optimum temperature for mycelial growth and sporulation was 25°C. The average time for initial sporulation at 15, 20 and 25°C was about 21, 10.4 and 8.8 days, respectively. Mycelial growth did not occur at temperatures above 35°C (Ignoffo *et al.*, 1976).

The fungus, *N.rileyi* was effective at 20^0C and 25^0C causing 80 and 71 per cent mortality of *H.armigera*, respectively. At 15^0C the disease progressed very slowly with larval mortality occurring in 12.28 days. Where as, at 20^0C to 25^0C the time gap was reduced to 6.12 days (Mohamed *et al.*, 1977).

The optimum field temperatures for normal growth and development of *N. rileyi* are 20°C to 30°C. High levels of conidia are correlated with periods of dry, gusty winds and dry foliage. A count of >1,50,000 conidia per slide per 2 hr exposure (1200–1400 hr) was encountered under this type of situation. The density of airborne conidia was less after rain (Kish and Allen, 1978).

A laboratory study showed that of constant temperature regimes of 25, 30, 35, 40 or 45°C, that of 25°C appeared to be optimum for the development of *N.rileyi* in 4^{th} instar larvae of the noctuid *Heliothis zea* (Gardner, 1985).

M.anisopliae:

Various isolates could grow at the different extremes of the temperature range, tropical ones at the hotter temperatures and temperate ones at the cooler temperatures. However, the most growth occurred at 25°C for 26 isolates. Upper temperature limits for growth varied from 28 to 37°C according to both fungal species and isolate. *M.anisopliae* isolates had rapid growth rates and tolerated a wide temperature range (8-11 to 35-37°C) (Fargues and maniania, 1992).

Of 7 temperatures, 28°C was the most favourable for growth and sporulation of *M.anisopliae*. The strain Ma-4 had the highest conidial production. *B.bassiana* could grow within the temperature range 20-28°C, but 25°C was the most suitable temperature for conidial production in all isolates, and 28°C was the most favourable temperature for growth and sporulation, respectively (Shashi-Sharma *et al.*, 1998).

Conidial production of *M.anisopliae* var. *acridum* on mycosed cadavers of *S.gregaria*, during 10 days incubation, was optimized at 96% relative humidity (RH), and at temperatures between 20 and 30°C, producing > 10^9

conidia per cadaver. Conidial yield was maximum at 25°C, when cadavers remained in contact with the damp substrate. Little sporulation occurred at temperatures of 15 and 40°C, regardless of RH, and no sporulation occurred at 10 or 45°C (Arthurs and Thomas, 2001).

The effect of temperature on the germination and growth of *M. anisopliae, M. anisopliae* var. acridum and *B.bassiana* was determined. The optimal temperature for the isolates was between 24 and 30 °C. The rate of growth of *B.bassiana* was greater than that of *M.anisopliae* at 24 °C; however, germination and growth of *M.anisopliae* var. acridum, was greater at 32 °C (Berlanga Padilla *et al.*, 2002).

Nine *M.anisopliae* var. anisopliae strains (KVL275, V245, V208, IMBST9601, IMBST9602, IMBST9609, IMBST9631, FAL376 and FAL538) were subjected to seven different temperatures between 10 and 37°C to study the effects of temperature on the germination of airborne conidia and the fungal growth behaviour on Sabouraud Dextrose Agar plates. All strains lost the ability to germinate at temperatures higher than 30°C. The optimum temperature range for germination was 22-30°C (Strasser *et al.*, 2003).

2.13.3 Humidity

N.rileyi:

High relative humidity (>90%) or free water (2-6 hrs dew) is very critical for sporulation, germination and invasion of host by *N.rileyi* (Getzin, 1961 and Allen *et al.*, 1971).

Although free water is very important, excess rainfall or long standing dew can be adverse. Excess moisture will reduce the density of air borne conidia from cadavers and prevents their dissemination by wind (Garcia and Ignoffo, 1977).

Cadavers exposed to overhead irrigation for 2 hrs (0.72" rainfall) lost 91 per cent of their conidia compared to those cadavers not exposed. The conidia which were not exposed to water were dislodged by air (Kish and Allen, 1978).

Fuxa (1984) observed reduced incidence of *N.rileyi* killed cadavers of *P.scabra, P.includens* and *A.gemmatalis* after heavy rainfall. This was due to washing of conidia into soil and prevention of aerial dispersion.

Initiation of sporulation is delayed and conidia production is reduced when *A. gemmatalis* cadavers infected with *N.rileyi* are exposed to water stress (relative humidity 25-30%) in the laboratory. Field observations also demonstrated that *N.rileyi* conidial viability remain high (> 75% germination) until 10 days on A. gemmatalis cadaver surface, acting as an inoculum source for this period (Sujii *et al*. 2002).

The mean larval population in the soyabean field increased up to the 3rd week of August, then declined significantly during the 4th week. The average larval mortality due to *B.bassiana* and/or *N.rileyi* ranged from 7.7% for larvae collected during the first week of August to 58.9% for larvae collected during the 4th week of August. The high relative humidity (>80%) and temperature of 23-31 degrees C prevailing in August were the most favourable for the rapid multiplication of the biological control agents (Sharma and Ansari, 2007).

M.anisopliae:

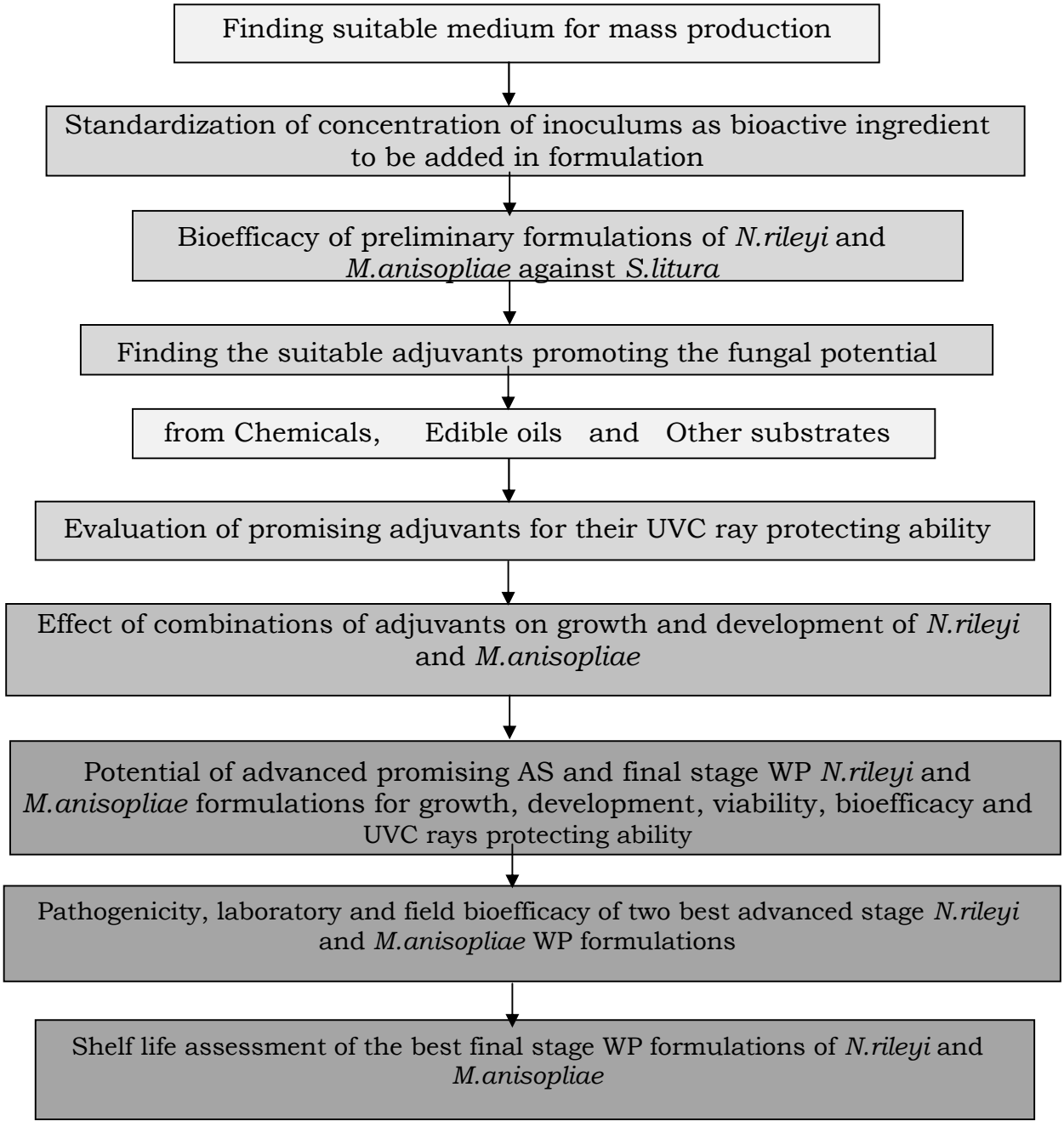
The environmental conditions likely to be encountered by the formulation in the field include high temperatures, low humidity and high light intensities. Formulated conidia of *M. flavoviridae* have been shown to be tolerant to high temperatures, for example 4 h at 55^{o}C causes minimal loss of viability (McClatchie *et al.,* 1994).

The effects of relative humidity (33, 53, 75, 85 and 98%) on the growth of *M.anisopliae* (Ma-1, Ma-2, Ma-3 and Ma-4), isolates were investigated in vitro. Observations on colony diameter were recorded after 20 days of inoculation. The relative humidity at 53% was most favourable to the growth of all isolates, resulting in a mean growth of 46.3 mm beyond this level, the growth significantly decreased. The viability of conidia decreased with increasing levels of RH (Shashi Sharma *et al.*, 2001).

3. MATERIAL AND METHODS

During the present study on development of wettable powder formulation of *Nomuraea rileyi* (Farlow) Samson and *Metarhizium anisolpiae* (Metschnikoff), the sequence of experiments conducted are given in the following flow chart.

Sequence of experimentation to develop the *N.rileyi* and *M.anisopliae* WP formulations.



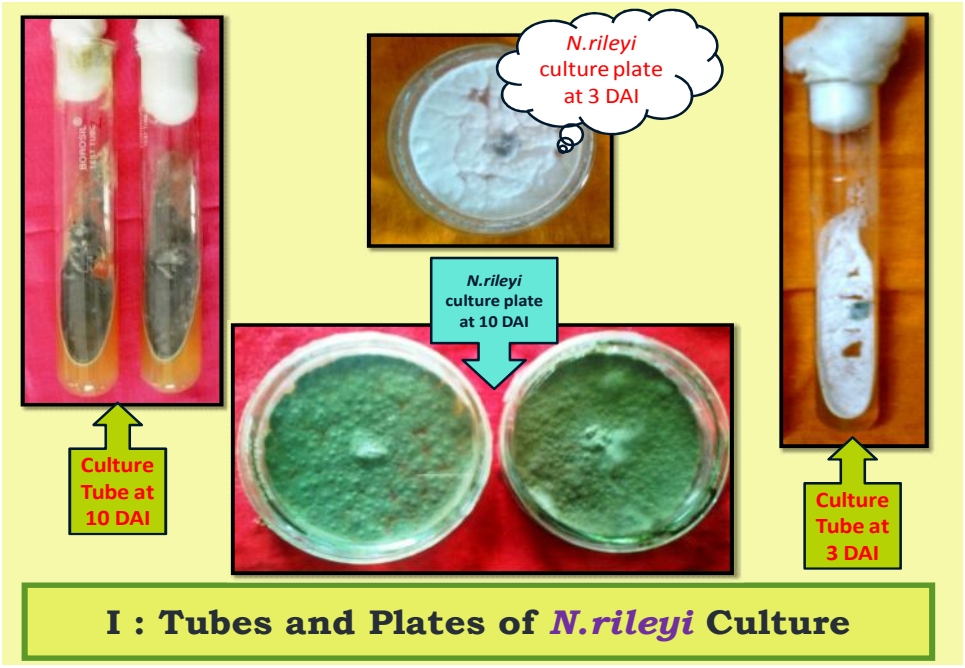


PLATE – V

Culture Tubes and Plates of *N.rileyi* and *M.anisopliae*

The laborotary evaluations were carried out in Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, M.P.K.V., Rahuri and field experiments were carried out at Agricultural Research Station, Niphad during 2009 to 2012.

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

3. 1 Material

3.1.1 Fungus culture

The pure fungus culture of *M.anisopliae* was available at Biocontrol Lab of the Department of Entomology, MPKV, Rahuri and *N.rileyi* was made, avaible from isolates in Biocontrol Lab of Entomological centre, College of Agriculture, Pune.

3.1.2 Medium

The medium used for multiplication and growth of the fungus was Sabourauds dextrose broth with yeast extract as find by media for mass production as in 4.1.1.

Table 1 : Adjuvants used in the formulation

Sr. No.	Adjuvants	Utility	Source
1.	Glycerol anhydrous (Glycerin)	Humectant, nutrient carrier, Osmotic protectant, Plasticizer	SRL, 26 Navketan Industrial premices Co-op. Society Ltd., Shanti nagar, Mahakali Caves road, Andheri (E),Mumbai-400 086.
2.	Tween 80 (Polyxyethylene sorbitan mono-oleate)	Synergists, Stabilizer	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
3.	Triton X-100 (polyethylene glycol-p-tertoctyl phenyl ether)	Emulsifier	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
4.	Boric acid powder	Lubricants, synergist	Manali Traders, S.V.Road, Malad (W), Mumbai-64.

5.	Carboxymethyl cellulose sodium salt (CMC)	Thickner, sticker, binder, stabilizer	Hi Media Lab. Pvt. Ltd., A-406, Bhaveshwar plaza, LBS marg, Mumbai-400 086.
6.	Magnesium sulphate ($MgSO_4$)	Synergists, Stabilizer	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
7.	Dextrose	Nutrients	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
8.	Yeast extract powder	For sporulation and fermentation	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
9.	Peptone bacteriological powder	Nitrogenous food source	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
10.	Malt extract powder	Nutrients	S.D.fine–Chem.Pvt.Ltd., Mumbai- 400 025
11.	Agar powder	Thickner and solidifier	SRL, 26 Navketan Industrial peremices Co-op. Society Ltd., Shanti nagar, Mahakali Caves road, Andheri (E),Mumbai-400 086.
12..	Groundnut oil	Antidessicant, humectant, nutrient, synergists, adhesive, encoater and sticker	Local Market
13.	Sunflower oil		
14.	Soybean oil		
15.	Mustard oil		
16.	Coconut oil		
17.	Ghee		
18.	Honey	Binder, stabilizer, nutrient, preservative, sticker, dispersing agent.	Dabur India Ltd., Solan (H.P.)
19.	Indigo	UV protectant	Local Market
20.	Turmeric powder	Synergist, stabilizer	Local Market
21.	Molasses	Sunscreen, wetter, sticker and nutrient	Local Market
22.	Milk	Humectant, emulsifier, nutrient carrier and sunscreen	Local Market
23.	Wheat flour	Feed source, binder and carrier	Local Market
24.	Corn flour		
25.	Sorghum flour		
26.	Bajra flour		
27.	Rice flour		

Table 2 : Test pest and target host

Sr. no.	Nomenclature of the pest	Test host
1	Leaf eating catterpillar, *Spodoptera litura* (Fabricius)	Soybean

3.1.3 Other Materials

Aluminium trays, inoculation needles, laminar flow cabinet, pertridishes, glass slides, glass bottles, tissue papers, sterilized cotton wool, test tubes, plastic vials, mechanical blender, occular and stage micrometer, Neubaur haemocytometer, wet and dry bulb thermometers, hygrometer and pH meter were made available in Biocontrol Laboratory of Entomology Department, M.P.K.V., Rahuri.

3.2 Methods

3.2.1 Development of WP formulations

3.2.1.1 Finding out most suitable medium for mass production of *N.rileyi and M.anisopliae*

The present study was conducted for evaluation of nine media of various nutrient sources on the basis of per cent surface coverage and biomass. Sabouraud's dextrose (SD) broth + 1%Yeast extract, Sabouraud's maltose (SM) broth + 1%Yeast extract, Potato peptone (PP) broth, Yeast extract glucose (YEG) broth, Potato dextrose (PD) broth, Potato maltose (PM) broth, Malt extract (ME) broth, Potato glucose (PG) broth and Potato dextrose (PD) broth + 1%Yeast extract were prepared. The each empty saline bottles were filled with 40 ml medium. As such different media bottles were sterilized under 15 lbs pressure at 121^0C for 15 minutes. Each bottle was inoculated with fungal culture ($2x10^8$cfu/ml) and incubated for 10 days at room temperature 25 + 2^oC. The individual fungal mat was separated using pre-weight Whatman No.1 filter paper. The observation on per cent surface coverage on 3,7,10 and 15 days and biomass on 10^{th} day were recorded (Hall and Bell, 1961).

3.2.1.2 Standardization of concentration of inoculum, *N.rileyi and M.anisopliae*

The fresh fungus cultured on Sabouraud's dextrose (SD) broth + Yeast extract medium, incubated for 10 days at 25 ± 2^0 C ,was harvested in a plastic container and grinded with sterilized blender for 3 minutes. Series of dilutions of the duly grinded fresh culture of *N.rileyi and M.anisopliae* from 10 to 90% concentration of bioactive ingradient (BAI) were made using distilled water as diluent. The stock samples were stored in 100 ml autoclaved sterilized saline bottle. Each preparation was evaluated for its potential for growth, development and viability of *N.rileyi* and *M.anisopliae* up to 10 days and bioefficacy of standardized aqua suspension (AS) 30% v/v of *N.rileyi* and *M.anisopliae.* The experiments were replicated thrice in C.R.D. For mortality data, it were corrected firstly by Abbotts formula (Abbott, 1925) and then by arc sin square root transformation (Gomez and Gomez, 1984). The experimental data were then subjected to statistical analysis.

Determination of quantity of bioactive ingredient (%) in the formulation:

Percentage of fungal mat using inoculum of *N.rileyi* and *M.anisopliae* in control and formulation product was calculated by adopting the following procedure given below :

The volume of harvested culture of *N.rileyi and M.anisopliae* from 25 saline bottles was measured and fungal mat separated from it and put on filter paper and weighted. The percent fungal mat w/v was calculated by using following formula :

$$\text{Per cent fungal mat (w/v)} = \frac{\text{Weight of 25 fungal mat}}{\text{Total volume of the culture from 25 bottles}} \times 100$$

3.2.1.3 Effect of various adjuvants with inoculum on growth and development of *N.rileyi* and *M.anisopliae*

Various formulations with adjuvants were made and tested for growth and development of *N.rileyi* and *M.anisopliae.*

3.2.1.3.1 The effect of chemical adjuvants

The test chemical adjuvants were glycerol (1.0, 2.0, 3.0 and 5.0%), triton-X-100 (0.03, 0.06, 0.12, 0.25 and 0.50%, boric acid (0.5, 1.0, 2.0, 3.0 and 5.0%), carboxymethyl cellulose (0.5, 0.75, 1.0, 1.25 and 1.50%) and magnesium sulphate (0.5, 1.0, 2.0, 3.0 and 5.0%).

These chemical adjuvants with different concenstrations were added to 100ml optimum concentration of 30% AS of *N. rileyi* ($1.97x10^9$cfu/ml) and *M.anisopliae* ($1.77x10^9$cfu/ml) in 500ml saline glass bottle and 31 liquid formulations were made.

The bottles were plugged with cotton wool and incubated at ambient temperature. One ml of the formulated liquid was added to 40 ml Sabouraud's dextrose broth with yeast extract medium in glass bottle and closed with cotton wool. The whole process was carried out in laminar flow cabinet. The observations on per cent surface coverage by fungus on 3rd, 7th and 10th days and fungal biomass on 10th day after inoculation were noted. The experimental data were subjected to statistical analysis. These experiments were carried out in CRD with three replications.

3.2.1.3.2 The effect of edible oils (vegetable oils)

To test edible oils as adjuvants comprising groundnut, sunflower, soybean, coconut and mustard oils each at 0.25, 0.50, 1.00 and 2.00 per cent and ghee 0.25, 0.50 and 1.0 per cent were mixed individually in optimum concentration of 30% AS of *N.rileyi* ($1.97x10^9$cfu/ml) and *M.anisopliae* ($1.77x10^9$ cfu/ml) in 500 ml saline glass bottle and 23 liquid formulations were prepared. Further procedure is explained under 3.2.1.3.1.

3.2.1.3.3 The effect of few other edible substrates

Honey (0.5, 1.0 and 2.0%), molasses (1.0, 2.0, 3.0 and 4.0%), milk (0.5, 1.0, 2.0 and 3.0%) and various flour viz. wheat, corn, sorghum, bajra and rice each at 1.0, 2.0, 3.0, 4.0 and 5.0% were mixed individually in

optimum concentration of 30% AS of *N.rileyi* ($1.97x10^9$ cfu/ml) and *M.anisopliae* ($1.77x10^9$cfu/ml) in 500ml saline glass bottle and 39 liquid formularies were prepared. Further procedure is explained under 3.2.1.3.1.

3.2.1.3.4 The effect of combination of chemical adjuvants

Optimum concentration of promising chemical adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-X-100 (0.03%), boric acid (2.0%) and carboxymethyl cellulose (0.50%) were selected and mixed with each other in combination with optimum concentration 30% AS of *N.rileyi* ($1.97x10^9$cfu/ml) and *M.anisopliae* ($1.77x10^9$cfu/ml) and 14 liquid formulations with multiple adjuvants and 5 liquid formulations with individual adjuvants were prepared. The 19 formulations were tested for growth and development of *N.rileyi* and *M.anisopliae*. Further procedure is explained under 3.2.1.3.1.

3.2.1.3.5 The effect of combination of chemicals and edible oils

Optimum concentration of promising chemical and oil adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-X-100 (0.03%), boric acid (2.0%) and carboxymethyl cellulose (0.50%) and oils as sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%) and ghee (0.5%) were selected and mixed with each other in combination with optimum concentrations 30% AS of *N.rileyi* ($1.97x10^9$cfu/ml) and *M.anisopliae* ($1.77x10^9$cfu/ml) and 39 liquid formulations with multiple adjuvants and 11 liquid formulationss with individual adjuvants were prepared. The 50 formulations were tested for growth and development of *N.rileyi* and *M.anisiopliae*. Further procedure is explained under 3.2.1.3.1.

3.2.1.3.6 The effect of combination of edible oils

Optimum concentration of edible oils as adjuvants compring sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%) and ghee (0.5%) were selected and mixed with each other in combination

with optimum concentrations 30% AS of *N. rileyi* ($1.97x10^9$ cfu/ml) and *M.anisopliae* ($1.77x10^9$ cfu/ml) and 13 liquid formulations with multiple adjuvants and 6 liquid formulations with individual adjuvants were prepared. The 19 formulations were tested for growth and development of *N.rileyi* and *M.anisopliae*. Further procedure is explained under 3.2.1.3.1.

3.2.1.3.7 The effect of combination of chemicals, oils and other substraes

Optimum concentration of various adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-x-100 (0.03%), boric acid (2.0%) and carboxyl methyl cellulose (0.50%), sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%), ghee (0.5%) and honey (1.0%) were selected and mixed with each other in combination with optimum concentrations 30% AS of *N.rileyi* ($1.97x10^9$cfu/ml) and *M.anisopliae* ($1.77x10^9$cfu/ml) and 17 formulations with multiple adjuvants and 12 formulations with individual adjuvants were prepared. The 29 formulations were tested for growth and development of *N.rileyi* and *M.anisiopliae*. Further procedure is explained under 3.2.1.3.1.

3.2.1.4 Effect of UVC rays on growth and development of inoculum in formulations of *N.rileyi* and *M.anisopliae.*

The 32 formulations of *N.rileyi* and *M.anisopliae* as per the 4.1.4. The adjuvants comprising glycerol (1.0, 2.0, 3.0 and 5.0%), tween-80 (0.5 and 1.0%), boric acid (1.0, 2.0 and 3.0%), carboxymethyl cellulose (0.5 and 1.0%), indigo (0.5 and 1.0%), turmeric (0.5 and 1.0%), molasses (0.5 and 1.0%), honey (1.0 and 2.0%), milk (1.0 and 2.0%), sunflower (0.5 and 1.0%), groundnut (0.5, 1.0 and 2.0%), mustard (0.5 and 1.0%), soybean (0.5 and 1.0%) and ghee (0.5 and 1.0%) and formulations without adjuvants were evaluated in C.R.D. with 3 replications for their UVC rays protectability along with *N.rileyi and M.anisopliae* 30% AS. Various concentrations of adjuvants were added to optimum concentration of *N.rileyi and M.anisopliae*

aqua suspension 30% v/v to prepare various formulations. Each formulations were kept in 50 ml glass beaker and such formulations were exposed to UVC rays for 10, 20, 30, 40, 50 minutes, 2, 3 and 5 hours.

One ml of such exposed formulation was added to 40 ml Sabouraud's dextrose (SD) broth + Yeast extract medium and observed for growth and development up to 10 days.

3.2.1.5 Potential of advanced test formulations of *N.rileyi and M.anisopliae*

3.2.1.5.1 Potential for growth, development and viability of test AS formulations of *N.rileyi* and *M.anisopliae*

The highly promising 10 formulations with honey, sunflower oil and ghee alone and formulations with combination with tween-80+ghee, glycerol+sunflower oil, glycerol+ghee, sunflower oil+ghee, tween-80+ glycerol+sunflower oil+carboxymethyl cellulose, tween-80+glycerol+honey, tween-80+glycerol+carboxymethyl cellulose and control without adjuvants (*N.rileyi* alone) of *N.rileyi* and 7 formulations with tweeen-80+ carboxymethyl cellulose, sunflower oil+ carboxymethyl cellulose, sunflower oil+honey,groundnut + boric acid, groundnut + carboxymethyl cellulose, groundnut + ghee, ghee + honey and control without adjuvants (*M.anisopliae* alone) of *M.anisopliae* were retested for their growth, development, viability and bioeffficacy against II and III instar larvae of *S.litura.*

3.2.1.5.2 Effect of UVC rays on advanced test AS formulations of *N.rileyi* and *M.anisopliae*

The promising 10 formulations of *N.rileyi* and 7 formulations of *M.anisopliae* as mentioned under 3.2.1.5.1 were tested for their UVC protecting ability.

Each formulations were kept in 50 ml glass beaker and such formulations were exposed to UVC rays for 10, 20, 30, 40, 50 minutes, 2, 3 and 5 hours.

One ml of such exposed formulation was added to 40 ml Sabouraud's dextrose (SD) broth + Yeast extract medium and observed for growth and development up to 10 days.

3.2.1.5.3 Bioefficacy of advanced test formulations of *N.rileyi* and *M.anisopliae*

The promising 10 formulations of *N.rileyi* and 7 formulations of *M.anisopliae* as mentioned under 3.2.1.5.1 were tested against II and III instar larvae of *S.litura*. One ml of each preparation was mixed in 99 ml of water and sprayed on II and III instar larvae of *S.litura*.

Laboratory experiment was carried out in Complete Randomized Design and three replications. Ten larvae were taken in a glass container along with castor leaves as food which were directly sprayed with 10 ml desired concentration of conidials suspension using hand atomizer and allowed to dry for about 15 minutes. Each larvae was transferred to a separate plastic vial (6 x 4cm) treated with antibiotics to avoid growth of other micro-organisms. Each vial containing moist filter paper at bottom with treated food. Fresh untreated castor leaves were provided to the larvae at every 24 hrs. Each treatment consisted of 10 larvae and replicated thrice. The treated larvae were incubated at room temperature at 25 $\pm$ 10 ^{o}C and RH of 70 $\pm$ 10%. The larval mortality was recorded at an interval of 24 hours up to 10 days. Percent mortality was calculated and corrected by formula given by Abbott, (1925). The data on cumulative per cent mortality obtained 10 days after inoculation (DAI) were subjected to Probit Analysis (Finney, 1971).

3.2.1.5.4 Potential for growth, development and viability of WP formulations of *N.rileyi* and *M.anisopliae*

The promising 10 formulations of *N.rileyi* and 7 formulations of *M.anisopliae* as mentioned under 3.2.1.5.1 of their WP formulations were tested for their growth, development and viability.

The viability index was worked out by designing following formula

$$\text{Viability index} = \frac{T\,(E \times F) - C\,(E \times F)}{C\,(E \times F)} \times 100$$

Where, T (E × F) = Biomass in treatment × cfu in treatment

C (E × F) = Biomass in control × cfu in control

3.2.1.5.5 Bio-efficacy of advanced test WP formulations of *N.rileyi* and *M.anisopliae*

The promising 10 formulations of *N.rileyi* and 7 formulations of *M.anisopliae* as mentioned under 3.2.1.5.1 of their WP formulations were tested against II and III instar larvae of *S.litura*. One ml of each preparation was mixed in 99 ml of water and sprayed on II and III instar larvae of *S.litura*. Further procedure is explained under 3.2.1.5.3.

3.2.1.6 Pathogenicity of each of two most promising final stage WP formulations of *N.rileyi* and *M.anisopliae* (LC_{50} and LT_{50} values) against *S.litura*

The bioassay of the two developed WP formulation of *N.rileyi* and *M.anisopliae* was carried out by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 g/l of water or at concentrations 0.01, 0.015, 0.02, 0.025, 0.03 and 0.04%, respectively) of each of the formulations and formulation without adjuvants. Further procedure is explained under 3.2.1.5.3 and calculated the LC_{50}, LC_{90} and LT_{50} of respective WP formulations.

3.2.1.7 Shelf life assessment of WP formulations at ambient conditions.

The samples of the advanced stage WP formulations of *N.rileyi* and *M.anisopliae* alongwith WP formulation of *N.rileyi* and *M.anisopliae* without adjuvants were evaluated for their shelf-life. The sample was drawn each of the formulations at 30 days interval upto 10 months and evaluated for growth, development and viability of the entomopathogen immediately after each of the sampling at 28± 2^0C. One gram of the WP formulation was added to 40ml Sabouraud's dextrose broth medium in glass bottle and closed with cotton wool. The whole process was carried out in laminar flow cabinet. The observations on per cent surface coverage and biomass developed by fungus on 10^{th} days after inoculation were noted. The experimental data were subjected to statistical analysis. These experiments were carried out in CRD with three replications.

The drawn samples were also tested for its colony forming unit (cfu) per gram simuntaneously with growth and biomass development. One gram of each of the product was drawn for monthly cfu count estimation by serial dilution technique for viability studies. The experiment was carried with 3 replications in Completely Randomized Design.

Testing cfu count of formulation of *N.rileyi* and *M.anisopliae*

The method suggested by Ming-Guang Feng *et al.* (1990) was used. The autoclaved Sabouraud's dextrose agar with yeast extract (SDA) medium in petridishes, (100 mm diameter) was inoculated with the help of micropipette by releasing 1 ml *N.rileyi* and *M.anisopliae* suspension prepared in the distilled water in laminar flow cabinet. Other petridishes with the medium were prepared in similar manner and inoculated with various dilutions in the series (10^1 to 10^{10} cfu/ ml) at 27 ± 1^0C. After 48 hrs from the 10 samples in each petridishes the numbers of colonies/petrideshes were counted and cfu/ml was calculated.

3.2.1.8 Phytotoxicity of WP formulations of *N.rileyi* and *M.anisopliae*

The observations in the field on soybean on phytotoxicity (*viz;* scorching, leaf injury on tips, wilting, vein clearing) of all the final stage formulations to soybean crop were recorded at 3,7and 15 DAT by spraying 0.01, 0.015, 0.02, 0.025, 0.03, 0.04 and 0.08 per cent concentration. The single spray of different WP formulations was undertaken on 22.08.2011.

Table 3 : Phytotoxicity score

Score	Percent crop health affected	
	Scorching of leaves (%)	Other symptoms
0	No adverse effect	
1	1-10	-
2	11-20	Slight yellowing
3	21-30	Slight reddening
4	31-40	Moderate reddening
5	41-50	Reddening and slight loosening
6	51-60	Reddening and moderate loosening
7	61-70	Loosening and drying
8	71-80	Slight drying
9	81-90	Partial drying
10	91-100	Complete drying of plant

3.2.1.9 Field efficacy of WP formulations of *N.rileyi* and *M.anisopliae*

Field experiments were conducted during *kharif* 2011 to evaluate the performance of wettable powder formulation of *N.rileyi* and *M.anisopliae* in

soybean against leaf eating catterpiller, *S.litura*. The experiments in soybean were conducted at Agricultural Research Station farm, Niphad.

Experiments were laid out in RBD with 12 treatments and three replications in 2011-12. The details of treatments are given in Table 2. The crop was raised following recommended agronomic practices except insecticide applications. Two sprays were given at an interval of 15 days using knapsack sprayer. The first spray was carried out at 40 days and 55 days after sowing.

Table 4 : Treatment details

Sr.No.	Treatments	Code name	BAI Conc.(%)	Dose g/l
1.	Formulation A & A1 *Nr/Ma* 5% WP	$N_{30}S_{1/1}$ & $M_{30}S_{1/1}C_{1/2}$	0.015	3.0
2.	Formulation A & A1 *Nr/Ma* 5% WP	$N_{30}S_{1/1}$ & $M_{30}S_{1/1}C_{1/2}$	0.02	4.0
3.	Formulation A & A1 *Nr/Ma* 5% WP	$N_{30}S_{1/1}$ & $M_{30}S_{1/1}C_{1/2}$	0.025	5.0
4.	Formulation A & A1 *Nr/Ma* 5% WP	$N_{30}S_{1/1}$ & $M_{30}S_{1/1}C_{1/2}$	0.03	6.0
5.	Formulation B & B1 *Nr/Ma* 5% WP	$N_{30}T_{1/1}G_{2/1}H_{1/1}$ & $M^{30}S_{1/1}H_{1/1}$	0.015	3.0
6.	Formulation B & B1 *Nr/Ma* 5% WP	$N_{30}T_{1/1}G_{2/1}H_{1/1}$ & $M^{30}S_{1/1}H_{1/1}$	0.02	4.0
7.	Formulation B & B1 *Nr/Ma* 5% WP	$N_{30}T_{1/1}G_{2/1}H_{1/1}$ & $M^{30}S_{1/1}H_{1/1}$	0.025	5.0
8.	Formulation B & B1 *Nr/Ma* 5% WP	$N_{30}T_{1/1}G_{2/1}H_{1/1}$ & $M^{30}S_{1/1}H_{1/1}$	0.03	6.0
9.	Formulation without adjuvant *Nr/Ma* 5% WP	-	0.02	4.0
10.	*Sl*NPV	Magic	-	1.0
11.	Quinolphos 25EC	Ekalux	0.05	2.0
12.	Untreated control	-	-	-

Experimental details

1. Crop	: Soybean
2. Location	: Agril. Research Station, MPKV, Niphad
3. Year	: 2011-12
4. Design	: RBD
5. No. of replications	: Three
6. No. of treatments	: Twelve
7. Date of sowing	: 14.07.2011
8. Date of spraying	: i) Ist spray at 45 days - 30.8.2011 and
	ii) IInd spray at 60 days- 13.9.2011
9. Variety	: JS-335
10. Spacing	: 30x10 cm
11. Plot size	: Gross 5.10 x 3.00 m Net 4.50 x 2.70m

Observations recorded

Observations on larval population were taken at 3 randomly selected spots of 1 meter in each treatment plot, leaving the border rows. Pre count of larval population was taken one day before spraying and post count were recorded at 3,7 and 14 DAS. The larval count of 14 DAS after first spray was considered as pre count for the second spray. The yield (kg/plot) obtained in individual treatment was recorded separately for assessing the effect of different treatments on yield. Data on yield (kg/plot) was converted into q/ha.

4. RESULTS AND DISCUSSION

The investigations on the development of wettable powder (WP) formulations of entomopathogenic fungi, *Nomuraea rileyi* and *Metarhizium anisopliae* were carried out during 2009-10 and 2010-11. The results of laboratory and field experiments are presented under the following main heading in this chapter and discussed in the light of available literature.

Development of WP formulation of *N.rileyi* and *M.anisopliae* comprised of series of experiments in link as under. The sequence of aspects of the study are:

1. Finding the suitable medium for mass production.
2. Standardization of bioactive ingredient for formulations.
3. Standardization of quantum of adjuvants.
4. Evaluation of primary and advanced stage formulations for growth, development, viability and UV protecting ability.
5. Pathogenicity and bioefficacy *in vivo* and *in vitro* and shelf-life of the most promising two formulations of each of the fungus.
6. Field efficacy and phytotoxicity of the two advanced stage WP formulations.

4.1 Development of WP formulations of *N.rileyi* and *M.anisopliae*

4.1.1 Suitability of different media for mass production

(Period: Feb.,March and June 2010 Av.Temp.(^{0}C) :Max.-32 ±1, Min.-17 ±1 Av.Humidity (%) :Morn.-88, Even.-32)

***N.rileyi* :**

The ultimate suitability of the medium for mass production of the entomopathogenic fungi was determined on the basis of growth, development and viability at 15 DAI, considering surface coverage, biomass development and cfu count, respectively. The surface coverage, biomass development and cfu count in the test media ranged from 28.33 to 100.0%, 1.04 to 6.10g and $2.33x10^8$ to $8.33x10^8$cfu/ml, respectively. The nine media of various nutrient

sources (Table 1) were evaluated to find out most suitable medium for growth, biomass and viability of *N.rileyi*.

Sabouraud's dextrose broth with yeast extract was significantly superior medium for the growth among all the nine test media. It showed maximum (61.67%) surface coverage at 3 DAI. It was at par with Sabouraud's maltose broth with yeast extract (58.33%) and potato dextrose broth with yeast extract (58.33%). At 7 DAI, the both media recorded cent per cent surface coverage. However, at 15 DAI Sabouraud's dextrose broth with yeast extract was most adequate as judged from significantly highest cfu count ($8.33x10^8$/ml) and biomass (6.10g) (Fig.1 and Plate-I). The next best media were Sabouraud's maltose broth with yeast extract and potato dextrose broth with yeast extract registering cfu count of $7.33x10^8$/ml and $5.67x10^8$/ml with the biomass of 5.63 and 4.20g, respectively

Table 1. Influence of the test media on growth, development and viability of *N.rileyi*

Tr No	Treatments	Surface coverage (%) on				Biomass (g/40 ml)	cfu (x 10^8/ ml)
		3 DAI	7 DAI	10 DAI	15 DAI		
T1	Sabouraud's dextrose broth +Yeast extract	61.67 (51.77)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	6.10	8.33 (2.97)**
T2	Sabouraud's maltose broth +Yeast extract	58.33 (49.78)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	5.63	7.33 (2.80)
T3	Potato peptone broth	11.67 (20.00)	23.33 (28.86)	21.67 (27.76)	41.67 (40.22)	1.47	3.67 (2.04)
T4	Yeast extract glucose broth	31.67 (34.27)	90.00 (71.56)	98.33 (82.51)	100.00 (90.00)	4.47	5.33 (2.42)
T5	Potato dextrose broth	28.33 (32.14)	66.67 (54.76)	96.67 (79.53)	100.00 (90.00)	2.97	3.67 (2.04)
T6	Potato maltose broth	20.00 (26.56)	43.33 (41.15)	63.33 (52.71)	71.67 (57.86)	1.60	3.33 (2.03)
T7	Malt extract	11.67 (20.00)	16.67 (24.12)	21.67 (27.76)	28.33 (32.14)	1.04	2.33 (1.68)
T8	Potato glucose broth	21.67 (27.76)	63.33 (52.71)	71.67 (57.86)	75.00 (60.00)	1.97	3.67 (2.04)
T9	Potato dextrose broth + Yeast extract	58.33 (49.78)	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	4.20	5.67 (2.48)
	S.E ±	**1.58**	**2.33**	**2.37**	**1.25**	**0.11**	**0.10**
	C.D.(P=0.05)	**4.69**	**6.92**	**7.05**	**3.70**	**0.34**	**0.29**

* Figures in parentheses indicate arcsin values. DAI = Days after inoculation

** Figures for cfu in parentheses are √n+0.5

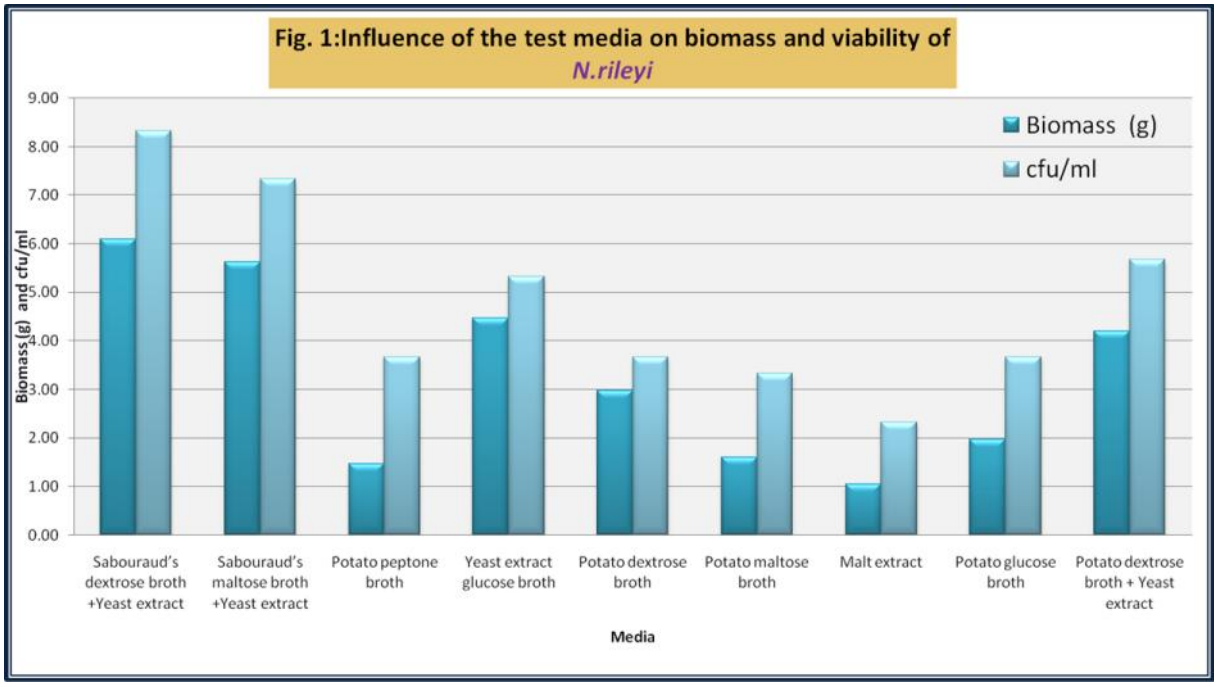
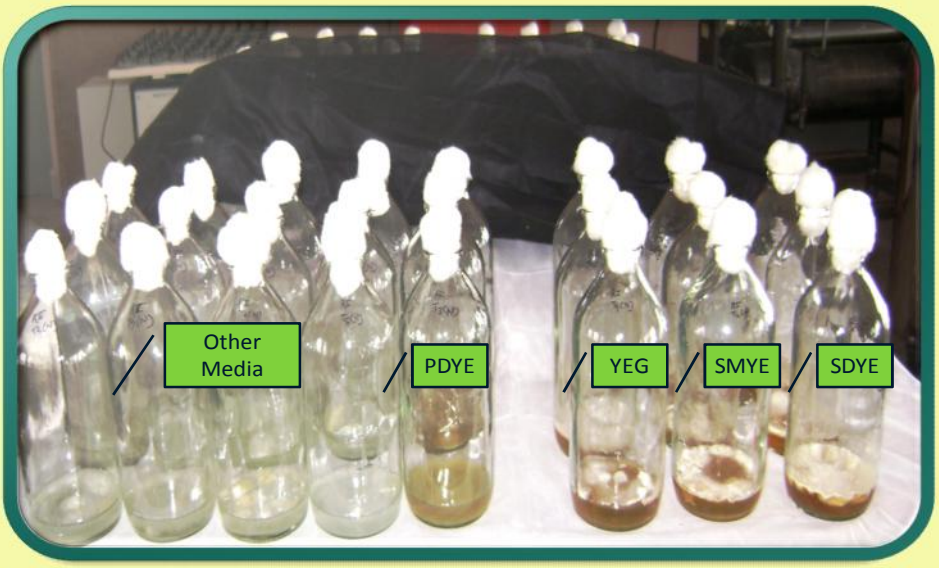


Fig. 1:Influence of the test media on biomass and viability of
N.rileyi
Biomass (g)
cfu/ml
Biomass (g) and cfu/ml
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Sabouraud's dextrose broth +Yeast extract
Sabouraud's maltose broth +Yeast extract
Potato peptone broth
Yeast extract glucose broth
Potato dextrose broth
Potato maltose broth
Malt extract
Potato glucose broth
Potato dextrose broth + Yeast extract
Media

I : Growth of *N.rileyi* on Test Media



II : Growth of *N.rileyi* on Test Media

PLATE – I

Growth of *N.rileyi* on test Media

The lowest per cent surface coverage at 3 (11.67%), 7 (16.67%), 10 (21.67%) and 15 (28.33%) DAI with least biomass (1.04g) and viability ($2.33x10^8$cfu/ml) at 15 DAI was recorded in malt extract. Thus, considering growth, development and viability of *N.rileyi* Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for the biomass production.

M.anisopliae :

The nine media of various nutrient sources (Table 2) were also evaluated to find out most suitable medium for growth, biomass and viability of *M.anisopliae.* The results were more or less similar to those obtained for *N.rileyi*. At 15 DAI, Sabouraud's dextrose broth with yeast extract proved to be superior which gave significantly highest cfu ($12.33x10^8$/ml) and biomass (7.20g) (Fig.2 and Plate-II). The next best medium was Sabouraud's maltose broth with yeast extract and potato dextrose broth with yeast extract which registering cfu count of ($10.33x10^8$ and $10.67x10^8$cfu/ml) and biomass (6.27 and 5.73g), respectively.

At 15 DAI, the lowest (48.33%) medium surface coverage and least biomass (1.57g) and cfu ($4.33x10^8$/ml) were registered in medium with malt extract. Thus, considering growth, development and viability of *M.anisopliae* Sabouraud's dextrose broth with yeast extract (SDY) emerged as the most potential medium for biomass production.

During the present investigation, Sabouraud's dextrose broth with yeast extract emerged as the most potential medium for biomass production for *N.rileyi* and *M.anisopliae.* It was in conformity with Manjula and Krishnamurthy (2005) who obtained excellent growth of the *N.rileyi* in medium with similar ingredients. Im *et al*. (1988) found that yeast extract was necessary for mycelial growth while dextrose was required for sporulation.

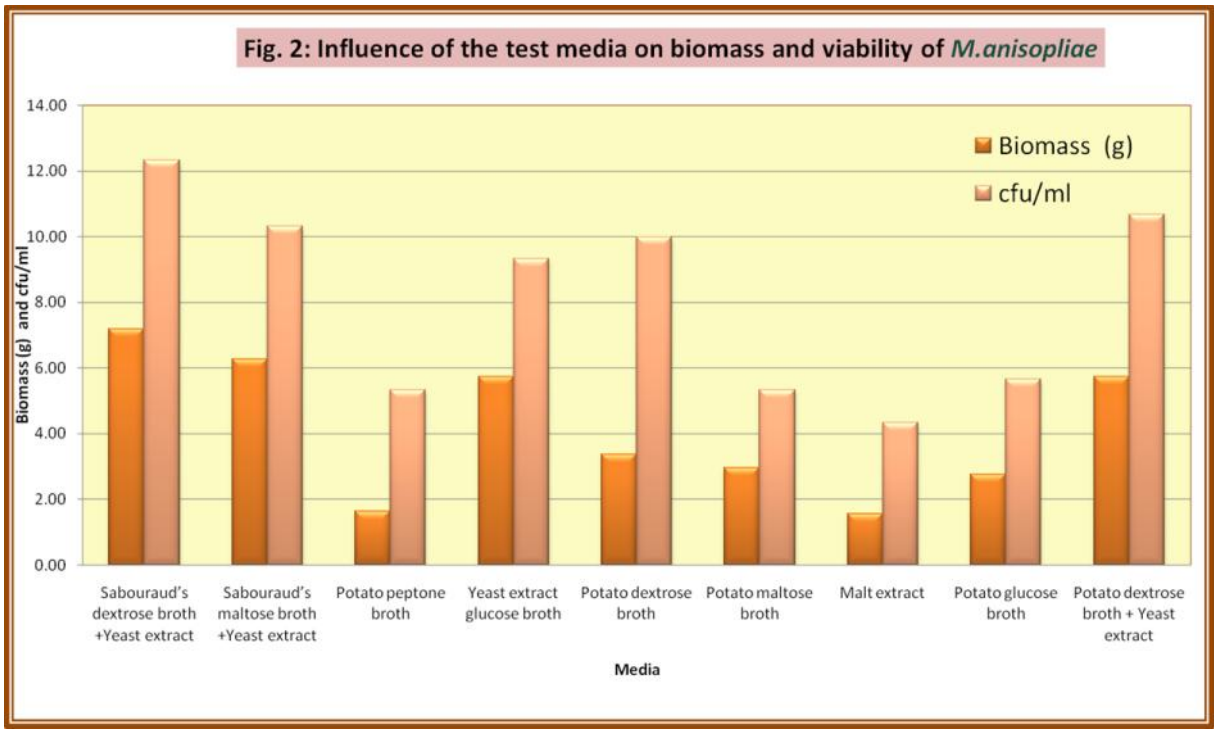
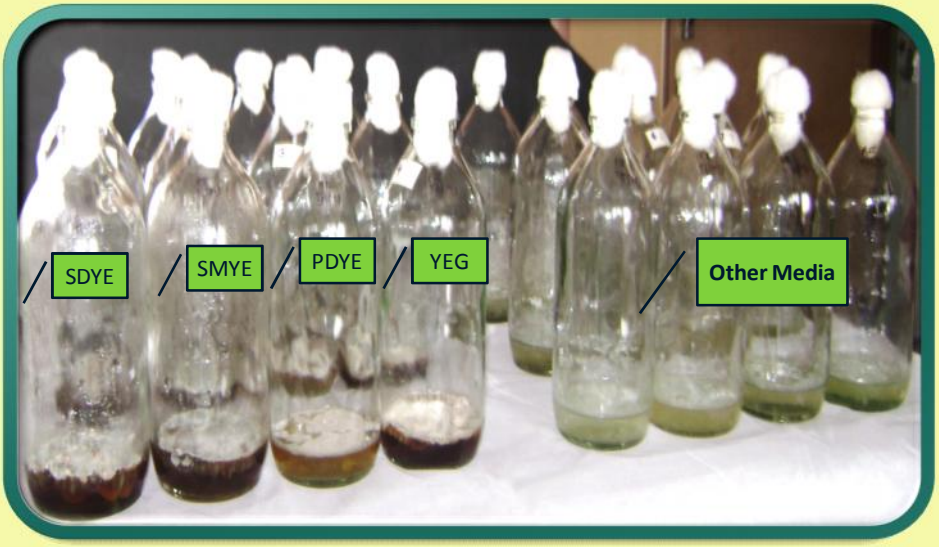


Fig. 2: Influence of the test media on biomass and viability of M.anisopliae
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Biomass (g) and cfu/ml
Biomass (g)
cfu/ml
Sabouraud's dextrose broth +Yeast extract
Sabouraud's maltose broth +Yeast extract
Potato peptone broth
Yeast extract glucose broth
Potato dextrose broth
Potato maltose broth
Malt extract
Potato glucose broth
Potato dextrose broth + Yeast extract
Media

I : Growth of *M.anisopliae* on Test Media



II : Growth of *M.anisopliae* on Test Media

PLATE – II

Growth of *M.anisopliae* on test media

Table 2. Influence of test media on growth, development and viability of *M.anisopliae*

Tr. No.	Treatments	Surface coverage (%) on				Biomass g/40ml medium	cfu (x 10^8/ml)
		3 DAI	7 DAI	10DAI	15 DAI		
T1	Sabourauds dextrose broth +Yeast extract	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	7.20	12.33 (3.58)**
T2	Sabourauds maltose broth +Yeast extract	98.33 (82.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	6.27	10.33 (3.29)
T3	Potato peptone broth	23.33 (28.86)	28.33 (32.14)	48.33 (44.03)	51.67 (45.97)	1.63	5.33 (2.42)
T4	Yeast extract glucose broth	93.33 (75.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	5.73	9.33 (3.13)
T5	Potato dextrose broth	93.33 (75.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	3.37	10.00 (3.24)
T6	Potato maltose broth	83.33 (65.88)	96.67 (79.53)	100.00 (90.00)	100.00 (90.00)	2.97	5.33 (2.42)
T7	Malt extract	23.33 (28.86)	26.67 (31.11)	40.00 (39.23)	48.33 (44.03)	1.57	4.33 (2.20)
T8	Potato glucose broth	85.00 (67.21)	95.00 (77.08)	100.00 (90.00)	100.00 (90.00)	2.77	5.67 (2.48)
T9	Potato dextrose broth + yeast extract	98.33 (82.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	5.73	10.67 (3.34)
	S.E ±	**1.94**	**1.28**	**0.64**	**0.45**	**0.07**	**0.09**
	C.D.(P=0.05)	**5.77**	**3.80**	**1.92**	**1.33**	**0.21**	**0.26**

* Figures in parentheses indicate arcsin values DAI = Days after inoculation
** Figures for cfu in parentheses are $\sqrt{n+0.5}$

Sabouraud's dextrose broth with yeast extract shown best performance for growth, development and viability of *M.anisopliae* in present studies which is in corroboration with the findings reported by Kulat *et al.* (2002), Shashi Sharma *et al.* (2002) and Pandey and Kanujia (2008). All the workers got highest sporulation and viability of *M.anisopliae* in the medium.

4.1.2 Standardization of concentration of *N.rileyi* and *M.anisopliae* as bioactive ingredient for the formulations

(Period : June 2010 Av.Temp.(^{0}C) :Max.-33 ±1, Min.-22 ±1 Av.Humidity (%) :Morn.-93, Even.-53)

4.1.2.1 Effect of inoculum concentrations on the growth and development

***N.rileyi* :**

The Sabouraud's dextrose broth with yeast extract grown *N.rileyi* and *M.anisopliae* cultures with 2x10^{9}cfu/ml and 17.67x10^{8}cfu/ml, respectively were used as stock bioactive ingredient to standardize its concentration in aqua suspension (AS) formulation. The growth was judged from per unit surface coverage while development was evaluated from biomass produced and viability from cfu/ml. The results revealed that the growth of *N.rileyi* increased with increase in concentration of inoculums in Sabouraud's dextrose broth with yeast extract. The highest (33.33%) growth in the form of per cent surface coverage was observed in *N.rileyi* (2x10^9) 90.0% concentration at 3 DAI (Table 3 and Plate-III). However, it was at par with *N.rileyi* (2x10^9) concentration 80.0, 70.0 and 60.0% (31.67% each), 40.0% (30.0%), 30.0% (28.33%) and 20.0% (28.33%). All the treatments (10.0 to 90.0 % inoculums) recorded cent per cent surface coverage at 7 and 10 DAI. *N.rileyi* (2x10^9) 90.0% produced highest biomass (11.17g). The biomass at 10 DAI was lowest 7.63g in concentration of 10.0%. However, it was at par with 30.0 to 80.0 % *N.rileyi* producing the fungal biomass of 10.57 to 11.07g, respectively. Maximum (21.67x10^8 cfu/ml) cfu count was registered in 50% concentration of *N.rileyi* aqua suspension. However, it was at par with that in 40% (20.67x10^{8}cfu/ml) and 30% (19.67x10^{8}cfu/ml) inoculums of aqua suspension (Fig.3).

The data in Table 3 clearly showed that at 10 DAI with increase in concentration of *N.rileyi* culture from 10% (pH 8.04) to 90% (pH 8.84) there was gradual increase in pH as compared the SDY medium pH (6.46) measured before adding the inoculum.

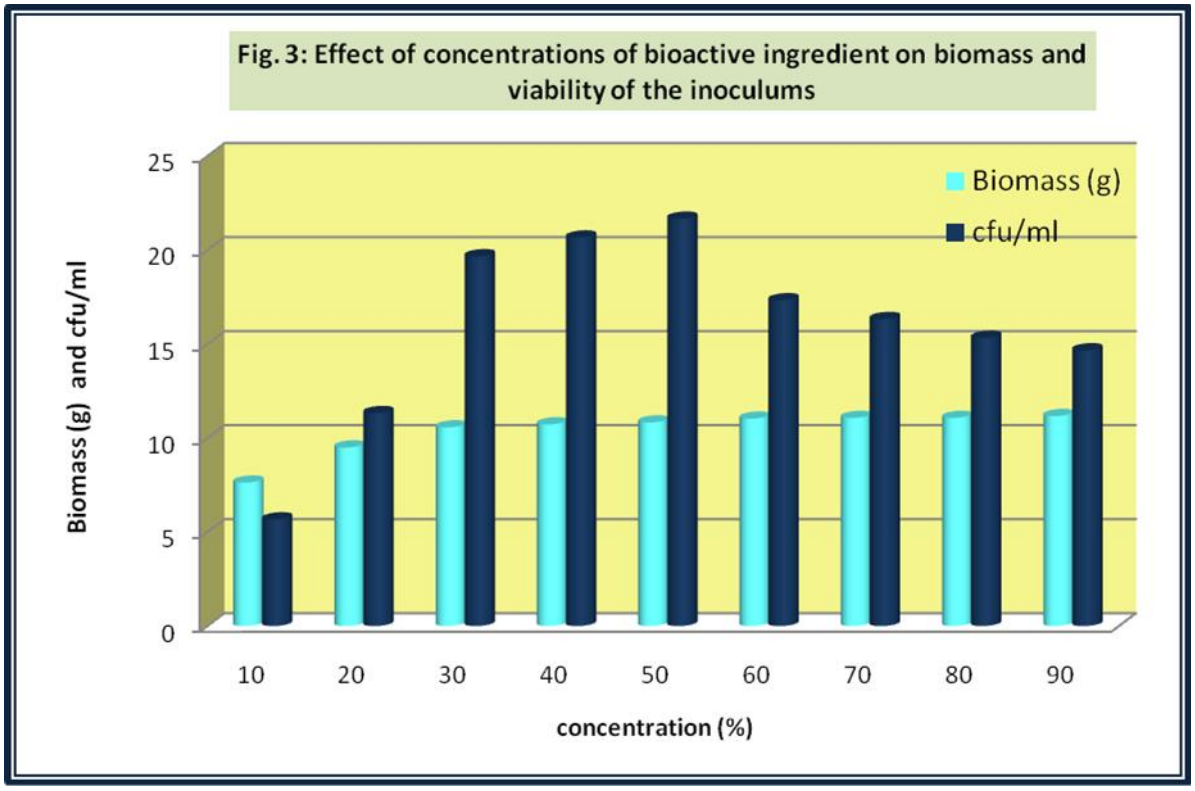


Fig. 3: Effect of concentrations of bioactive ingredient on biomass and viability of the inoculums
Biomass (g)
cfu/ml
Biomass (g) and cfu/ml
0
5
10
15
20
25
10
20
30
40
50
60
70
80
90
concentration (%)

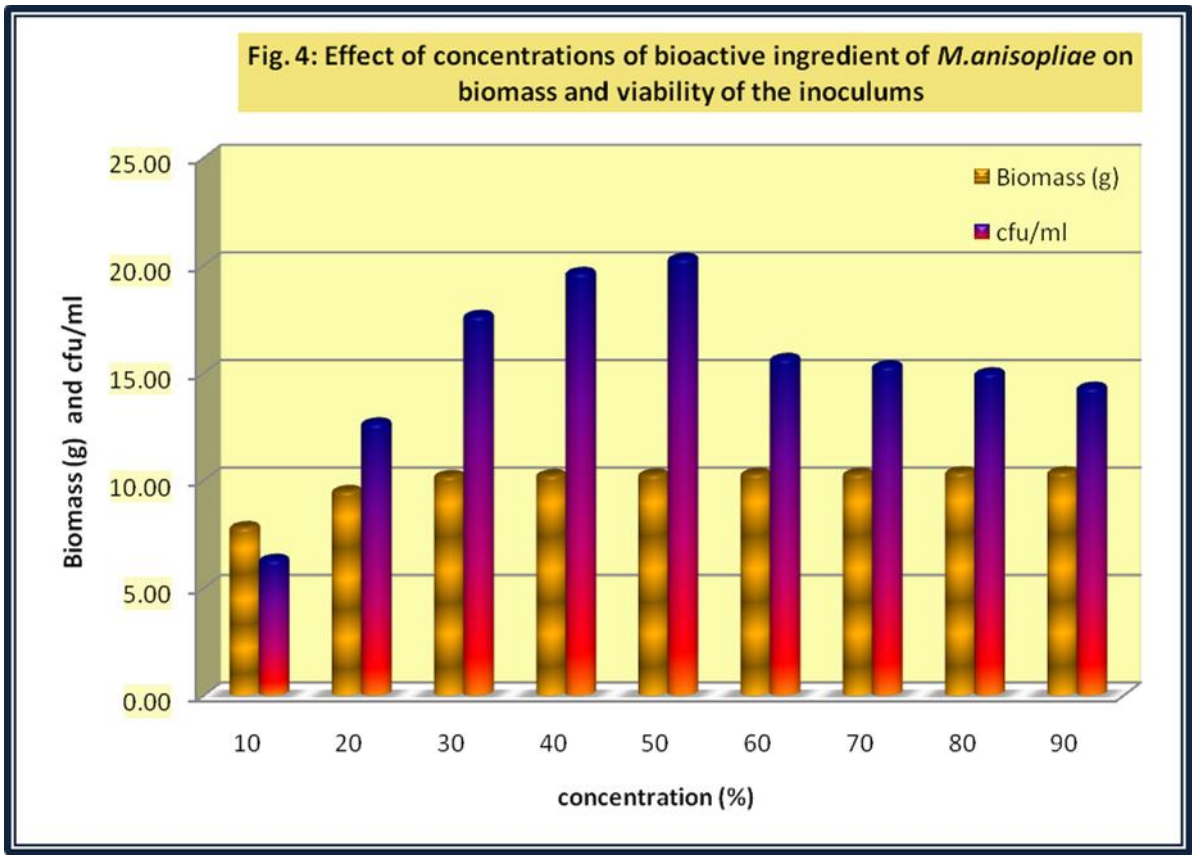


Fig. 4: Effect of concentrations of bioactive ingredient of M.anisopliae on biomass and viability of the inoculums
Biomass (g)
cfu/ml
Biomass (g) and cfu/ml
0.00
5.00
10.00
15.00
20.00
25.00
10
20
30
40
50
60
70
80
90
concentration (%)

Table 3. Effect of concentrations of bioactive ingredient of *N.rileyi* on growth and development in SDY medium

Tr. No.	*N. rileyi* (2x10^9 cfu/ml) conc.(%)	Surface coverage (%) on			Biomass g/40 ml medium	cfu (x10^8/ml)	pH at 10 DAI
		3 DAI	7 DAI	10 DAI			
T1	10.0	21.67 (27.76)*	100.00 (90.00)	100.00 (90.00)	7.63	5.67 (2.48)**	8.04
T2	20.0	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	9.50	11.33 (3.44)	8.07
T3	30.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	10.57	19.67 (4.49)	8.13
T4	40.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	10.73	20.67 (4.60)	8.21
T5	50.0	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	10.83	21.67 (4.71)	8.45
T6	60.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.03	17.33 (4.22)	8.64
T7	70.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.07	16.33 (4.10)	8.75
T8	80.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	11.07	15.33 (3.98)	8.79
T9	90.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	11.17	14.67 (3.89)	8.84
	S.E ±	**1.42**	**-**	**-**	**0.26**	**0.10**	**-**
	C.D. (P=0.05)	**4.21**	**-**	**-**	**0.78**	**0.29**	**-**

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
** Figures for cfu in parentheses are √n+0.5

***M.anisopliae* :**

The data on effect of various concentrations of the entomopathogenic fungus on growth and development are presented in Table 4 and depicted in Fig.4. The results revealed that the growth of *M.anisopliae* increased with increase in concentration of inoculums in sabouraud's dextrose broth with yeast extract. The highest (96.67%) growth in the form of per cent surface coverage was observed in 90% concentration of *M.anisopliae* (17.67x10^8) at 3 DAI. However, it was at par with 40 to 80% *M.anisopliae* which showed 86.67 to 95.0% surface coverage. The 10 to 90% of *M.anisopliae* recorded cent per cent surface coverage at 7 DAI. The treatment concentration 90 and 80% produced highest biomass (10.40g). It was at par with the treatment 70, 60, 50, 40 and 30% fungal culture which produced the biomass of 10.33, 10.33, 10.30, 10.27 and 10.23g, respectively. The lowest biomass (7.85g) was registered in 10% concentration (Plate-III). Maximum (20.33x10^{8}cfu/ml) cfu count was observed in 50% concentration of *M.anisopliae* aqua suspension.

However, it was at par with 40% (19.67x10^{8}cfu/ml) and 30% (17.67x10^{8}cfu/ml) of *M.anisopliae* aqua suspension.

Table 4. Effect of concentrations of bioactive ingredient of *M.anisopliae* on growth and development in SDY medium

Tr. No.	*M. anisopliae* (1.77x10^{9}cfu/ml Conc. (%)	Surface coverage (%) on			Biomass g/40ml medium	Cfu/ml (x10^8)	pH at 10 DAI
		3 DAI	7 DAI	10 DAI			
T1	10.0	65.00 (53.73)*	100.00 (90.00)	100.00 (90.00)	7.85	6.33 (2.61)**	8.88
T2	20.0	68.33 (55.73)	100.00 (90.00)	100.00 (90.00)	9.53	12.67 (3.63)	8.86
T3	30.0	68.33 (55.73)	100.00 (90.00)	100.00 (90.00)	10.23	17.67 (4.26)	8.84
T4	40.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.27	19.67 (4.49)	8.82
T5	50.0	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	10.30	20.33 (4.56)	8.82
T6	60.0	95.00 (77.08)	100.00 (90.00)	100.00 (90.00)	10.33	15.67 (4.02)	8.84
T7	70.0	95.00 (77.08)	100.00 (90.00)	100.00 (90.00)	10.33	15.33 (3.98)	8.84
T8	80.0	95.00 (77.08)	100.00 (90.00)	100.00 (90.00)	10.40	15.00 (3.94)	8.86
T9	90.0	96.67 (79.53)	100.00 (90.00)	100.00 (90.00)	10.40	14.33 (3.85)	8.86
	S.E ±	**3.95**	**-**	**-**	**0.09**	**0.11**	**-**
	C.D.(P=0.05)	**11.74**	**-**	**-**	**0.28**	**0.32**	**-**

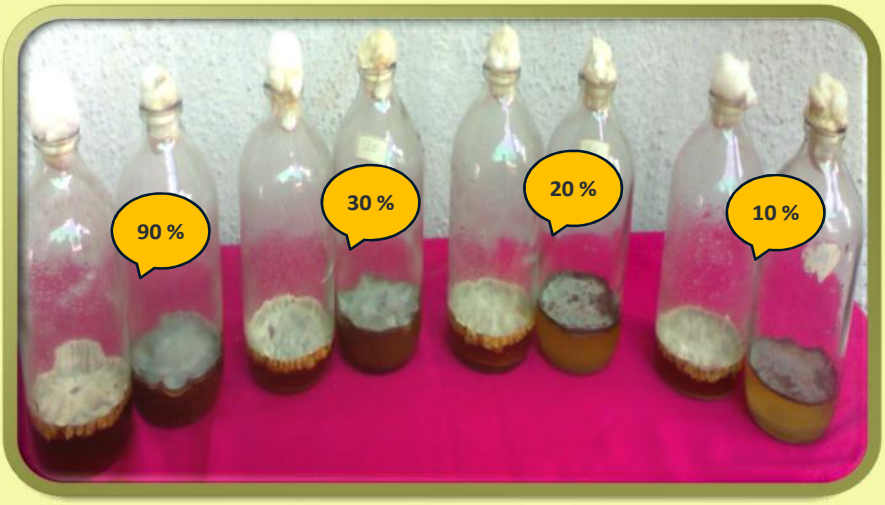
*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
** Figures for cfu in parentheses are √n+0.5

The pH of culture medium SDY (6.46) increased to 8.82 to 8.88 at 10 DAI by addition of *M.anisopliae* culture from 10 to 90%. There was no relation between per cent culture and the pH measured at 10 DAI.

There is no published literature on optimization of concentration of the bioactive ingredient in aqua suspension formulation of entomopathogenic fungus.

Thus, it showed narrow difference in biomass development in the at par treatments. It indicated that the increase in concentration from 1 to 9 times, there was no proportionate increase in biomass produced. It established that the constant quantity of medium with increased pressure of inoculum determined the quantity of biomass produced. So, increase in concentration of the inoculum in same quantity (40 ml) of medium could not produced proportionately higher biomass of the inoculum on the basis of the

I : Growth of *N.rileyi* as influence by concentration of inoculum



II : Growth of *M.anisopliae* as influence by concentration of inoculum

PLATE – III

Growth of *N.rileyi* and *M.anisopliae* as influence by concentration of inoculums

result. The concentration of 30.0 % was considered optimum for formulating the product having cfu of $2x10^9$/ml of *N.rileyi* and $17.67x10^8$/ml of *M.anisopliae*.

Determination of per cent bioactive ingredient in the formulation of *N.rileyi* and *M.anisopliae* :

***N.rileyi*:**

Total weight of 25 fungal mats of *N.rileyi* harvested after 10 DAI= 160.0g

Total volume of 25 fungal culture bottles of *N.rileyi* at 10 DAI= 960ml

So, required quantity of bioactive ingredient culture for 1kg or 1 litre formulation @ 30% : 300 ml

Quantity of bioactive ingredient (fungal mat) @ 16.67 X 300/100= 50.01

Thus, the quantity of wet fungal mat in 1000g : 50.01g

So, the bioactive ingredient per cent in the WP formulation = 5%

***M.anisopliae* :**

Total weight of 25 fungal mats of *M.anisopliae* harvested after 10 DAI= 152.50g

Total volume of 25 fungal culture bottles of *N.rileyi* at 10 DAI= 915ml

So, required quantity of bioactive ingredient culture for 1kg or 1 litre formulation @ 30% : 300 ml

Quantity of bioactive ingredient (fungal mat) @ 16.67 X 300/100= 50.01

Thus, the quantity of wet fungal mat in 1000g : 50.01g

So, the bioactive ingredient per cent in the WP formulation = 5%

4.1.2.2 Influence of concentrations on bioefficacy against *S.litura*

The results of the bioefficacy of standardised aqua suspension (30% v/v) of *N. rileyi* and *M.anisopliae* against II and III instar larvae of *S.litura* are presented in Table 5 to 8.

The II instar larvae of *S.litura*

***N.rileyi* :**

The mortality of *S.litura* at 5, 7 and 10 DAT ranged from 6.67 to 46.67, 16.67 to 63.33 and 36.67 to 90.0 per cent; respectively against zero kill in untreated control. The trend of mortality was almost same in all observations. Results (Table 5) revealed that the highest (46.67%) mortality was observed in the concentration of $1.8x10^7$cfu/ml and found to be significantly superior to all the treatments at 5 DAT. However, it was at par with $1.6x10^7$cfu /ml (43.11%) and $1.4x10^7$cfu/ml (36.67%).

Table 5. Influence of concentrations of 30% (v/v) of *N.rileyi* culture on bioefficacy against II instar larvae of *S.litura*

Tr.No.	*N.rileyi* conc. (cfu/ml)	Larval mortalitly (%)		
		5 DAT	7 DAT	10 DAT
T1	$2x10^6$	6.67 (15.00)*	16.67 (24.12)	36.67 (37.29)
T2	$4x10^6$	20.00 (26.56)	33.33 (35.24)	43.33 (41.15)
T3	$6x10^6$	23.33 (28.86)	33.33 (35.24)	56.67 (48.85)
T4	$8x10^6$	26.67 (31.11)	36.67 (37.29)	56.67 (48.85)
T5	$1.0x10^7$	30.00 (33.21)	40.00 (39.23)	60.00 (50.77)
T6	$1.2x10^7$	33.33 (35.24)	43.33 (41.15)	63.33 (52.71)
T7	$1.4x10^7$	36.67 (37.29)	46.67 (43.11)	66.67 (54.76)
T8	$1.6x10^7$	43.33 (41.15)	53.33 (46.89)	83.33 (65.88)
T9	$1.8x10^7$	46.67 (43.11)	63.33 (52.71)	90.00 (71.56)
T10	Untreated control	0.0 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E +	**2.57**	**2.38**	**2.19**
	C.D.(P=0.05)	**7.69**	**7.14**	**7.29**

*Figures in parentheses indicate arcsin values DAT = Days after treatment

The mortality of larvae at 10 DAT was in the range of 36.67 to 90.0 per cent. The treatment with $1.8x10^6$cfu/ml (90.0%) was superior over rest of the treatments except that with $1.6x10^7$cfu/ml (83.33%). Thus, it was observed that there was increase in mortality of *S.litura* larvae with increase in concentration and duration of exposure. Next promising and at par treatments with optimum mortality were $1.0x10^7$, $1.6x10^7$ and $8x10^6$cfu/ml.

***M.anisopliae* :**

The mortality of *S.litura* at 5, 7 and 10 DAT ranged from 6.67 to 40.0, 13.33 to 53.33 and 26.67 to 83.33 per cent, respectively against zero kill in untreated control. Various concentrations of *M.anisopliae* were evaluated against second instar larvae of *S.litura* (Table 6). The highest of 40.0 per cent mortality was observed in the concentration of $1.8x10^7$cfu/ml and found to be significantly superior to all the treatments at 5 DAT. However, it was at par with $1.6x10^7$cfu/ml (36.67%), $1.2x10^7$ and $1.4x10^7$cfu/ml (26.67% each).

Table 6. Influence of concentrations of 30% of *M.anisopliae* culture on bioefficacy against II instar larvae of *S.litura*

Tr. No.	*M.anisopliae* conc. (cfu/ml)	Larval mortalitly (%)		
		5 DAT	7 DAT	10 DAT
T1	$2x10^6$	6.67 (15.00)*	13.33 (21.39)	26.67 (31.11)
T2	$4x10^6$	16.67 (24.12)	23.33 (28.86)	33.33 (35.24)
T3	$6x10^6$	16.67 (24.12)	23.33 (28.86)	50.00 (45.00)
T4	$8x10^6$	20.00 (26.56)	30.00 (33.21)	53.33 (46.89)
T5	$1.0x10^7$	23.33 (28.86)	33.33 (35.24)	53.33 (46.89)
T6	$1.2x10^7$	26.67 (31.11)	36.67 (37.29)	60.00 (50.77)
T7	$1.4x10^7$	26.67 (31.11)	40.00 (39.23)	63.33 (52.71)
T8	$1.6x10^7$	36.67 (37.29)	46.67 (43.11)	73.33 (58.89)
T9	$1.8x10^7$	40.00 (39.23)	53.33 (46.89)	83.33 (65.88)
T10	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±	**2.75**	**1.90**	**1.90**
	C.D.(P=0.05)	**8.16**	**5.66**	**5.66**

*Figures in parentheses indicate arcsin values. DAT = Days after treatment

The mortality of larvae at 10 DAT, was in the range of 26.67 to 83.33 per cent. The treatment with $1.8x10^7$cfu/ml (83.33%) was superior over rest of the treatments. The next promising treatment was $1.6x10^7$cfu/ml (73.33%). Thus, it was observed that there was increase in mortality of *S.litura* larvae with increase in concentration and time of exposure. Next promising and at par treatments with optimum concentration were $1.4x10^7$ and $1.2x10^7$cfu/ml.

The III instar larvae of *S.litura*

***N.rileyi* :** The concentrations of $2x10^6$ to $1.8x10^7$cfu/ml were evaluated against the III instar larvae of *S.litura* (Table 7).

Table 7. Influence of concentrations of 30% of *N.rileyi* culture on bioefficacy against III instar larvae of *S.litura*

Treat. No.	*N.rileyi* conc. (cfu/ml)	Larval mortalitly (%) at		
		5 DAT	7 DAT	10 DAT
T1	$2x10^6$	3.33 (10.47)*	13.33(21.39)	30.00(33.21)
T2	$4x10^6$	13.33(21.39)	23.33(28.86)	36.67(37.29)
T3	$6x10^6$	16.67(24.12)	26.67(31.11)	50.00(45.00)
T4	$8x10^6$	20.00(26.56)	30.00(33.21)	50.00(45.00)
T5	$1.0x10^7$	23.33(28.86)	33.33(35.24)	53.33(46.89)
T6	$1.2x10^7$	26.67(31.11)	36.67(37.29)	56.67(48.85)
T7	$1.4x10^7$	30.00(33.21)	40.00(39.23)	60.00(50.77)
T8	$1.6x10^7$	36.67(37.29)	46.67(43.11)	76.67(61.14)
T9	$1.8x10^7$	40.00(39.23)	53.33(46.89)	86.67(68.61)
T10	Untreated control	0.00(0.00)	0.00(0.00)	0.00(0.00)
	S.E ±	**2.91**	**2.18**	**2.68**
	C.D.(P=0.05)	**8.73**	**6.55**	**8.03**

*Figures in parentheses indicate arcsin values DAT = Days after treatment

All the concentrations showed significantly higher mortality than untreated control in all the observations. The treatment with highest concentration of $1.8x10^7$ cfu /ml was most promising, recording the mortality of 86.67 % at 10 DAT. However, the concentration of 1.6 $x10^7$cfu/ml (76.67%) was at par to it. The next promising and at par treatments were $6x10^6$ to $1.4x10^7$ cfu/ml resulting in 50 to 60 per cent kill of the pest. Few caterpillars in higher concentrations showed external mycosis of the entomopathogenic fungi. However, internal fungal growth was observed in body fluid of the cadaver.

***M.anisopliae* :**

The bioefficacy of $2x10^6$ to $1.8x10^7$cfu/ml concentrations were evaluated against III instar larvae of *S.litura* (Table 8). All the concentrations showed significantly higher mortality than untreated control in all the observations. The treatment with highest concentration $1.8x10^7$cfu/ml was most promising, recording mortality of 80.0 per cent at 10 DAT. The treatment with $1.6x10^7$cfu/ml (66.67%) was found next to it at 10 DAT. Few caterpillars in higher concentrations showed external mycosis of the entomopathogenic fungi. However, internal fungal growth was observed in body fluid of the cadaver.

Table 8. Influence of concentrations of 30% of *M.anisopliae* culture on bioefficacy against III instar larvae of *S.litura*

Sr. No.	*M. anisopliae* conc. (cfu/ml)	Larval mortalitly (%)		
		5 DAT	7 DAT	10 DAT
T1	$2x10^6$	3.33 (10.47)*	10.00 (18.44)	26.67 (31.11)
T2	$4x10^6$	10.00 (18.44)	20.00 (26.56)	33.33 (35.24)
T3	$6x10^6$	13.33 (21.39)	23.33 (28.86)	46.67 (43.11)
T4	$8x10^6$	16.67 (24.12)	26.67 (31.11)	50.00 (45.00)
T5	$1.0x10^7$	20.00 (26.56)	30.00 (33.21)	50.00 (45.00)
T6	$1.2x10^7$	23.33 (28.86)	33.33 (35.24)	53.33 (46.89)
T7	$1.4x10^7$	26.67 (31.11)	36.67 (37.29)	56.67 (48.85)
T8	$1.6x10^7$	33.33 (35.24)	43.33 (41.15)	66.67 (54.76)
T9	$1.8x10^7$	36.67 (37.29)	50.00 (45.00)	80.00 (63.44)
T10	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±	**2.48**	**1.55**	**1.76**
	C.D.(P=0.05)	**7.38**	**4.60**	**5.22**

*Figures in parentheses indicate arcsin values. DAT = Days after treatment

The mortality of *S.litura* increased with increase in concentrations of *N.rileyi*. Vimladevi (1994), Kulkarni and Lingappa (2002) and Manjula and Krishnamurthy (2005) reported similar observations for bioefficacy of *N.rileyi* against *S.litura*. Dayakar and Kanujia (2003) observed that susceptibility of *S.litura* to *N.rileyi* and *M.anisopliae* decreased with increase in the age, as it

was also experienced in present study. However, the concentration is required to be optimised. Considering statistically at par treatments, *N.rileyi* $8x10^6$ and $1.0x10^7$cfu/ml gave 56.67 and 60.0 per cent mortality of the II instar larvae against the requirement of $1.2x10^7$ and $1.4x10^7$cfu/ml to get equal mortality of III instar caterpillar of *S.litura*. So, concentration of $6x10^6$ and $1.2x10^7$cfu/ml could be considered optimum for II and III instar larvae to get more than 50 per cent mortality.

4.1.3 Influence of adjuvants with inoculum on growth and development of *N.rileyi* and *M.anisopliae*

(Period: Aug. and Sep. 2010 Av.Temp.(^{0}C) :Max.-30 ±1,Min.-21 ±1 Av.Humidity (%) :Morn.-92, Even.-65)

4.1.3.1 Chemical adjuvants

The chemical adjuvants comprising glycerol, tween-80, triton-x-100, boric acid and magnesium sulphate with different concentrations in the standardized aqua suspension (30% v/v) of *N.rileyi* and *M.anisopliae* culture grown for 10 DAI were evaluated for growth and development of *N.rileyi* and *M.anisopliae* are presented in Table 9 and 10.

***N.rileyi* :**

The results presented in Table 9 indicated that the differences in surface coverage and biomass production in treatment with the concentrations of the adjuvants were significant at 3, 7 and 10 DAI. The surface coverage by *N.rileyi* in the treatment with boric acid 2.0 per cent was highest (46.67%).It was followed by boric acid 1.0 per cent (40.0%), glycerol 3.0 per cent (36.67%) and boric acid 0.5 per cent (36.67%) which were at par with each other. The treatment with triton-X-100 and tween-80 prevented the growth of the fungus at 3 DAI. The treatment without adjuvant recorded highest (30%) surface coverage at 3 DAI.

However, out of 32 treatments all over at 7 DAI, except the treatments with various concentrations of tween-80, triton-X-100, boric acid 5.0, CMC 1.50, magnesium sulphate 3.00, magnesium sulphate 5.0 per cent and control (*N.r.*alone) recorded cent per cent and significantly highest surface coverage than rest of the treatments.

At 10 DAI, the treatment with triton-X-100 1.0 per cent was extremely detrimental to the *N.rileyi* as evidenced from only 10 per cent surface coverage when all the rest of treatments including control without adjuvant showed cent per cent surface coverage.

Glycerol : Glycerol 2.0 per cent produced highest (9.97g) fungal biomass and was at par with glycerol 3.0 and 5.0 per cent recording 9.93 and 9.90g fungal biomass, respectively. In view of uncomparable surface coverage the adjuvants were evaluated on the basis of biomass development.

Tween-80 : All the treatments with Tween-80 0.03 to 1.0 per cent produced fungal biomass. Tween-80 0.50 per cent produced maximum (8.83 g) biomass. It was followed by tween-80 0.25 (8.30g), 1.0 per cent (8.0g), 0.12 per cent (7.50g) and 0.06 per cent (7.10g) and 0.03 per cent (7.0g).

Fungal biomass production increased upto concentration of 0.06 to 0.50 per cent tween-80 and decreased thereafter by increase in the concentration. Hence, the optimum concentration of tween-80 was 0.5 per cent for further study of combination of adjuvants in the aqua suspension formulation.

Triton-X-100 : The treatments with triton-X-100 0.06 and 0.12 per cent recorded highest (7.90g) fungal biomass which were at par to that with triton-X-100 0.03 per cent (7.73g) .

Thus, biomass production increased as concentration of triton-X-100 0.03 to 0.12 per cent and decreased over further increased in concentration of adjuvant. Therefore, the optimum concentration of triton-X-100 0.03 per cent was selected for further evaluation of combination of adjuvants.

Boric acid : All the treatments with boric acid recorded cent percent surface coverage by the fungus at 7 DAI except the treatment with boric acid 5.0 per cent (83.33%). At 10 DAI, the treatment with boric acid 2.0 per cent registered highest (9.80g) fungal biomass, followed by boric acid 3.0, 5.0, 1.0 and 0.5 per cent (9.63, 9.47, 9.35 and 9.30g), respectively.

The increase in concentration of boric acid upto 2.0 per cent increased the production of fungal biomass but after that it decreased. So, 2.0 per cent of boric acid was as adjuvant selected for further study.

Table 9. Effect of chemical adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatment	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*N.r.*+ Glycerol	1.0	18.33 (25.33)*	100.00 (90.00)	100.00 (90.00)	9.40
T2	*N.r.*+ Glycerol	2.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.97
T3	*N.r.*+ Glycerol	3.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	9.93
T4	*N.r.*+ Glycerol	5.0	20.00 (26.56)	100.00 (90.00)	100.00 (90.00)	9.90
T5	*N.r.*+ Tween-80	0.03	0.00 (0.00)	38.33 (38.23)	100.00 (90.00)	7.00
T6	*N.r.*+ Tween-80	0.06	0.00 (0.00)	46.67 (43.11)	100.00 (90.00)	7.10
T7	*N.r.*+ Tween-80	0.12	0.00 (0.00)	50.00 (45.00)	100.00 (90.00)	7.50
T8	*N.r.*+ Tween-80	0.25	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.30
T9	*N.r.*+ Tween-80	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.83
T10	*N.r.*+ Tween-80	1.00	0.00 (0.00)	58.33 (49.79)	90.00 (71.56)	8.00
T11	*N.r.*+ Triton-X-100	0.03	0.00 (0.00)	25.00 (30.00)	100.00 (90.00)	7.73
T12	*N.r.*+ Triton-X-100	0.06	0.00 (0.00)	30.00 (33.21)	100.00 (90.00)	7.90
T13	*N.r.*+ Triton-X-100	0.12	0.00 (0.00)	30.00 (33.21)	100.00 (90.00)	7.90
T14	*N.r.*+ Triton-X-100	0.25	0.00 (0.00)	20.00 (26.56)	100.00 (90.00)	7.00
T15	*N.r.*+ Triton-X-100	0.50	0.00 (0.00)	20.00 (26.56)	100.00 (90.00)	6.47
T16	*N.r.*+ Triton-X-100	1.00	0.00 (0.00)	6.67 (15.00)	10.00 (18.44)	0.90
T17	*N.r.*+ Boric Acid	0.5	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	9.30
T18	*N.r.*+ Boric Acid	1.0	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.35
T19	*N.r.*+ Boric Acid	2.0	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	9.80
T20	*N.r.*+ Boric Acid	3.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	9.63
T21	*N.r.*+ Boric Acid	5.0	23.33 (28.86)	83.33 (65.88)	100.00 (90.00)	9.47
T22	*N.r.*+ Carboxy methyl Cellulose	0.5	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	9.87

Table 9 Contd.....

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	3 DAI	3 DAI	
T23	*N.r.*+ Carboxy methyl Cellulose	0.75	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	9.80
T24	*N.r.*+ Carboxy methyl Cellulose	1.0	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)	9.60
T25	*N.r.*+ Carboxy methyl Cellulose	1.25	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	9.23
T26	*N.r.*+ Carboxy methyl Cellulose	1.5	20.00 (26.56)	90.00 (71.56)	90.00 (71.56)	7.80
T27	*N.r.*+ Magnesium Sulphate	0.5	20.00 (26.56)	100.00 (90.00)	100.00 (90.00)	5.80
T28	*N.r.*+ Magnesium Sulphate	1.0	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	6.07
T29	*N.r.*+ Magnesium Sulphate	2.0	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.20
T30	*N.r.*+ Magnesium Sulphate	3.0	30.00 (33.21)	93.33 (75.00)	100.00 (90.00)	5.93
T31	*N.r.*+ Magnesium Sulphate	5.0	25.00 (30.00)	91.67 (73.26)	100.00 (90.00)	5.77
T32	Control (*N.r.* alone)		30.00 (33.21)	46.67 (43.11)	100.00 (90.00)	7.43
	S.E ±		**1.05**	**2.50**	**1.35**	**0.15**
	C.D.(P=0.05)		**2.97**	**7.09**	**3.82**	**0.43**

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
N.r. = Nomuraea rileyi

CMC : 0.5 to 1.25 per cent CMC registered the cent per cent surface coverage at 7 DAI. At 10 DAI, CMC 0.5 per cent recorded highest (9.87g) fungal biomass. However, it was at par with CMC 0.75 per cent (9.80g), and CMC 1.0 per cent (9.60g). In view of inverse trend in CMC concentration and the biomass production of CMC 0.5 per cent was choosen for further studies of combinations of adjuvants.

The magnesium sulphate as adjuvant was detrimental for development (5.80 to 6.20g) as compared to the control (7.43g).

***M.anisopliae* :**

Data presented in Table 10 revealed the significant differences in surface coverage and biomass production of *M.anisopliae.*

Glycerol : At 3 DAI, the fungus culture with glycerol from 1.0 to 5.0 per cent showed significant growth of the mycoagent. Glycerol 1.0 per cent recorded highest (93.33%) surface coverage. It was followed by glycerol 2.0 per cent (85.0%). At 7 and 10 DAI, all the treatments of glycerol showed cent per cent surface coverage of the medium. At 10 DAI, glycerol 3.0 per cent produced highest (8.17g) biomass which was at par with glycerol 2.0 per cent (7.97g).

Tween 80: Treatments of tween-80 at 0.03 to 0.12 per cent did not show growth at 3 DAI. Tween-80 0.50 per cent recorded highest (13.33%) surface coverage among comprising tween-80 trearments at 3 DAI. At 7 DAI, tween-80 0.50 and 1.0 per cent recorded cent per cent surface coverage; followed by tween-80 0.25 per cent (40.0%), tween-80 0.12, 0.06 and 0.03 per cent (36.67% each).

The fungal biomass at 10 DAI, was maximum (7.47 g) in tween-80 0.50 per cent. It was followed by tween-80 1.0 per cent (7.0g), tween-80 0.25 per cent (6.47g), tween-80 0.12 per cent (6.43g) and tween-80 0.06 per cent (6.37g) and tween-80 0.03 per cent (6.27g).

Fungal biomass production increased upto concentration of 0.06 to 0.50 per cent tween-80 and decreased thereafter by increase in the concentration. Hence, the optimum concentration of tween-80 was 0.5 per cent for further study of combination of adjuvants in the aqua suspension formulation.

Triton-X-100 : Among the various concentration of triton-X-100 the growth of fungus was not observed on 3 DAI. At 7 DAI, the treatment with triton-X-100 0.06, 0.12 and 0.25 per cent showed highest (28.33%) surface coverage. It was followed by triton-X-100 0.03 per cent (18.33%). Triton-X-100 0.50 and 1.0 per cent was extremely detrimental to *M.anisopliae* as evidenced from no growth and development of the fungus. Triton-X-100 0.03 per cent produced highest (6.80g) biomass. It was followed by triton-X-100 0.06, 0.12 and 0.25 percent, (6.50g 4.80 and 4.40g), respectively.

Thus, biomass production decreased as concentration of triton-X-100 from 0.03 to 0.25 per cent increased. The biomass was not observed at the

higher concentrations of 0.50 and 1.0 per cent. Therefore, the optimum concentration of triton-X-100 0.03 per cent was selected for further evaluation of utility of combination of adjuvants.

Boric acid: The treatments at 0.5 to 5.0 per cent with boric acid at 3 DAI recorded 10.0 to 16.67 per cent surface coverage by fungal mycoagent. The highest (16.67%) per cent surface coverage was recorded in boric acid 2.0 per cent. It was followed by boric acid 3.0, 5.0, 1.0 and 0.5 per cent (15.0, 15.0, 11.67 and 10.0%). All the treatments with boric acid recorded cent per cent surface coverage by fungus at 7 and 10 DAI. At 10 DAI boric acid 3.0 per cent registered highest (8.17g) fungal biomass production. However, it was at par with boric acid 2.0 per cent (7.97g)

The increase in concentration of boric acid upto 2.0 per cent increased the production of fungal biomass but after that it decreased. So, 2.0 per cent of boric acid was as adjuvant selected for further study.

Carboxymethyl cellulose (CMC): In various concentration of CMC ranging from 0.5 to 1.5 per cent, registered the cent per cent surface coverage at 7 and 10 DAI. At 10 DAI, CMC 0.5 per cent recorded highest of 9.0g fungal biomass. However, it was at par with CMC 0.75 per cent (8.83g). It was followed by CMC 1.0 per cent (8.77g). In view of inverse trend in CMC concentration and the biomass production of CMC 0.5 per cent was choosen for further studies of combinations of adjuvants.

Magnesium sulphate: The magnesium sulphate as adjuvant was detrimental for development (4.90 to 5.27g) as compared to the control without adjuvant (7.43g).

Overall on the basis of performance of the concentrations of chemical adjuvants for production of biomass of *N.rileyi* and *M.anisopliae,* glycerol 2.0%, boric acid 2.0%, CMC 0.5% and tween-80 0.5% resulting in 8.83 to 9.97g and 7.47 to 9.0g biomass per 40 ml medium, respectively, emerged as best adjuvants for further study on development of the aqua suspension formulation. The 0.03% triton-X-100 which produced 7.73 and 6.80g biomass of *N.rileyi* and *M.anisoplie* was also considered as dispersing and spreading agent for the formulation.

Table 10. Effect of chemical adjuvants with inoculum in *M. anisopliae* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*M.a.*+ Glycerol	1.0	93.33 (75.00)*	100.00 (90.00)	100.00 (90.00)	6.30
T2	*M.a.*+ Glycerol	2.0	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	7.97
T3	*M.a.*+ Glycerol	3.0	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	8.17
T4	*M.a.*+ Glycerol	5.0	73.33 (58.89)	100.00 (90.00)	100.00 (90.00)	7.47
T5	*M.a.*+ Tween 80	0.03	0.00 (0.00)	36.67 (37.29)	100.00 (90.00)	6.27
T6	*M.a.*+ Tween 80	0.06	0.00 (0.00)	36.67 (37.29)	100.00 (90.00)	6.37
T7	*M.a.*+ Tween 80	0.12	0.00 (0.00)	36.67 (37.29)	100.00 (90.00)	6.43
T8	*M.a.*+ Tween 80	0.25	11.67 (20.00)	40.00 (39.23)	100.00 (90.00)	6.47
T9	*M.a.*+ Tween 80	0.50	13.33 (21.39)	100.00 (90.00)	100.00 (90.00)	7.47
T10	*M.a.*+ Tween 80	1.00	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	7.00
T11	*M.a.*+ Triton X 100	0.03	0.00 (0.00)	18.33 (25.33)	100.00 (90.00	6.80
T12	*M.a.*+ Triton X 100	0.06	0.00 (0.00)	28.33 (32.14)	100.00 (90.00	6.50
T13	*M.a.*+Triton X 100	0.12	0.00 (0.00)	28.33 (32.14)	96.67 (79.53)	4.80
T14	*M.a.*+Triton X 100	0.25	0.00 (0.00)	28.33 (32.14)	96.67 (79.53)	4.40
T15	*M.a.*+Triton X 100	0.50	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
T16	*M.a.*+Triton X 100	1.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
T17	*M.a.*+ Boric Acid	0.5	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	7.03
T18	*M.a.*+ Boric Acid	1.0	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	7.37
T19	*M.a.*+ Boric Acid	2.0	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	7.97
T20	*M.a.*+ Boric Acid	3.0	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	8.17
T21	*M.a.*+ Boric Acid	5.0	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	7.90
T22	*M.a.*+ Carboxymethyl Cellulose	0.5	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	9.00
T23	*M.a.*+ Carboxymethyl Cellulose	0.75	18.33 (25.33)	100.00 (90.00)	100.00 (90.00)	8.83
T24	*M.a.*+ Carboxymethyl Cellulose	1.0	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	8.77
T25	*M.a.*+ Carboxymethyl Cellulose	1.25	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	7.57
T26	*M.a.*+ Carboxymethyl Cellulose	1.5	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.90
T27	*M.a.*+ Magnesium sulphate	0.5	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	5.07
T28	*M.a.*+ Magnesium sulphate	1.0	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	5.27

Table 10 Contd.....

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T29	*M.a.*+ Magnesium sulphate	2.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	5.23
T30	*M.a.*+ Magnesium sulphate	3.0	28.33 (32.39)	100.00 (90.00)	100.00 (90.00)	5.23
T31	*M.a.*+ Magnesium sulphate	5.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	4.90
T32	Control. (*M.a.*alone)	-	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	6.23
	S.E ±		**1.17**	**0.75**	**0.99**	**0.07**
	C.D.(P=0.05)		**3.31**	**2.12**	**2.79**	**0.21**

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
M.a. = Metarhizium anisopliae

The literature on impact of the promising adjuvants on growth and biomass development is lacking for the fungus. The report of Campbell *et al.* (1978) about best sporulation of *M.anisopliae* and that of Silva *et al.* (2005) regarding more growth of *M.anisopliae* with high lipase activity by addition of tween-80 supported the utility of glycerol and tween-80 for development and viability of *N.rileyi* in present study.

4.1.3.2 Edible oils (vegetable oils) as adjuvants

***N.rileyi* :**

Effect on growth : Data presented in Table 11 revealed that at 3 DAI, all the edible oils irrespective of their concentrations showed significantly higher growth of *N.rileyi* at 3, 7 and 10 DAI, except that in mustard oil 0.25 and 0.5 per cent. The surface coverage ranged from 31.67 to 93.33 per cent in oil supplements against 30.0 per cent in control. At 7 DAI, all treatments except control and at 10 DAI all the treatments showed cent per cent surface coverage.

Effect on biomass / 40 ml medium : In view cent per cent surface coverage, biomass production remained as parameter to decide best oil adjuvants. SFO 2% was the best adjuvant which resulted in maximum biomass of 12.50g. However, it was at par to the treatment with *N.r.*+SFO 1.0% (12.43g), *N.r.*+GH 1.0% (11.80g) and *N.r.*+GH 0.5% (11.77g).

Table 11. Effect of edible oils as adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc.(%) of adjuvant	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*N.r.*+ GNO	0.25	75.00 (60.00)*	100.00 90.00)	100.00 (90.00)	9.03
T2	*N.r.*+ GNO	0.5	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.00
T3	*N.r.*+ GNO	1.0	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.10
T4	*N.r.*+ GNO	2.0	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.17
T5	*N.r.*+ SFO	0.25	63.33 (52.71)	100.00 (90.00)	100.00 (90.00)	10.00
T6	*N.r.*+ SFO	0.5	68.33 (55.73)	100.00 (90.00)	100.00 (90.00)	11.43
T7	*N.r.*+ SFO	1.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.43
T8	*N.r.*+ SFO	2.0	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	12.50
T9	*N.r.*+ SBO	0.25	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.00
T10	*N.r.*+ SBO	0.50	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	10.03
T11	*N.r.*+ SBO	1.0	58.33 (49.79)	100.00 (90.00)	100.00 (90.00)	10.13
T12	*N.r.*+ SBO	2.0	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	10.17
T13	*N.r.*+ CNO	0.25	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	9.00
T14	*N.r.*+ CNO	0.5	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	9.20
T15	*N.r.*+ CNO	1.0	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	9.33
T16	*N.r.*+ CNO	2.0	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	9.37
T17	*N.r.*+ MUO	0.25	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	8.00
T18	*N.r.*+ MUO	0.5	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	8.97
T19	*N.r.*+ MUO	1.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	8.93
T20	*N.r.*+ MUO	2.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	9.00
T21	*N.r.*+ GH	0.25	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.50
T22	*N.r.*+ GH	0.5	91.67 (73.26)	100.00 (90.00)	100.00 (90.00)	11.77
T23	*N.r.*+ GH	1.0	93.33 (75.00)	100.00 (90.00)	100.00 (90.00)	11.80
T24	Control (*N.r.* alone)	-	30.00 (33.21)	45.00 (42.13)	100.00 (90.00)	7.40
	S.E +		**2.20**	**-**	**-**	**0.26**
	C.D.P=0.05)		**6.24**	**--**	**--**	**0.76**

*Figures in parentheses indicate arcsin values DAI = Days after inoculation *N.r.* = *Nomuraea rileyi* GNO=Groundnut oil SFO= Sunflower oil SBO= Soybean oil CNO=Coconut oil MUO=Mustard oil GH=Ghee

The next highly promising and at par treatments were *N.r.*+GH 0.50%, *N.r.*+SFO 0.5% (11.43g), *N.r.*+GNO 2.0% (11.17g), *N.r.*+GNO 1.0% (11.10g). The biomass in rest of the treatments ranged from 8.0 to 11.0g; when it was 7.40g in control.

***M.anisopliae* :**

Effect on growth : Data presented in Table 12 revealed that all the edible oils irrespective of their concentrations showed significantly higher growth of *M.anisopliae* at 3, 7 and 10 DAI. The surface coverage at 3 DAI ranged from 30.0 to 86.67 per cent in oil treatments against 26.67 per cent in control. At 7 DAI and 10 DAI, all the treatments showed cent per cent surface coverage.

Effect on biomass / 40 ml medium : SFO 2% was the best adjuvant resulting in maximum biomass of 8.40g. However, it was at par with *M.a.*+SFO 1.0% (8.37g), *M.a.*+GNO 1.0% (8.23g), *M.a.*+GNO 2.0% (8.23g), *M.a.*+GNO 0.5% (8.20g), *M.a.*+GH 1.0% (8.20g) and *M.a.*+GH 0.5% (8.07g). The next highly promising adjuvants was SFO 0.5% (8.0g). The biomass in rest of the treatments ranged from 6.40 to 7.53g; when it was 6.30g in control.All the edible oils showed increase in growth and development of *N.rileyi* and *M.anisopliae* with increase in concentration of oils.

In view of at par biomass development in lower concentrations of oil than their higher concentrations, the lower concentrations were considered optimum and more appropriate adjuvant. Accordingly, SFO 1.0%, GH 0.5%, GNO 0.5%, SBO 0.5%, MUO 0.5% and CNO 1.0% were most promising to economise the adjuvants. These showed 8.97 to 12.43g and 6.50 to 8.37g biomass developed of *N.rileyi* and *M.anisopliae,* respectively.

The published literature on impact of oils on growth and biomass production is meagre. Kaur *et al.* (1999) found that sunflower oil and groundnut oil accelerated spore germination and growth of *B.bassiana*. The mycelial weight increased with increase in concentration of oils. The reports are in conformity with the increased biomass development of *N.rileyi* by addition oils of sunflower and groundnut in present study. The results of promising performance of ghee could not be compared as the work on it seemed to be pioneer.

Table 12. Effect of edible oils as adjuvants with inoculum in *M.anisopliae* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*M.a.*+ GNO	0.25	30.00 (33.21)*	100.00 (90.00)	100.00 (90.00)	7.10
T2	*M.a.*+ GNO	0.5	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	8.20
T3	*M.a.*+ GNO	1.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	8.23
T4	*M.a.*+ GNO	2.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	8.23
T5	*M.a.*+ SFO	0.25	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.03
T6	*M.a.*+ SFO	0.5	66.67 (54.76)	100.00 (90.00)	100.00 (90.00)	8.00
T7	*M.a.*+ SFO	1.0	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	8.37
T8	*M.a.*+ SFO	2.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	8.40
T9	*M.a.*+ SBO	0.25	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	6.33
T10	*M.a.*+ SBO	0.50	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	6.50
T11	*M.a.*+ SBO	1.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	6.60
T12	*M.a.*+ SBO	2.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	6.63
T13	*M.a.*+ CNO	0.25	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.57
T14	*M.a.*+ CNO	0.5	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	6.80
T15	*M.a.*+ CNO	1.0	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	7.50
T16	*M.a.*+ CNO	2.0	61.67 (51.77)	100.00 (90.00)	100.00 (90.00)	7.53
T17	*M.a.*+ MUO	0.25	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	6.40
T18	*M.a.*+ MUO	0.5	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	7.20
T19	*M.a.*+ MUO	1.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.30
T20	*M.a.*+ MUO	2.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.33
T21	*M.a.*+ GH	0.25	65.00 (53.73)	100.00 (90.00)	100.00 (90.00)	7.50
T22	*M.a.*+ GH	0.5	71.67 (57.86)	100.00 (90.00)	100.00 (90.00)	8.07
T23	*M.a.*+ GH	1.0	75.00 (60.00)	100.00 (90.00)	100.00 (90.00)	8.20
T24	Control(*M.a.*alone)	-	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	6.30
	S.E ±		**1.52**	**-**	**-**	**0.13**
	C.D.(P=0.05)		**4.30**	**-**	**-**	**0.38**

*Figures in parentheses are arcsin values DAI= Days after inoculation
M.a = Metarhizium anisopliae GNO=Groundnut oil SFO= Sunflower oil SBO= Soybean oil CNO=Coconut oil MUO=Mustard oil GH=Ghee

4.1.3.3 Other edible substrates as adjuvants

The other edible substrates comprising honey, molasses, milk, wheat, sorghum, bajra, rice and corn flour and turmeric and their various concentrations were tested for their effect on growth and development of *N.rileyi* and *M.anisopliae* in aqua suspension.

***N.rileyi* :**

Effect on growth : The results on impact of some edible adjuvants on growth and development of *N.rileyi* are presented in Table 13.

Table 13. Effect of other edible substrates as adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*N.r.*+ Honey	0.5	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	11.27
T2	*N.r.*+ Honey	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	11.67
T3	*N.r.*+ Honey	2.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	11.77
T4	*N.r.*+ Molasses	1.0	28.33 (32.74)	56.67 (48.85)	60.00 (50.77)	6.63
T5	*N.r.*+ Molasses	2.0	30.00 (33.21)	71.67 (57.86)	75.00 (60.00)	6.87
T6	*N.r.*+ Molasses	3.0	30.00 (33.21)	95.00 (77.08)	98.33 (82.51)	6.93
T7	*N.r.*+ Molasses	4.0	30.00 (33.21)	98.33 (82.51)	100.00 (90.00)	6.90
T8	*N.r.*+ Milk	0.5	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	7.70
T9	*N.r.*+ Milk	1.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.90
T10	*N.r.*+ Milk	2.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.90
T11	*N.r.*+ Milk	3.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.87
T12	*N.r.*+ Wheat flour	1.0	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	7.83
T13	*N.r.*+ Wheat flour	2.0	18.33 (25.33)	100.00 (90.00)	100.00 (90.00)	7.90
T14	*N.r.*+ Wheat flour	3.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.97
T15	*N.r.*+ Wheat flour	4.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	7.97
T16	*N.r.*+ Wheat flour	5.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	7.87
T17	*N.r.*+ Corn flour	1.0	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	7.93
T18	*N.r.*+Corn flour	2.0	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	8.00

Table 13 contd .....

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T19	*N.r.*+Corn flour	3.0	65.00 (53.73	100.00 (90.00)	100.00 (90.00)	8.03
T20	*N.r.*+ Corn flour	4.0	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	7.97
T21	*N.r.*+ Corn flour	5.0	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	7.90
T22	*N.r.*+ Sorghum flour	1.0	20.00 (26.56)	100.00 (90.00)	100.00 (90.00)	6.90
T23	*N.r.*+ Sorghum flour	2.0	41.67 (40.22)	100.00 (90.00)	100.00 (90.00)	7.10
T24	*N.r.*+ Sorghum flour	3.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	7.43
T25	*N.r.*+ Sorghum flour	4.0	61.67 (51.77)	100.00 (90.00)	100.00 (90.00)	7.63
T26	*N.r.*+ Sorghum flour	5.0	68.33 (55.73)	100.00 (90.00)	100.00 (90.00)	7.70
T27	*N.r.*+ Bajra flour	1.0	18.33 (25.33)	100.00 (90.00)	100.00 (90.00)	7.53
T28	*N.r.*+ Bajra flour	2.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	7.07
T29	*N.r.*+ Bajra flour	3.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.17
T30	*N.r.*+ Bajra flour	4.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.10
T31	*N.r.*+ Bajra flour	5.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	7.10
T32	*N.r.*+ Rice flour	1.0	43.33 (41.15)	100.00 (90.00)	100.00 (90.00)	7.67
T33	*N.r.*+ Rice flour	2.0	48.33 (44.03)	100.00 (90.00)	100.00 (90.00)	7.77
T34	*N.r.*+ Rice flour	3.0	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	7.80
T35	*N.r.*+ Rice flour	4.0	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	7.87
T36	*N.r.*+ Rice flour	5.0	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	7.87
T37	*N.r.*+ Turmeric	0.5	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	5.77
T38	*N.r.*+ Turmeric	1.0	20.00 (26.56)	100.00 (90.00)	100.00 (90.00)	5.03
T39	*N.r.*+ Turmeric	1.5	16.67 (24.12)	18.33 (25.33)	43.33 (41.15)	4.67
T40	Control (*N.r.*alone)	-	30.00 (33.21)	46.67 (43.11)	100.00 (90.00)	7.30
	S.E ±		**1.29**	**0.96**	**0.97**	**0.04**
	C.D.(P=0.05)		**3.62**	**2.70**	**2.74**	**0.10**

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation *N.r. = Nomuraea rileyi*

The trend of growth of *N.rileyi* at 3, 7 and 10 DAI in the treatments was more or less similar to previous experiments. All the adjuvant treatments allowed

cent per cent surface coverage at 10 DAI except turmeric 1.5% and molasses 1 to 3%.

Effect on biomass/ 40 ml medium : The differences in biomass produced by the treatments were significant at 10 DAI. Honey 2.0 and 1.0 per cent was the best adjuvant which showed higher (11.67 to 11.77g) biomass. Except adverse effect of turmeric, molasses, sorghum flour 1 to 2 per cent and bajra flour (4.67 to 7.20g) rest of the treatments showed more fungal biomass (7.43 to 8.03g) than control (7.30g). However, the increase in biomass was not satisfactory in the substrate except honey.

***M.anisopliae* :**

Effect on growth : The results on impact of some edible adjuvants on growth and development of *M.anisopliae* are presented in Table 14.

Table 14. Effect of other edible substrates as adjuvants with inoculum in *M.anisopliae* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T1	*M.a.*+ Honey	0.5	83.33 (65.88)*	100.00 (90.00)	100.00 (90.00)	8.90
T2	*M.a.*+ Honey	1.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	9.10
T3	*M.a.*+ Honey	2.0	91.67 (73.26)	100.00 (90.00)	100.00 (90.00)	9.20
T4	*M.a.*+ Molasses	1.0	5.00 (12.92)	73.33 (58.89)	100.00 (90.00)	7.53
T5	*M.a.*+ Molasses	2.0	5.00 (12.92)	75.00 (60.00)	100.00 (90.00)	7.63
T6	*M.a.*+ Molasses	3.0	3.33 (10.47)	65.00 (53.73)	100.00 (90.00)	7.63
T7	*M.a.*+ Molasses	4.0	5.00 (12.92)	66.67 (54.76)	100.00 (90.00)	7.53
T8	*M.a.*+ Milk	0.5	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	6.40
T9	*M.a.*+ Milk	1.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	6.70
T10	*M.a.*+ Milk	2.0	48.33 (44.03)	100.00 (90.00)	100.00 (90.00)	6.83
T11	*M.a.*+ Milk	3.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	6.90
T12	*M.a.*+ Wheat flour	1.0	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	6.57
T13	*M.a.*+ Wheat flour	2.0	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	6.67
T14	*M.a.*+ Wheat flour	3.0	73.33 (58.89)	100.00 (90.00)	100.00 (90.00)	7.00

Table 14 Contd……

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium
			3 DAI	7 DAI	10 DAI	
T15	*M.a.*+ Wheat flour	4.0	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	7.20
T16	*M.a.*+ Wheat flour	5.0	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	7.23
T17	*M.a.*+ Corn flour	1.0	20.00 (26.56)	100.00 (90.00)	100.00 (90.00)	7.13
T18	*M.a.*+ Corn flour	2.0	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	7.27
T19	*M.a.*+ Corn flour	3.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	7.40
T20	*M.a.*+ Corn flour	4.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	7.43
T21	*M.a.*+ Corn flour	5.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	7.47
T22	*M.a.*+ Sorghum flour	1.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	6.33
T23	*M.a.*+ Sorghum flour	2.0	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	6.33
T24	*M.a.*+ Sorghum flour	3.0	61.67 (51.77)	100.00 (90.00)	100.00 (90.00)	6.43
T25	*M.a.*+ Sorghum flour	4.0	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	6.57
T26	*M.a.*+ Sorghum flour	5.0	61.67 (51.77)	100.00 (90.00)	100.00 (90.00)	6.57
T27	*M.a.*+ Bajra flour	1.0	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.60
T28	*M.a.*+ Bajra flour	2.0	31.67 (34.27)	100.00 (90.00)	100.00 (90.00)	6.70
T29	*M.a.*+ Bajra flour	3.0	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	6.73
T30	*M.a.*+ Bajra flour	4.0	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	6.67
T31	*M.a.*+ Bajra flour	5.0	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	6.67
T32	*M.a.*+ Rice flour	1.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	6.80
T33	*M.a.*+ Rice flour	2.0	43.33 (41.15)	100.00 (90.00)	100.00 (90.00)	6.90
T34	*M.a.*+ Rice flour	3.0	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	6.93
T35	*M.a.*+ Rice flour	4.0	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	7.00
T36	*M.a.*+ Rice flour	5.0	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	7.03
T37	*M.a.*+ Turmeric	0.5	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	6.70
T38	*M.a.*+ Turmeric	1.0	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	6.70
T39	*M.a.*+ Turmeric	1.5	13.33 (21.39)	100.00 (90.00)	100.00 (90.00)	6.17
T40	Control (*M.a.*alone)	-	26.67 (31.11)	100.00 (90.00)	100.00 (90.00)	6.30
	S.E ±		**1.75**	**0.55**	**-**	**0.06**
	C.D.(P=0.05)		**4.92**	**1.55**	**-**	**0.16**

*Figures in parentheses indicate arcsin values DAI = Days after inoculation *M.a.*= *Metarhizium anisopliae*

The trend of growth of *M.anisopliae* at 3, 7 and 10 DAI in the treatments was more or less similar to previous observed for *N.rileyi*. All the adjuvant treatment allowed cent per cent surface coverage at 7 DAI except molasses 1 to 4%.

Effect on biomass/ 40 ml medium : The differences in biomass produced by the treatments were significant at 10 DAI. Honey 1.0 and 2.0 per cent was the best nutrient source which showed highest (9.10 to 9.20g) biomass.

These results established that honey 0.5, 1.0 and 2.0 per cent was superior among other edible adjuvants to formulate *N.rileyi* and *M.anisopliae*. Hence, honey 1.0 per cent was considered as optimum as nutrient source giving 11.67g and 9.10g biomass of *N.rileyi* and *M.anisopliae*, respectively.

Results of honey as promising adjuvant for biomass development of both entomopathogenic fungus could not be compared for want of published information.

4.1.5 Effect of UVC rays on growth and development of inoculum in formulation of *N.rileyi* and *M.anisopliae*

(Period: Sep.to Dec. 2010, Jan.2011 Av.Temp.(^{0}C):Max.-28 ±1, Min.- 9 ±1 Av.Humidity (%) :Morn.-90, Even.-35)

4.1.4.1 Effect of 10 to 50 minutes and 1 to 5 hours UVC rays on growth of the mycoagents

N.rileyi and *M.anisopliae* liquid cultures with various adjuvants exposed to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours and observations on per cent surface coverage by the fungal growth on the medium although noted at 3,7 and 10 days after exposure results at 10 DAI are presented in Table 15 and 16 to avoid complications in interpretation of the voluminous data.

***N.rileyi* : Effect on growth:**

UVC exposure –10 minutes: At 10 DAI, all the treatments with various adjuvants recorded cent per cent growth of fungus except the treatments with tween-80 0.5 per cent (48.33%), tween-80 1.0 per cent (60.0%) and

control (*N.r.*alone) (98.33%), respectively. Overall 10 minutes UVC rays exposure was harmless to *N. rileyi.* Detrimental effect of tween-80 might be attributed to breaking surface tension on *N.rileyi* spores due to its wetting agent action which might had permitted adverse impact of UVC rays.

UVC exposure –20, 30, 40 and 50 minutes: The trend of results was almost same in all observations except glycerol 1 and 2% comprising treatments which showed slightly low (96.67 to 98.33%), moderately low (83.33 to 95%) and (81.67 to 90%) at 1 to 5% concentrations by the exposure for 20, 30 and 40, 50 minutes, respectively. Tween-80 was consistently harmful to the fungal growth on UVC ray exposure, when all the treatments showed cent per cent medium surface coverage.

UVC exposure –2, 3 and 5 hrs : The trend of results was almost similar to that in previous observations. Glycerol 1 to 5% and Tween-80 0.5 and 1% resulted in 78.33 to 90.0, 78.33 to 86.67, 76.67 to 81.67 and 21.67 to 30, 18.33 to 26.67 and 16.67 to 25.0 per cent growth by 2, 3 and 5 hrs exposure, respectively, against complete surface coverage by rest of the treatments. The formulation of *N.r.*alone (without UVC rays exposure) recorded cent per cent surface coverage of medium.

Table 15. Influence of *N.rileyi* with adjuvants on growth of *N.rileyi* exposed to UVC rays

Tr. No.	Treatments	Conc. (%)of adj.	Surface coverage (%) on 10 DAI in UVC exposure upto							
			10 min	20 min	30 min	40min	50 min	2 hrs	3 hrs	5 hrs
T1	*N.r.*+ GLY	1.0	100.0 (90.0)*	100.00 (90.00)	98.33 (82.51)	83.33 (65.88)	81.67 (64.67)	80.00 (63.44)	78.33 (62.24)	76.67 (61.14)
T2	*N.r.*+ GLY	2.0	100.0 (90.0)	100.00 (90.00)	98.33 (82.51)	95.00 (77.08)	90.00 (71.56)	90.00 (71.56)	86.67 (68.61)	81.67 (64.67)
T3	*N.r.*+ GLY	3.0	100.0 (90.0)	100.00 (90.00)	100.00 (90.00)	90.00 (71.56)	85.00 (67.21)	83.33 (65.88)	81.67 (64.67)	78.33 (62.24)
T4	*N.r.*+ GLY	5.0	100.0 (90.0)	100.00 (90.00)	96.67 (79.53)	85.00 (67.21)	83.33 (65.88)	78.33 (62.24)	78.33 (62.24)	76.67 (61.14)
T5	*N.r.*+ TW-80	0.5	48.33 (44.03)	45.00 (42.13)	43.33 (41.15)	33.33 (35.24)	33.33 (35.24)	21.67 (27.76)	18.33 (25.33)	16.67 (24.12)
T6	*N.r.*+ TW-80	1.0	60.00 (50.77)	58.33 (49.78)	55.00 (47.87)	50.00 (45.00)	43.33 (41.15)	30.00 (33.21)	26.67 (31.11)	25.00 (30.00)
T7	*N.r.*+ BA	1.0	100.0 (90.0)	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)
T8	*N.r.*+ BA	2.0	100.0 (90.0)	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)
T9	*N.r.*+ BA	3.0	100.0 (90.0)	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)
T10	*N.r.*+ CMC	0.5	100.0 (90.0)	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)

Table 15 Contd.....

Tr. No.	Treatments	Conc. (%)of adj.	Surface coverage (%) on 10 DAI in UVC exposure upto							
			10 min	20 min	30 min	40min	50 min	2 hrs	3 hrs	5 hrs
T11	*N.r.*+ CMC	1.0	100.0 (90.0)	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)
T12	*N.r.*+ Indigo	0.5	100.0 (90.0)	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)
T13	*N.r.*+ Indigo	1.0	100.0 (90.0)	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)
T14	*N.r.*+ Turmeric	0.5	100.0 (90.0)	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)
T15	*N.r.*+ Turmeric	1.0	100.0 (90.0)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	91.67 (73.26)	91.67 (73.26)
T16	*N.r.*+ Molasses	1.0	100.0 (90.0)	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)
T17	*N.r.*+ Molasses	2.0	100.0 (90.0)	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)
T18	*N.r.*+HO	0.5	100.0 (90.0)	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)
T19	*N.r.*+HO	1.0	100.0 (90.0)	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)
T20	*N.r.*+ Milk	1.0	100.0 (90.0)	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)
T21	*N.r.*+ Milk	2.0	100.0 (90.0)	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)
T22	*N.r.*+ SFO	0.5	100.0 (90.0)	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)
T23	*N.r.*+ SFO	1.0	100.0 (90.0)	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)
T24	*N.r.*+ GNO	0.5	100.0 (90.0)	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)
T25	*N.r.*+ GNO	1.0	100.0 (90.0)	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)
T26	*N.r.*+ GNO	2.0	100.0 (90.0)	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)
T27	*N.r.*+ SBO	0.5	100.0 (90.0)	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)
T28	*N.r.*+ SBO	1.0	100.0 (90.0)	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)
T29	*N.r.*+ MUO	0.5	100.0 (90.0)	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)
T30	*N.r.*+ MUO	1.0	100.0 (90.0)	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)
T31	*N.r.*+ GH	0.5	100.0 (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)
T32	*N.r.*+ GH	1.0	100.0 (90.0)	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)
T33	Control (*N.r.*alone)	-	100.0 (90.0)	100.00 (90.00)	100.00 (90.00)	93.33 (75.00)	90.00 (71.56)	81.67 (64.67)	78.33 (62.24)	71.67 (57.86)
T34	Control (*N.r.*alone) (W.UVC)	-	100.0 (90.0)	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)
	S.E +		**0.27**	**0.25**	**1.19**	**0.55**	**0.67**	**0.75**	**0.62**	**0.75**
	C.D (P=0.05)		**0.76**	**0.70**	**3.37**	**1.56**	**1.89**	**2.12**	**1.74**	**2.12**

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
N.r. = Nomuraea rileyi W. UVC=without UVC TW= Tween-80
SFO = Sunflower oil GLY= Glycerol CNO = Coconut oil
TX = Triton-X-100 GRO = Groundnut oil BA = Boric acid
SBO = Soybean oil MUO = Mustard oil GH = Ghee
CMC = Carboxymethyl Cellulose

***M.anisopliae* :**

Effect on growth : UVC exposure-10 minutes : At 10 DAI, treatments except tween-80 1.0 per cent (80.0%), turmeric 1.0 per cent (83.33%) and control (90.0%) recorded cent per cent surface coverage by fungus in culture medium (Table 16).

UVC exposure- 20, 30, 40 and 50 minutes : At 10 DAI, the treatments except tween-80 0.5 per cent (61.67%), tween-80 1.0 per cent (43.33%), turmeric 1.0 per cent (81.67%), molasses 1.0 per cent (93.33%), molasses 2.0 per cent (97.67%) and control (76.67%) showed cent per cent surface coverage by fungus. More or less similar trend of growth was seen by the exposure for 30 and 40 minutes. Tween-80 0.5 and 1.0%, CMC 1.0%, turmeric 0.5 and 1.0% and molasses 1.0 and 2.0% resulted in 41.67, 30.0, 85.0, 95.0, 75.0, 93.33 and 85.0 per cent growth; respectively, at 10 DAI by 50 minutes UVC rays exposure. Tween-80, turmeric and molasses were consistently harmful to the fungal growth on UVC ray exposure, when all the treatments showed cent per cent medium surface coverage.

UVC exposure- 2, 3 and 5 hrs : The trend of results was almost similar to that in previous observations. At 10 DAI, among the treatments with various chemical adjuvants, fungus culture with boric acid 2.0, indigo 0.5 and 1.0 per cent covered cent per cent growth of fungus. Glycerol 1.0 and 2.0 per cent recorded 95.0 per cent surface coverage among its various concentrations. The treatments with nutrient sources, honey and milk recorded cent per cent growth of fungus in culture medium at 10 DAI. Among the treatments with various oils showed cent per cent surface coverage of mycoagent whereas control (*M.a.*alone) recorded 55.0 per cent surface coverage by fungus on medium.

At exposure to 3 hours UVC ray, fungus culture with adjuvants showed 11.67 to 100 per cent growth whereas control recorded only 50.0 per cent growth of fungus. The fungus culture with indigo, boric acid 2.0 per cent, honey, milk, sunflower oil, groundnut oil and ghee were covered cent per cent surface coverage of fungus.

Table 16. Influence of *M.anisopliae* with adjuvants on growth of *M.anisopliae* exposed to UVC rays

Tr. No.	Treat ments	Con. (%) of adj.	Surface coverage (%) on 10 DAI in UVC exposure upto							
			10 min	20min	30min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*M.a.*+ GLY	1.0	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.08)	93.33 (75.00)	90.00 (71.56)
T2	*M.a.*+ GLY	2.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.08)	95.00 (77.08)	95.00 (77.08)
T3	*M.a.*+ GLY	3.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	88.33 (70.00)	90.00 (71.56)	90.00 (71.56)
T4	*M.a.*+ GLY	5.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	75.00 (60.00)	75.00 (60.00)	75.00 (60.00)
T5	*M.a.*+ TW-80	0.5	100.00 (90.00)	61.67 (51.77)	65.00 (53.73)	43.33 (41.15)	41.67 (40.22)	35.00 (36.27)	31.67 (34.27)	30.00 (33.21)
T6	*M.a.*+ TW-80	1.0	80.00 (63.44)	43.33 (41.15)	43.33 (41.15)	33.33 (35.24)	30.00 (33.21)	11.67 (20.00)	11.67 (20.00)	10.00 (18.44)
T7	*M.a.* +BA	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.08)	95.00 (77.08)	95.00 (77.08)	95.00 (77.08)
T8	*M.a.*+ BA	2.0	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)
T9	*M.a.*+ BA	3.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)
T10	*M.a.*+ CMC	0.5	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	98.33 (82.51)	95.00 (77.08)	95.00 (77.08)
T11	*M.a.*+ CMC	1.0	100.00 (90.00)	100.00 (90.00)	88.33 (70.00)	86.67 (68.61)	85.00 (67.21)	85.00 (67.21)	85.00 (67.21)	80.00 (63.44)
T12	*M.a.*+ Indigo	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 (90.000
T13	*M.a.*+ Indigo	1.0	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)
T14	*M.a.*+ Turmeric	0.5	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.08)	91.67 (73.26)	86.67 (68.61)	85.00 (67.21)
T15	*M.a.*+ Turmeric	1.0	83.33 (65.88)	81.67 (64.67)	80.00 (63.44)	76.67 (61.14)	75.00 (60.00)	75.00 (60.00)	75.00 (60.00)	58.33 (49.79)
T16	*M.a.*+ Molasses	1.0	100.00 (90.00)	93.33 (75.00)	93.33 (75.00)	93.33 (75.00)	93.33 (75.00)	55.00 (47.87)	55.00 (47.87)	55.00 (47.87)
T17	*M.a.*+ Molasses	2.0	100.00 (90.00)	91.67 (73.26)	91.67 (73.26)	85.00 (67.21)	85.00 (67.21)	43.33 (41.15)	41.67 (40.22)	40.00 (39.23)
T18	*M.a.*+ HO	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)
T19	*M.a.*+ HO	1.0	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)
T20	*M.a.*+ Milk	1.0	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)
T21	*M.a.*+ Milk	2.0	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)
T22	*M.a.*+ SFO	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)
T23	*M.a.*+ SFO	1.0	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)
T24	*M.a.*+ GNO	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)
T25	*M.a.*+ GNO	1.0	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)
T26	*M.a.*+ GNO	2.0	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)
T27	*M.a.*+ SBO	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)	96.67 (79.53)	96.67 (79.53)
T28	*M.a.*+ SBO	1.0	100.00 (90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	96.67 (79.53)	95.00 (77.08)
T29	*M.a.*+ MUO	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)	98.33 (82.51)	98.33 (82.51)
T30	*M.a.*+ MUO	1.0	100.00 (90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	95.00 (77.080	98.33 (82.51)
T31	*M.a.*+ GH	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)	95.00 (77.08)

Table 16 Contd.....

Tr. No.	Treatments	Con. (%) of adj.	Surface coverage (%) on 10 DAI in UVC exposure upto							
			10 min	20min	30min	40 min	50 min	2 hrs	3 hrs	5 hrs
T32	*M.a.*+ GH	1.0	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)	90.00 (71.56)
T33	Control (*M.a.*alone)	-	90.00 (71.56)	76.67 (61.14)	75.00 (60.00)	65.00 (53.73)	65.00 (53.73)	55.00 (47.87)	50.00 (45.00)	50.00 (45.00)
T34	Control (*M.a.*alone) (W.UVC)	-	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)
	S.E ±		**1.31**	**0.93**	**0.95**	**1.85**	**1.05**	**1.07**	**1.82**	**1.28**
	C.D (P=0.05)		**3.70**	**2.63**	**2.68**	**5.23**	**2.96**	**3.01**	**5.14**	**3.60**

*Figures in parentheses are arc sin values.
M.a.= Metarhizium anisopliae TW= Tween-80 DAI = Days after inoculation
SFO = Sunflower oil
GLY= Glycerol CNO = Coconut oil TX = Triton-X-100
GRO = Groundnut oil BA = Boric acid SBO = Soybean oil
CMC = Carboxymethyl Cellulose MUO = Mustered oil GH = Ghee W.UVC = Without UVC

Fungus culture with boric acid 2.0, indigo 0.5 and 1.0, honey 0.5 and 1.0, milk 1.0 and 2.0, sunflower oil 0.5 and 1.0, groundnut oil 0.5, 1.0 and 2.0 registered cent per cent growth of fungus at 10 DAI by exposure to UVC ray for 5 hours.

4.1.4.2 Effect of UVC rays on biomass development of *N.rileyi* and *M.anisopliae*

The data on biomass produced by the mycoagent of *N.rileyi* and *M.anisopliae* with various adjuvants in culture medium after UVC rays for 10 to 50 minutes, 2, 3 and 5 hours are presented in Table 17. The differences of biomass production in different treatments were significant and trend of performance of adjuvants was more or less similar to that observed for surface coverage.

***N.rileyi* :**

UVC exposure- 10 to 50 minutes : The biomass produced by the fungus in treatments with *N.r.*+indigo 0.5% was 5.50g. It emerged significantly superior to rest of the treatments, except at par treatments with *N.r.*+SFO 1.0 per cent (5.40g), *N.r.*+SFO 0.5% (5.37g) and *N.r.*+indigo 1.0% (5.33g). The next promising treatments were *N.r.*+BA 2.0% (5.27g), *N.r.*+GNO 0.5% (5.20g), *N.r.*+GLY 3.0% (5.13g), *N.r.*+BA 3.0 per cent (5.10g) and *N.r.*+GLY 2.0

per cent (5.10g). The lowest (3.40g) biomass was recorded in control (*N.r.* alone)

Table 17. Effect of UVC treatment on biomass production by *N.rileyi* in the presence of some adjuvants

Tr. No.	Treatments	Conc. (%) of Adj.	Biomass (g) produced after indicated exposure							
			10m	20m	30m	40m	50m	2 hrs	3hrs	5 hrs
T1	*N.r.*+GLY	1.0	4.83	4.77	4.63	4.53	4.50	4.07	4.00	3.80
T2	*N.r.*+GLY	2.0	5.10	5.00	4.80	4.67	4.63	4.20	4.17	3.90
T3	*N.r.*+GLY	3.0	5.13	4.83	4.73	4.60	4.47	4.20	4.03	3.80
T4	*N.r.*+GLY	5.0	4.83	4.67	4.53	4.50	4.53	4.30	4.00	3.80
T5	*N.r.*+TW-80	0.5	3.57	3.50	3.43	3.27	3.20	2.97	2.80	2.60
T6	*N.r.*+TW-80	1.0	3.45	3.30	3.00	3.10	3.00	2.70	2.60	2.40
T7	*N.r.*+BA	1.0	5.07	5.03	4.87	4.93	4.87	4.67	4.53	4.30
T8	*N.r.*+BA	2.0	5.27	4.97	4.97	4.97	4.93	4.73	4.57	4.40
T9	*N.r.*+BA	3.0	5.10	5.10	5.03	4.93	4.87	4.63	4.47	4.27
T10	*N.r.*+CMC	0.5	4.27	4.27	4.07	4.10	4.10	3.97	3.87	3.70
T11	*N.r.*+CMC	1.0	4.13	4.10	4.07	3.93	3.70	3.50	3.40	3.23
T12	*N.r.*+Indigo	0.5	5.50	5.20	5.00	4.93	4.87	4.67	4.43	4.23
T13	*N.r.*+Indigo	1.0	5.33	5.20	4.97	4.87	4.70	4.47	4.40	4.13
T14	*N.r.*+Turmeric	0.5	4.67	4.63	4.10	3.97	3.93	3.67	3.40	3.13
T15	*N.r.*+Turmeric	1.0	4.47	4.20	3.93	3.83	3.77	3.57	3.30	2.93
T16	*N.r.*+Molasses	1.0	4.20	4.00	3.97	3.90	3.77	3.47	3.37	3.17
T17	*N.r.*+Molasses	2.0	4.37	4.03	3.87	3.73	3.73	3.37	3.13	3.03
T18	*N.r.*+Honey	0.5	4.80	4.63	4.53	4.57	4.40	4.07	4.00	3.97
T19	*N.r.*+Honey	1.0	4.90	4.70	4.60	4.60	4.50	4.17	4.10	4.07
T20	*N.r.*+Milk	1.0	4.70	4.57	4.63	4.57	4.50	4.33	4.20	4.07
T21	*N.r.*+Milk	2.0	4.63	4.77	4.77	4.67	4.63	4.43	4.30	4.10
T22	*N.r.*+SFO	0.5	5.37	5.27	5.20	5.20	5.10	5.10	4.97	4.87
T23	*N.r.*+SFO	1.0	5.40	5.50	5.27	5.27	5.27	5.20	5.03	4.97
T24	*N.r.*+ GNO	0.5	5.20	5.13	4.97	4.97	4.97	4.93	4.83	4.77
T25	*N.r.*+ GNO	1.0	5.10	5.30	5.17	5.13	5.03	4.97	4.93	4.87
T26	*N.r.*+GNO	2.0	5.10	5.27	5.13	5.10	5.10	4.97	4.90	4.77
T27	*N.r.*+SBO	0.5	4.63	4.73	4.63	4.67	4.57	4.47	4.37	4.30
T28	*N.r.*+SBO	1.0	4.50	4.63	4.53	4.47	4.40	4.30	4.23	4.10
T29	*N.r.*+MUO	0.5	4.70	4.63	4.53	4.27	4.37	4.07	3.93	3.73
T30	*N.r.*+MUO	1.0	4.67	4.43	4.37	4.30	4.27	4.13	4.00	3.77
T31	*N.r.*+GH	0.5	4.80	4.63	4.53	4.47	4.37	4.23	4.07	3.87
T32	*N.r.*+GH	1.0	4.83	4.77	4.73	4.67	4.37	4.17	3.97	3.93
T33	Control (*N.r. a*lone)	-	3.40	3.20	3.10	3.00	2.90	2.50	2.40	1.80
T34	Control (*N.r. a*lone) (W.UVC)	-	6.90	6.90	7.20	7.20	7.30	7.30	7.00	7.30
	S.E ±		**0.06**	**0.05**	**0.05**	**0.05**	**0.06**	**0.07**	**0.06**	**0.13**
	C.D(P=0.05)		**0.18**	**0.15**	**0.15**	**0.14**	**0.18**	**0.19**	**0.18**	**0.36**

DAI = Days after inoculation *N.r. = Nomuraea rileyi* W.UVC=without UVC
TW= Tween-80 SFO = Sunflower oil GLY= Glycerol CNO = Coconut oil
TX = Triton-X-100 GNO = Groundnut oil BA = Boric acid SBO = Soybean oil
CMC = Carboxymethyl Cellulose MUO = Mustered oil GH = Ghee

After 20 minutes UVC rays exposure the significantly maximum (5.50g) biomass developed in *N.r.*+SFO 1.0 per cent. The next promising and at par treatments were *N.r.*+GNO 1.0% (5.30g), *N.r.*+SFO 0.50% (5.27g), *N.r.*+GNO 2.0% (5.27g), *N.r.*+indigo 0.5 and 1.0% (5.20g). The treatments in decending order of superiority for the biomass were *N.r.*+GNO 0.5% (5.13g), *N.r.*+BA 3.0% (5.10g), *N.r.*+GLY 2.0% (5.00g) and *N.r.*+BA 2.0% (4.97g). The fungus culture with adjuvant *N.r.*+tween 80 and *N.r.*+molasses produced least biomass (3.30 to 4.03g) when the fungus culture without adjuvants produced 3.20g biomass in culture medium.

After 30 minutes UVC rays exposure, the treatment with adjuvants *N.r.*+SFO 1.0 per cent produced 5.27g biomass. It was significantly highest than rest of treatments except at par treatments with *N.r.*+SFO 0.5 per cent (5.20g), *N.r.*+GNO 1.0% and *N.r.*+GNO 0.5% (5.17 and 4.97g) for production of biomass. The results of 40 min. UVC rays exposure were more or less similar to that of 30 minutes UVC rays exposure.

After 50 minutes of UVC exposure, SFO 1.0% produced significantly maximum (5.27g) biomass over the rest of the treatments with and without adjuvants. It recorded more or less doubled biomass over control (2.90g). The next promising treatments for biomass development were *N.r.*+SFO 0.5% (5.10g), *N.r.*+GNO 1.0% (5.03g), *N.r.*+GNO 0.5% (4.97g) and *N.r.*+BA 2.0% (4.93g).

UVC exposure 2 hours : There were significant differences among the treatments for production of fungal biomass. The adjuvant *N.r.*+SFO 1.0% (5.20g) showed its superiority for biomass production over the adjuvants (2.70 to 5.10g) and control (2.50g). However, it was at par with *N.r.*+SFO 0.5% (5.10g). *N.r.*+Tween-80, *N.r.*+CMC, *N.r.*+turmeric and *N.r.*+molasses produced least biomass (2.70 to 3.97g) when control produced 2.50g biomass. Among the chemical adjuvants biomass production was 2.70 to 4.73g. The other edible substrate produced biomass in 3.73 to 4.63g. The results of 3 hours UVC rays exposure were more or less similar to that of 2 hours UVC rays exposure.

UVC exposure 5 hours : There were significant differences among treatments for fungal biomass production. The treatment with adjuvant *N.r.*+SFO 1.0% produced highest (4.97g) biomass when it was in rest of the treatments (2.40 to 4.87g) against 1.80g in control (*N.r.* alone). The next promising treatments were *N.r.*+SFO 0.5% (4.87g), *N.r.*+GNO 1.0% (4.87g) and *N.r.*+GNO 2.0% (4.77g). The control *N.r.*alone without UVC exposure produced 7.30g of fungal biomass (Fig.5).

***M.anisopliae* : Effect on growth :**

UVC exposure 10 to 50 minutes : The biomass produced in sunflower oil 1.0 per cent was 5.77g which emerged significantly superior to remaining treatments. However, it was at par with *M.a.*+groundnut oil 2.0 per cent (5.50g), *M.a.*+boric acid 2.0 per cent (5.43g), *M.a.*+sunflower oil 0.5 per cent (5.40g), *M.a.*+boric acid 3.0 per cent (5.37g), *M.a.*+groundnut oil 1.0 per cent (5.37g), *M.a.*+boric acid 1.0 per cent (5.33g), *M.a.*+soybean oil 1.0 per cent (5.30g), *M.a.*+milk 2.0 per cent (5.27g), *M.a.*+soybean oil 0.5 (5.23g), *M.a.*+groundnut oil 0.5 per cent (5.20g), *M.a.*+mustard oil 1.0 per cent (5.13g) and *M.a.*+molasses 2.0 per cent (5.10g). The lowest biomass (2.97g) in treatments with control was recorded (Table 18).

After 20 minutes UVC rays exposure, significantly maximum biomass (5.73g) with *M.a.*+sunflower oil 1.0 per cent was registered. The next treatments in their decending order were *M.a.*+sunflower oil 0.5 per cent (5.37g), *M.a.*+groundnut oil 2.0 per cent (5.33g), *M.a.*+groundnut oil 1.0 per cent (5.27g), *M.a.*+boric acid 2.0 per cent (5.23g), *M.a.*+soybean oil 1.0 per cent (5.20g) and *M.a.*+milk 2.0 per cent (5.17g). Among the treatments with various adjuvants, the fungus culture with adjuvants produced fungal biomass which ranged from 3.07 to 5.73g.

After 30 minutes UVC rays exposure, sunflower oil 1.0 per cent produced 5.30g fungal biomass which was significantly highest than rest of the treatments. However, it was at par with *M.a.*+sunflower oil 0.5 per cent (5.13g) and *M.a.*+groundnut oil 2.0 per cent (5.13g).

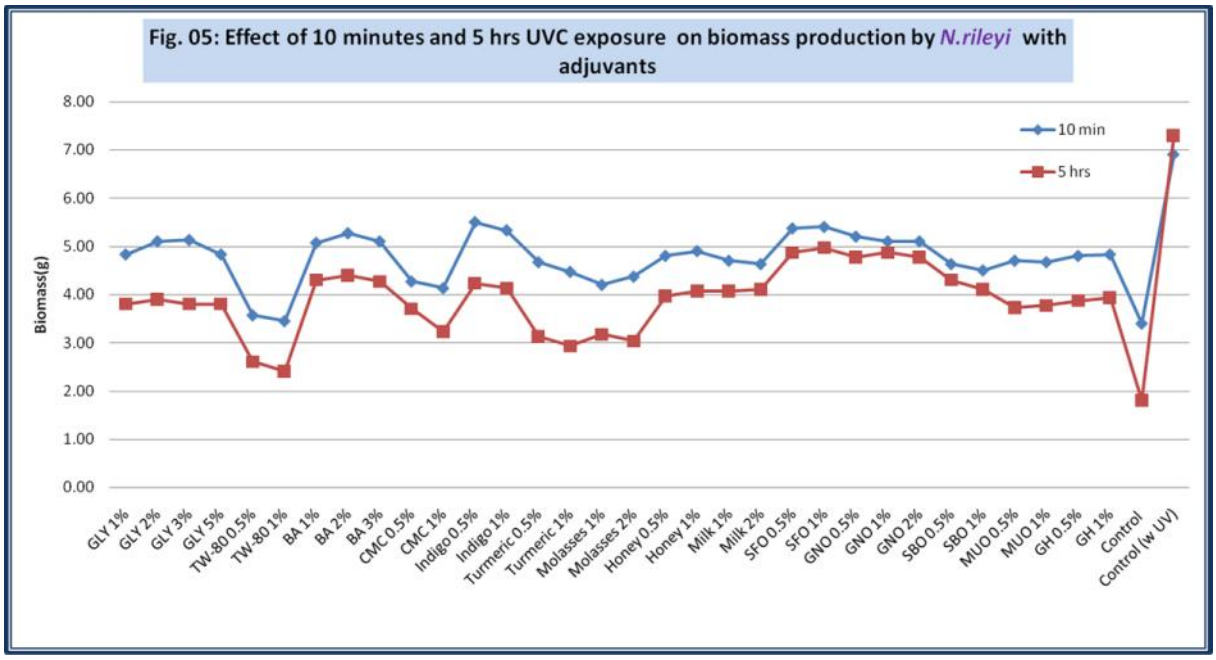


Fig. 05: Effect of 10 minutes and 5 hrs UVC exposure on biomass production by N.rileyi with adjuvants
Biomass(g)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
10 min
5 hrs
GLY 1%
GLY 2%
GLY 3%
GLY 5%
TW-80 0.5%
TW-80 1%
BA 1%
BA 2%
BA 3%
CMC 0.5%
CMC 1%
Indigo 0.5%
Indigo 1%
Turmeric 0.5%
Turmeric 1%
Molasses 1%
Molasses 2%
Honey 0.5%
Honey 1%
Milk 1%
Milk 2%
SFO 0.5%
SFO 1%
GNO 0.5%
GNO 1%
GNO 2%
SBO 0.5%
SBO 1%
MUO 0.5%
MUO 1%
GH 0.5%
GH 1%
Control
Control (w UV)

Table 18. Effect of UVC treatment on biomass production by *M.anisopliae* in the presence of some adjuvants

Tr. No.	Treatments	Conc. (%)	Biomass (g) produced after indicated exposure							
			10min	20min	30min	40min	50min	2hrs	3hrs	5hrs
T1	*M.a.*+GLY	1.0	4.20	3.97	3.67	3.60	3.53	3.50	3.30	3.20
T2	*M.a.*+GLY	2.0	4.33	4.20	4.00	3.43	3.40	3.40	3.30	3.30
T3	*M.a.*+GLY	3.0	4.37	4.30	3.97	3.40	3.40	3.73	3.57	3.40
T4	*M.a.*+GLY	5.0	4.37	4.17	3.97	3.47	3.40	3.40	3.37	3.20
T5	*M.a.*+TW-80	0.5	4.17	3.90	3.77	2.97	2.97	2.67	2.27	1.97
T6	*M.a.*+TW-80	1.0	3.30	3.67	2.87	2.67	2.43	1.20	0.80	0.70
T7	*M.a.*+BA	1.0	5.33	5.07	4.77	3.97	3.93	3.80	3.80	3.70
T8	*M.a.*+BA	2.0	5.43	5.23	5.07	4.90	4.73	4.70	4.63	4.57
T9	*M.a.*+BA	3.0	5.37	4.93	4.80	4.10	4.03	4.00	3.70	3.47
T10	*M.a.*+CMC	0.5	3.97	3.97	3.77	3.50	3.50	3.47	3.47	3.40
T11	*M.a.*+CMC	1.0	4.20	3.97	3.77	3.40	3.33	3.30	3.30	3.30
T12	*M.a.*+Indigo	0.5	4.90	4.70	4.33	4.30	4.30	4.23	4.27	4.17
T13	*M.a.*+Indigo	1.0	4.03	4.73	4.37	4.37	4.37	4.30	4.23	4.13
T14	*M.a.*+Turmeri	0.5	3.83	3.63	3.30	3.20	3.23	3.20	3.17	2.90
T15	*M.a.*+Turmeri	1.0	3.27	3.07	2.60	1.97	1.80	1.70	1.70	1.60
T16	*M.a.*+Molasse	1.0	5.00	4.90	4.87	4.27	4.20	2.93	2.87	2.70
T17	*M.a.*+Molasse	2.0	5.10	5.00	4.87	4.30	4.30	2.90	2.70	2.70
T18	*M.a.*+HO	0.5	4.40	4.33	4.00	3.90	3.80	3.70	3.60	3.50
T19	*M.a.*+HO	1.0	4.87	4.77	4.53	4.10	4.00	3.90	3.80	3.70
T20	*M.a.*+Milk	1.0	4.93	4.77	4.53	4.17	4.17	4.30	4.20	4.03
T21	*M.a.*+Milk	2.0	5.27	5.17	4.83	4.63	4.47	4.47	4.33	4.07
T22	*M.a.*+SFO	0.5	5.40	5.37	5.13	5.10	5.03	5.00	5.00	4.90
T23	*M.a.*+SFO	1.0	5.77	5.73	5.30	5.10	5.03	5.03	5.00	5.00
T24	*M.a.*+ GNO	0.5	5.20	5.17	4.90	4.80	4.80	4.77	4.73	4.73
T25	*M.a.*+ GNO	1.0	5.37	5.27	5.07	5.00	5.00	4.77	4.73	4.67
T26	*M.a.*+GNO	2.0	5.50	5.33	5.13	4.90	4.90	4.90	4.73	4.60
T27	*M.a.*+SBO	0.5	5.23	5.07	4.73	4.70	4.73	4.77	4.50	4.37
T28	*M.a.*+SBO	1.0	5.30	5.20	4.73	4.53	4.43	4.40	4.30	4.07
T29	*M.a.*+MUO	0.5	5.03	4.97	4.77	4.50	4.40	4.33	4.30	4.27
T30	*M.a.*+MUO	1.0	5.13	4.97	4.67	4.33	4.23	4.20	4.13	3.97
T31	*M.a.*+GH	0.5	5.00	4.83	4.37	4.30	4.23	4.20	4.07	3.93
T32	*M.a.*+GH	1.0	5.03	4.93	4.57	4.33	4.23	4.00	3.90	3.83
T33	Control (*M.a.*alone)	-	2.97	2.83	2.63	2.40	2.37	2.33	2.30	2.23
T34	Control (*M.a.*alone) (W.UVC)	-	6.50	6.40	6.40	6.50	6.50	6.50	6.60	6.50
	S.E ±		**0.08**	**0.05**	**0.08**	**0.06**	**0.08**	**0.05**	**0.07**	**0.05**
	C.D(P=0.05)		**0.23**	**0.15**	**0.24**	**0.17**	**0.23**	**0.16**	**0.21**	**0.14**

DAI = Days after inoculation *M.a. = Metarhizium anisopliae* W.UVC= without UVC
MUO = Mustard oil GH = Ghee, TW = Tween-80
TX= Triton-X-100 CNO = Coconut oil BA = Boric acid
CMC = Carboxymethyl Cellulose GLY = Glycerol GNO = Groundnut oil
SFO = Sunflower oil SBO = Soybean oil HO = Honey

Among treatments of fungus culture with chemical adjuvants, the biomass production was ranged from 2.87 to 5.07g. The least biomass of 2.60g in treatment with turmeric 1.0 per cent was produced.The results of 40 and 50 minutes UVC rays exposure were more or less similar to that of 30 minutes UVC rays exposure. The fungus culture with sunflower oil 0.5 and 1.0 per cent produced significantly highest fungal biomass of 5.10 and 5.03g at 40 and 50 minutes exposure, respectively.

UVC exposure 2, 3 and 5 hours : After 2 hours UVC rays exposure, sunflower oil 1.0 per cent (5.03g) showed its superiority for biomass production to remaining treatments with adjuvants (1.20g to 5.00g) and control (2.33g). *M.a.*+Tween-80, *M.a.*+turmeric and *M.a.*+molasses produced least biomass (1.20 to 3.20g), whereas control produced 2.33g biomass. The results of 3 hours UVC rays exposure was more or less similar to that of 2 hours UVC rays exposure.

After 5 hours exposure to UVC rays, the fungal culture with *M.a.*+sunflower oil 1.0 per cent produced maximum (5.0g) biomass to rest of the treatments.

However, it was at par with *M.a.*+sunflower oil 0.5 per cent (4.90g). The next effective treatment for fungal biomass production was *M.a.*+groundnut oil 0.5 per cent (4.73g), *M.a.*+groundnut oil 1.0 per cent (4.67g) and *M.a.*+groundnut oil 2.0 per cent (4.53g). The control *M.a.*alone without UVC exposure produced 6.50g of fungal biomass (Fig.6).

It is indicated that surface coverage and biomass produced by *N.rileyi* and *M.anisopliae* with or without adjuvants in culture medium after exposure to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours decreased with increase in exposure period. The adjuvants reacted variably for their UVC rays protecting capacity for *N.rileyi* and *M.anisopliae.* However, higher concentrations of the adjuvants were better than their lower ones except turmeric and CMC. Among the various oils, sunflower and groundnut oil, among chemical adjuvant glycerol 2.0 per cent, CMC 0.50 per cent, boric

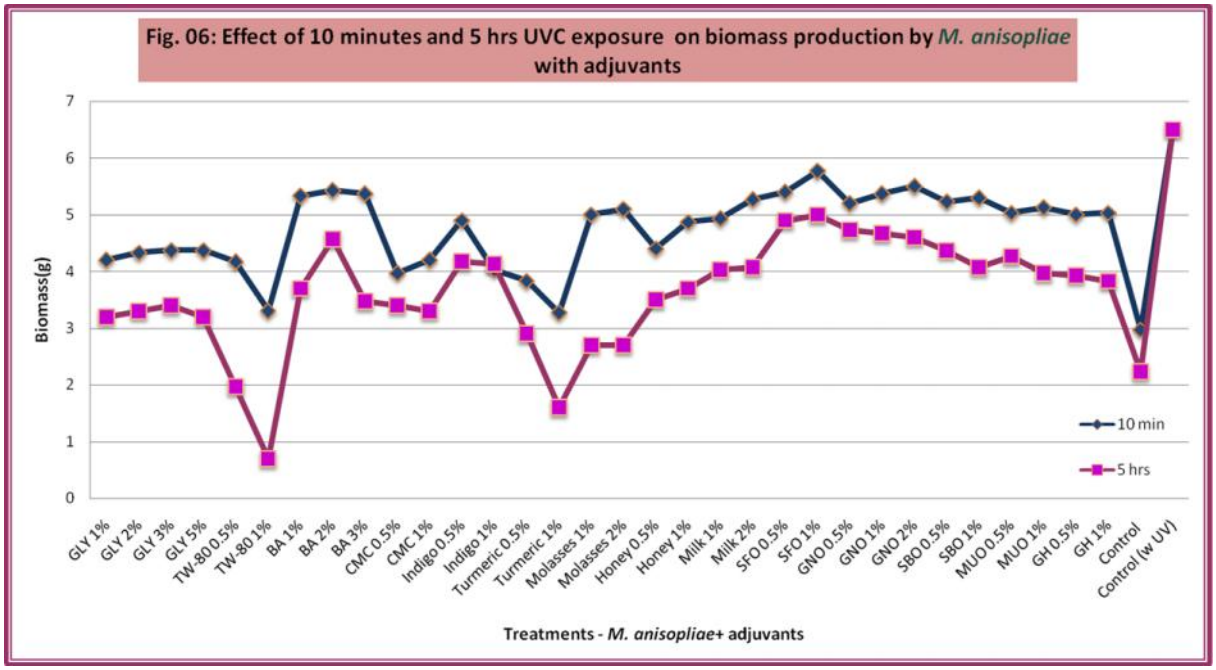


Fig. 06: Effect of 10 minutes and 5 hrs UVC exposure on biomass production by M. anisopliae with adjuvants
Biomass(g)
0
1
2
3
4
5
6
7
10 min
5 hrs
GLY 1%
GLY 2%
GLY 3%
GLY 5%
TW-80 0.5%
TW-80 1%
BA 1%
BA 2%
BA 3%
CMC 0.5%
CMC 1%
Indigo 0.5%
Indigo 1%
Turmeric 0.5%
Turmeric 1%
Molasses 1%
Molasses 2%
Honey 0.5%
Honey 1%
Milk 1%
Milk 2%
SFO 0.5%
SFO 1%
GNO 0.5%
GNO 1%
GNO 2%
SBO 0.5%
SBO 1%
MUO 0.5%
MUO 1%
GH 0.5%
GH 1%
Control
Control (w UV)
Treatments - M. anisopliae+ adjuvants

acid and among nutrient sources honey, milk act as appreciable UVC protectant.

According to Hunt *et al.* (1994), the chemical sunscreen incorporated in oil formulations of the *Metarhizium spp. g*ave protection after solar radiation of 2 hrs but increased exposure upto 5hrs failed to offer protection. Moore *et al.* (1993) pointed out that the conidial viability of *Metarhizium spp.* decreased with increased UV exposure. Similarly Alves *et al.* (1998) reported that germination of *Metarhizium anisopliae* decreased with increasing exposure time to solar radiation. Peanut oil enhanced the conidial tolerance against UV light for upto 6 hrs of exposure compared to unformulated and tween-80. Reduction in relative per cent culturability of *M.anisopliae* with increased UV exposure from 1 to 8 hrs was reported by Braga *et al.* (2001b). Rangel and Roberts (2007) reported that any carbon source plus 1% NaCl or KCl with high alkalinity had the highest UVB tolerance. Francisco *et al.* (2008) found that conidia of *M.anisopliae* with oil emulsion had higher survival after 3hrs of UV exposure. These findings are in line with the present investigation.

4.1.6 Influence of multiple adjuvants with inoculum on growth and development of *N.rileyi* and *M.anisopliae*

(Period: Sep.to Dec. 2010 and Jan.,Feb.2011 Av.Temp.(^{0}C) :Max.-30 ±1,Min.-10 ±1 Av.Humidity (%) :Morn.-90, Even.-32)

4.1.5.1 The influence of combination of chemical adjuvants

The combinations of glycerol, tween-80, triton-X-100, boric acid and carboxymethyl cellulose added in aqua suspension of the mycoagent of *N.rileyi* and *M.anisopliae* were evaluated.

***N.rileyi* :**

Effect on growth : The results on growth and development of *N.rileyi* during 10 days are presented in Table 19 and depicted in Fig.7.

At 3 DAI, for surface coverage T8-*N.r.*+GLY+BA and T9-*N.r.*+GLY+CMC (90% each) were significantly superior to remaining treatments (0.0 to 40%). However, T10-*N.r.*+BA+CMC was at par with it recording 86.67 per cent surface coverage. T15-*N.r.*+TW and T16-*N.r.*+TX, which completely prevented the growth of the fungus upto 3 days.

Table 19. Effect of combinations of chemical adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adjuvant	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*N.r.*+ TW + TX	0.5 + 0.03	10.00 (18.44)*	66.67 (54.76)	100.00 (90.00)	9.37	8.22
T2	*N.r.*+ TW + GLY	0.5 + 2.0	6.67 (15.00)	70.00 (56.79)	100.00 (90.00)	8.07	8.53
T3	*N.r.*+ TW + BA	0.5 + 2.0	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.53	7.54
T4	*N.r.*+ TW + CMC	0.5 + 0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.63	7.52
T5	*N.r.*+ TX + GLY	0.03 + 2.0	6.67 (15.00)	83.33 (65.88)	100.00 (90.00)	8.97	8.58
T6	*N.r.*+ TX + BA	0.03 + 2.0	10.00 (18.44)	28.33 (32.14)	100.00 (90.00)	9.80	7.35
T7	*N.r.*+ TX + CMC	0.03 + 0.5	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	8.20	7.37
T8	*N.r.*+ GLY + BA	2.0 + 2.0	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	9.03	7.50
T9	*N.r.*+ GLY + CMC	2.0 + 0.5	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	10.10	8.36
T10	*N.r.*+ BA + CMC	2.0 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.90	8.12
T11	*N.r.*+ TW + TX + GLY	0.5 + 0.03 +2	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	10.20	8.03
T12	*N.r.*+ TW+GLY+BA +CMC	0.5+2+2+0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	11.20	7.70
T13	*N.r.*+ TW+GLY+CMC	0.5+2+0.5	8.33 (16.78)	53.33 (46.89)	100.00 (90.00)	12.17	8.10
T14	*N.r.*+ TW+GLY+BA	0.5+2+2	6.67 (15.00)	63.33 (52.71)	100.00 (90.00)	10.97	7.42
T15	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.83	7.78
T16	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.80	7.26
T17	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.85
T18	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.15
T19	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.97	7.71
T20	Control (*N.r.*alone)	-	30.00 (33.21)	41.67 (40.22)	100.00 (90.00)	7.70	8.48
	SE +		**1.88**	**3.20**	**-**	**0.07**	**-**
	C.D.(P=0.05)		**5.37**	**9.15**	**-**	**0.21**	**-**

*Figures in parentheses are arcsin values. DAI = Days after inoculation
N.r. = Nomuraea rileyi TW = Tween-80
GLY= Glycerol BA = Boric acid
TX = Triton-X-100 CMC = Carboxymethyl Cellulose

At 7 DAI, the growth and development of fungus ranged from 10 to 100 per cent in all treatments. The treatments with T3-*N.r.*+TW+BA, T4-*N.r.*+TW+CMC, T8-*N.r.*+GLY+BA, T9-*N.r.*+GLY+CMC, T10-*N.r.*+BA+CMC,

Fig.7 : Effect of combination of chemical adjuvants with inoculum in *N.rileyi* 30% AS on biomass of the mycoagent

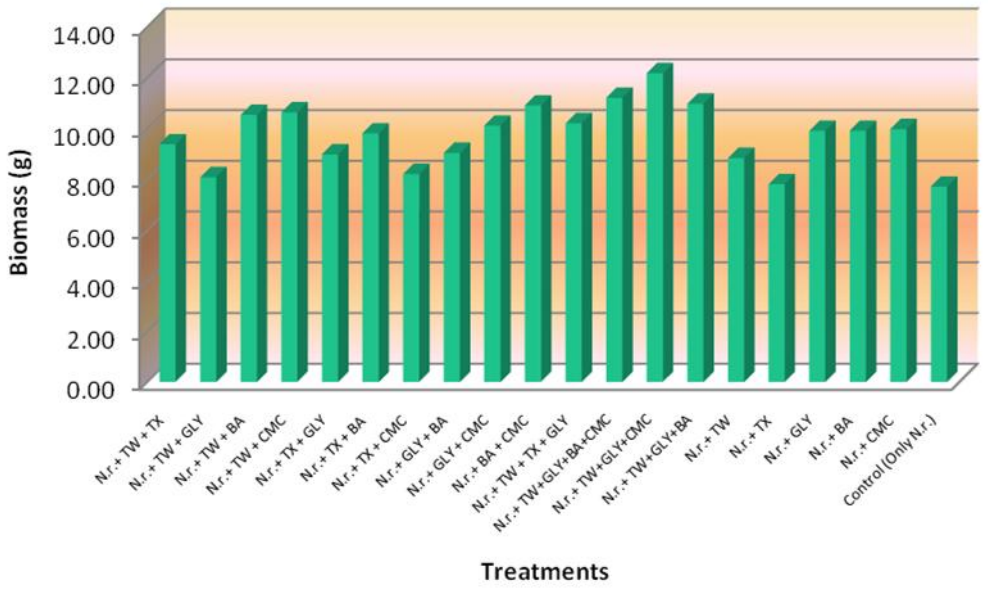
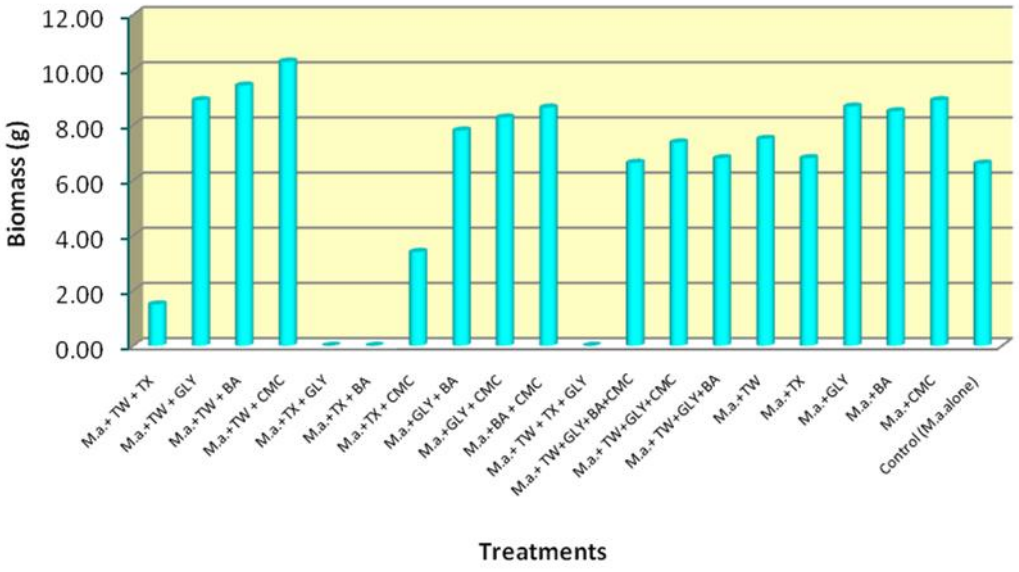


Fig. 8: Effect of combination of chemical adjuvants with inoculum in *M.anisopliae* 30% AS on biomass of the mycoagent



T12-*N.r.*+TW+GLY+BA+CMC, T17-*N.r.*+GLY, T18-*N.r.*+BA and T19-*N.r.*+CMC recorded significantly higher surface coverage (100%). At 10 DAI, all the treatments covered cent per cent surface of the medium.

Effect on biomass : Corresponding observations on biomass produced in gram per 40 ml liquid medium showed that treatment T13-*N.r.*+TW+GLY+CMC proved its superiority, producing 12.17g fungal biomass. It was followed by T12-*N.r.*+TW+GLY+BA+CMC (11.20g). Other adjuvant in combinations and alone developed 7.80 to 10.97g biomass. The control (*N.r.*alone) could produced 7.70g biomass. It was least among all the treatments.

The pH of the fungal culture developed from 20 treatment formulations ranged from 7.15 in adjuvant with 2% Boric acid to 8.58 in adjuvant with Triton-X-100 0.03% +Glycerol 2% registering biomass of 9.90 and 8.97g, respectively. The pH of formulation producing maximum biomass (12.17g) was 8.10 comparing adjuvants Tween-80 0.5% +Glycerol 2% +CMC 0.5%, when it was 8.48 in control producing biomass of 7.70g per 40ml medium. These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

***M.anisopliae* :**

Effect on growth : The results on growth and development of the mycoagent during 10 days are presented in Table 20.

Observations on surface coverage (%) at 3 and 7 and 10 DAI registered significant differences for the growth and development. At 3 DAI, T17-*M.a.*+GLY (80%) were significantly superior to rest of the treatments for the surface growth. Among the treatments with multiple adjuvants T9-

M.a.+GLY+CMC recorded maximum (16.67%) surface coverage by the mycoagent. The growth in treatments with T1-*M.a.*+TW+TX, T2-*M.a.*+TW+GLY, T5-*M.a.*+TX+GLY, T6-*M.a.*+TX+BA, T7- *M.a.*+TX+CMC, T11-*M.a.*+TW+TX+GLY and T16-*M.a.*+TX was completely prevented.

At 7 DAI, T8-*M.a.*+GLY+BA, T9-*M.a.*+GLY+CMC, T15-*M.a.*+TW, T17-*M.a.*+GLY, T18-*M.a.*+BA, T19-*M.a.*+CMC and T20-control (*M.a.*alone) showed cent per cent growth of the mycoagent. It was nil in treatments with T1-*M.a.*+TW+TX, T5-*M.a.*+TX+GLY, T6-*M.a.*+TX+BA, T7-*M.a.*+TX+CMC and T11-*M.a.*+TW+TX+GLY. At 10 DAI, the treatments except those with T1-*M.a.*+Tw+Tx (10.0%), T5-*M.a.*+Tx+Gl (0%), T6-*M.a.*+Tx+Bo (0%), T7-*M.a.*+Tx+CMC (0%) and T11-*M.a.*+Tw+Tx+Gl (0%) covered cent per cent surface coverage by mycoagent.

Effect on biomass and pH : The treatment with T4-*M.a.*+TW+CMC proved its superiority by producing 10.30g fungal biomass (Fig.8). It was followed by T19-*M.a.*+CMC (9.93g), T3-*M.a.*+TW+BA (9.43g) and T2- *M.a.*+TW+GLY (8.90g). T5-*M.a.*+TX+GLY, T6-*M.a.*+TX+BA and T11-*M.a.*+TW+TX+GLY were detrimental for the biomass development whereas, T1-*M.a.*+TW+TX (1.50g) and T7-*M.a.*+TX+CMC (3.40g) produced negligible amount of biomass, when ever T20-control (*M.a.*alone) produced 6.60g biomass.

The pH of *M.anisopliae* cultures developed from inoculums in the formulations varied broadly from 4.12 to 8.89 against biomass range of 0 to 10.30 g per 40 ml medium (Table 20). It could be realized from the pH figure that pH declined less than 5 in Triton-X-100 comprising formulations. It adversely affected the fungal development as evidenced from 0 to 3.40g biomass against 6.63 to 10.30g biomass with pH of 5.21 to 7.10.

On the basis of cent per cent surface coverage as well as highest (12.17 and 10.30g) biomass production of the mycoagents the treatment with T13-*N.r.*+TW+GLY+CMC (12.17g) and T4-*M.a.*+TW+CMC (10.30g) were considered most promising formulations of *N.rileyi* and *M.anisopliae.* There is no published literature on similar line of work.

Table 20. Effect of combination of chemical adjuvants with inoculum in *M.anisopliae* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatment	Conc. (%) of adj.	Surface coverage (%)			Biomass in g/40ml medium	PH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*M.a.*+ TW + TX	0.5 +0.03	0.00 (0.00)*	0.00 (0.00)	10.00 (18.44)	1.50	4.70
T2	*M.a.*+TW + GLY	0.5 +2.0	0.00 (0.00)	23.33 (28.86)	100.00 (90.00)	8.90	6.25
T3	*M.a.*+TW + BA	0.5 +2.0	6.67 (15.00)	36.67 (37.29)	100.00 (90.00)	9.43	5.60
T4	*M.a.*+TW + CMC	0.5 +0.5	5.00 (12.92)	91.67 (73.26)	100.00 (90.00)	10.30	7.10
T5	*M.a.*+TX + GLY	0.03 +2.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	4.47
T6	*M.a.*+TX + BA	0.03 +2.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	4.12
T7	*M.a.*+TX + CMC	0.03 +0.5	0.00 (0.00)	0.00 (0.00)	43.33 (41.15)	3.40	4.14
T8	*M.a.*+GLY + BA	2.0 + 2.0	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	7.80	8.50
T9	*M.a.*+GLY + CMC	2.0 + 0.5	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	8.27	8.80
T10	*M.a.*+BA + CMC	2.0 + 0.5	5.00 (12.92)	83.33 (65.88)	100.00 (90.00)	8.63	5.70
T11	*M.a.*+TW+TX+GLY	0.5+0.03 +2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00	4.30
T12	*M.a.*+TW+ GLY+BA+CMC	0.5+2+2 +0.5	5.00 (12.92)	30.00 (33.21)	100.00 (90.00)	6.63	5.21
T13	*M.a.*+TW +GLY+CMC	0.5+2+0.5	6.67 (15.00)	83.33 (65.88)	100.00 (90.00)	7.37	7.15
T14	*M.a.*+TW +GLY+BA	0.5+2+2	5.00 (12.92)	26.67 (31.11)	100.00 (90.00)	6.80	6.24
T15	*M.a.*+TW	0.50	13.33 (21.39)	100.00 (90.00)	100.00 (90.00)	7.50	8.89
T16	*M.a.*+TX	0.03	0.00 (0.00)	21.67 (27.76)	100.00 (90.00)	6.80	4.42
T17	*M.a.*+GLY	2.00	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	8.67	7.40
T18	*M.a.*+BA	2.00	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	8.50	8.59
T19	*M.a.*+CMC	0.50	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	8.90	8.30
T20	Control (*M.a.*alone)		10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	6.60	8.00
	S.E +		**1.35**	**2.82**	**1.51**	**0.09**	**-**
	C.D(P=0.05)		**3.88**	**8.07**	**4.31**	**0.27**	**-**

*Figures in parentheses are arcsin values.
M.a. = Metarhizium anisopliae
TX= Triton-X-100
GLY = Glycerol
DAI = Days after inoculation
BA = Boric acidTW = Tween-80
CMC = Carboxymethyl Cellulose

4.1.5.2 The influence of combinations of chemicals and oils adjuvants

The adjuvant combination treatments comprised of duly evaluated promising concentration of glycerol, tween-80, triton-X-100, boric acid and

carboxymethyl cellulose; edible oils, sunflower, coconut, mustard, groundnut, soybean oil and ghee. These were added in aqua suspension of *N.rileyi* and *M.anisopliae* to get advanced test formulations and inoculated in the SDY culture medium. The results on the influence of the multiple adjuvant treatments on growth and development of the mycoagent up to 10 days are presented in Table 21 and 22.

***N.rileyi* : Effect on growth :** Significant differences in surface coverage in multiple and single adjuvant, the treatments were registered, at 3 and 7 DAI (Table 21). At 3 DAI, T23-*N.r.*+GH+BA, T24-*N.r.*+GH+CMC, T45-*N.r.*+SFO recorded signiciantly highest per cent surface growth (86.67%) over remaining treatments (5 to 80%) except T50-*N.r.*+GH (85.0%). It was followed by T48-*N.r.*+GNO (80.00%) which was at par with T50-*N.r.*+GH.

Effect on biomass and pH : T18-*N.r.*+GLY+GH produced highest (12.07g) fungal biomass. It was at par with T6- *N.r.*+TW+GH (11.90), T33-*N.r.*+TW+GLY+SFO+CMC (11.87g) and T13- *N.r.*+GLY+SFO (11.83g). The next promising treatments in their descending order of superiority for biomass production were T50- *N.r.*+GH, T38- *N.r.*+TW+GLY+GRO+CMC and T39-*N.r.*+TW+GLY+SBO+CMC which produced 11.70, 11.30 and 11.30g biomass, respectively.

The least growth and development of the fungus was observed in T2-*N.r.*+TW+CNO and T3- *N.r.*+TW+MUO (5% each). Nevertheless, the triton-X-100 alone or with other adjuvants was found to be detrimental to the fungal growth and development. It was evident from less production of fungal biomass (7.73 to 8.50g). The biomass in control was 7.60g which was significantly lower than adjuvant comprising treatments except that of T41-*N.r.*+TX (7.73g).

The pH of the fungal culture developed from 51 treatment formulations ranged from 7.10 in treatment T26 *N.r.*+ TW + TX + GLY + SFO +CNO to 8.81 in T37 *N.r.*+ TW + GLY+ GH registering biomass of 8.17 and 10.67g, respectively. The pH of formulation producing maximum biomass (12.07g) was 7.58 comparing treatment adjuvants Glycerol 2% +GH 0.5%, when it was 8.40 in control producing biomass of 7.60g per 40 ml medium.

Table 21. Effect of combinations of multiple chemicals and oils with inoculum in *N.rileyi* 30% AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH
			3 DAI	7 DAI	10 DAI		
T1	*N.r.*+ TW + SFO	0.5 + 1.0	6.67 (15.00)*	88.33 (70.00)	100.00 (90.00)	10.83	7.45
T2	*N.r.*+ TW + CNO	0.5 + 1.0	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.23	8.34
T3	*N.r.*+ TW + MUO	0.5 + 0.5	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	9.73	7.56
T4	*N.r.*+ TW + GNO	0.5 + 0.5	6.67 (15.00)	66.67 (54.76)	100.00 (90.00)	11.10	7.75
T5	*N.r.*+ TW + SBO	0.5 + 0.5	8.33 (16.78)	93.33 (75.00)	100.00 (90.00)	11.00	7.22
T6	*N.r.*+ TW + GH	0.5 + 0.5	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.90	7.69
T7	*N.r.*+ TX + SFO	0.03 + 1.0	5.00 (12.92)	46.67 (43.11)	100.00 (90.00)	8.50	7.90
T8	*N.r.*+ TX + CNO	0.03 + 1.0	6.67 (15.00)	50.00 (45.00)	100.00 (90.00)	8.38	8.15
T9	*N.r.*+ TX + MUO	0.03 + 0.5	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	8.33	7.90
T10	*N.r.*+ TX+ GNO	0.03 + 0.5	6.67 (15.00)	28.33 (32.14)	100.00 (90.00)	8.32	7.92
T11	*N.r.*+ TX + SBO	0.03 + 0.5	5.00 (12.92)	28.33 (32.14)	100.00 (90.00)	8.07	8.30
T12	*N.r.*+ TX + GH	0.03 + 0.5	6.67 (15.00)	28.33 (32.14)	100.00 (90.00)	8.47	7.45
T13	*N.r.*+ GLY + SFO	2.0 + 1.0	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.83	7.44
T14	*N.r.*+ GLY + CNO	2.0 + 1.0	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.90	7.60
T15	*N.r.*+ GLY + MUO	2.0 + 0.5	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.30	8.05
T16	*N.r.*+ GLY + GNO	2.0 + 0.5	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	11.17	7.47
T17	*N.r.*+ GLY + SBO	2.0 + 0.5	15.00 (22.79)	83.33 (65.88)	100.00 (90.00)	11.17	7.28
T18	*N.r.*+ GLY + GH	2.0 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.07	7.58
T19	*N.r.*+ SFO+ BA	1.0 + 2.0	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	9.90	7.52
T20	*N.r.*+ SFO+ CMC	1.0 + 0.5	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	9.93	7.53
T21	*N.r.*+ GNO + BA	0.5 + 2.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	9.27	7.63
T22	*N.r.*+ GNO + CMC	0.5 + 0.5	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	11.20	7.74
T23	*N.r.*+ GH + BA	0.5 + 2.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	9.70	7.80
T24	*N.r.*+ GH + CMC	0.5 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.63	7.97
T25	*N.r.*+ TW + TX + GLY + SFO	0.5 + 0.03 +2+ 1	6.67 (15.00)	33.33 (35.24)	100.00 (90.00)	8.50	7.67
T26	*N.r.*+ TW + TX + GLY + SFO +CNO	0.5 + 0.03 +2 + 1 + 1	6.67 (15.00)	31.67 (34.27)	100.00 (90.00)	8.17	7.10
T27	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO	0.5+0.03+2 +1+1+0.5	8.33 (16.78)	31.67 (34.27)	100.00 (90.00)	8.50	7.50

Table 21 Contd.....

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH
			3 DAI	7 DAI	10 DAI		
T28	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO	0.5+0.03 +2+1+1 +0.5+0.5	6.67 (15.00)	43.33 (41.15)	100.00 (90.00)	8.33	7.28
T29	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO+SBO	0.5+0.03+2 +1+1+0.5 +0.5+o.5	6.67 (15.00)	40.00 (39.23)	100.00 (90.00)	8.50	7.70
T30	*N.r.*+ TW + TX + GLY + SFO +CNO+MUO+GNO +SBO+GH	0.5+0.03+2 +1+1+0.5 +0.5+o.5+0.5	6.67 (15.00)	68.33 (55.73)	100.00 (90.00)	8.40	7.21
T31	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO +SBO+GH+BA	0.5+0.03+2 +1+1+0.5+ 0.5+o.5+o.5+2	8.33 (16.78)	53.33 (46.89)	100.00 (90.00)	8.40	7.25
T32	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO +SBO+GH+BA+CMC	0.5+0.03+2 +1+1+0.5+0.5+ o.5+o.5+2+0.5	8.33 (16.78)	33.33 (35.24)	100.00 (90.00)	8.33	7.31
T33	*N.r.*+ TW+GLY +SFO+CMC	0.5+2+1 +0.5	8.33 (16.78)	96.67 (79.53)	100.00 (90.00)	11.87	7.21
T34	*N.r.*+TW+GLY +CNO+CMC	0.5+2+1 +0.5	6.67 (15.00)	80.00 (63.44)	100.00 (90.00)	11.00	7.20
T35	*N.r.*+TW+GLY +MUO+CMC	0.5+2+0.5 +0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.57	7.52
T36	*N.r.*+ TX+GLY +SFO+CMC	0.03+2+1+0.5	8.33 (16.78)	23.33 (28.86)	100.00 (90.00)	10.07	8.19
T37	*N.r.*+ TW+GLY+GH	0.5+2+0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.67	8.81
T38	*N.r.*+TW+GLY +GNO+CMC	0.5+2+0.5+0.5	5.00 (12.92)	86.67 (68.61)	100.00 (90.00)	11.30	7.75
T39	*N.r.*+TW+GLY +SBO+CMC	0.5+2+0.5 +0.5	10.00 (18.44)	96.67 (79.53)	100.00 (90.00)	11.30	7.69
T40	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.80	7.78
T41	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.73	7.26
T42	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.80	7.85
T43	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.75	7.15
T44	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.90	7.71
T45	*N.r.*+ SFO	1.00	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.40	7.16
T46	*N.r.*+ CNO	1.00	46.67 (43.11)	90.00 (71.56)	100.00 (90.00)	9.70	7.26
T47	*N.r.*+ MUO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	10.30	7.52
T48	*N.r.*+ GNO	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.00	7.63
T49	*N.r.*+ SBO	0.50	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	10.20	7.33
T50	*N.r.*+ GH	0.50	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.70	7.21
T51	Control (*N.r.*alone)		30.00 (33.21)	46.67 (43.11)	100.00 (90.00)	7.60	8.40
	SE ±		**1.70**	**2.91**	**-**	**0.09**	**-**
	C.D. (P=0.05)		**4.77**	**8.16**	**NS**	**0.25**	**-**

*Figures in parentheses are arcsin value DAI = Days after inoculation *N.r.* = *Nomuraea rileyi* GH = Ghee
TW = Tween-80 SFO = Sunflower oil GLY= Glycerol CNO = Coconut oil TX = Triton-X-100 GNO = Groundnut oil
BA = Boric acid SBO= Soybean oil CMC=Carboxymethyl Cellulose MUO = Mustered oil

These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

***M.anisopliae* :**

Effect on growth : Significant differences among the various combination treatments and adjuvants alone were registered for medium surface coverage by *M.anisopliae* at 3, 7 and 10 DAI (Table 22). At 3 DAI, T22-GNO+CMC recorded significantly highest (91.67%) surface coverage. The next promising treatments were T45- *M.a.*+SFO (81.67%), T20-*M.a.*+SFO+CMC (80.00%), T42- *M.a.*+GLY (75.00%) and T50- *M.a.*+GH (70.00%).

The growth of fungus in treatment combinations with triton-X-100 and oils was prevented at 3 and 7 DAI, while it was negligible (6.67 to 30.0%) at 10 DAI.

At 7 DAI, out of 51 treatments, the treatment with T16-*M.a.*+GLY+GNO, T17-*M.a.*+GLY+SBO, T18-*M.a.*+GLY+GH, T20-*M.a.*+SFO+CMC, T21- *M.a.*+GNO+BA, T22- *M.a.*+GNO+CMC, T23-*M.a.*+GH+BA, T24- *M.a.*+GH+CMC, T40- *M.a.*+TW, T41- *M.a.*+TX, T42-*M.a.*+GLY, T43- *M.a.*+BA, T44- *M.a.*+CMC, T45- *M.a.*+SFO, T46- *M.a.*+CNO, T47- *M.a.*+MUO, T48- *M.a.*+GNO, T49- *M.a.*+SBO, T50- *M.a.*+GH and T51-control (*M.a.*alone) recorded cent per cent surface coverage. The least (16.67%) surface coverage in treatment T33- TW+GLY+SFO+CMC was recorded. At 10 DAI, the treatments except with triton-X-100 in combination with oils and chemicals (T7 to T12 ; T25 to T32 and T36) recorded cent per cent coverage of surface by the mycoagent.

Table 22. Effect of combinations of chemicals and oils as adjuvants with inoculum in *M.anisopliae* 30% AS on growth and biomass of mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH
			3 DAI	7 DAI	10 DAI		
T1	*M.a.*+TW + SFO	0.5 + 1.0	10.00 (18.44)*	80.00 (63.44)	100.00 (90.00)	8.90	8.34
T2	*M.a.*+TW + CNO	0.5 + 1.0	15.00 (22.79)	83.33 (65.88)	100.00 (90.00)	7.90	8.88
T3	*M.a.*+TW + MUO	0.5 + 0.5	6.67 (15.00)	36.67 (37.29)	100.00 (90.00)	8.73	6.88
T4	*M.a.*+TW + GNO	0.5 + 0.5	6.67 (15.00)	23.33 (28.86)	100.00 (90.00)	8.77	5.63
T5	*M.a.*+TW + SBO	0.5 + 0.5	6.67 (15.00)	45.00 (42.13)	100.00 (90.00)	8.57	8.26
T6	*M.a.*+TW + GH	0.5 + 0.5	6.67 (15.00)	76.67 (61.14)	100.00 (90.00)	8.73	8.16
T7	*M.a.*+TX + SFO	0.03 + 1.0	0.00 (0.00)	0.00 (0.00)	30.00 (33.21)	3.03	4.20
T8	*M.a.*+TX + CNO	0.03 + 1.0	0.00 (0.00)	0.00 (0.00)	28.33 (32.14)	2.60	4.15
T9	*M.a.*+TX + MUO	0.03 + 0.5	0.00 (0.00)	0.00 (0.00)	11.67 (20.00)	1.73	4.20
T10	*M.a.*+TX + GNO	0.03 + 0.5	0.00 (0.00)	0.00 (0.00)	11.67 (20.00)	1.50	4.08
T11	*M.a.*+TX + SBO	0.03 + 0.5	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	1.57	4.09
T12	*M.a.*+TX + GH	0.03 + 0.5	0.00 (0.00)	0.00 (0.00)	6.67 (15.00)	1.17	4.21
T13	*M.a.*+GLY + SFO	2.0 + 1.0	23.33 (28.86)	96.67 (79.53)	100.00 (90.00)	7.83	8.34
T14	*M.a.*+GLY + CNO	2.0 + 1.0	5.00 (12.92)	91.67 (73.26)	100.00 (90.00)	8.27	7.34
T15	*M.a.*+GLY + MUO	2.0 + 0.5	10.00 (18.44)	80.00 (63.44)	100.00 (90.00)	8.87	7.72
T16	*M.a.*+GLY + GNO	2.0 + 0.5	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	9.63	8.45
T17	*M.a.*+GLY + SBO	2.0 + 0.5	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	8.50	8.26
T18	*M.a.*+GLY + GH	2.0 + 0.5	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	8.70	8.59
T19	*M.a.*+SFO + BA	1.0 + 2.0	8.33 (16.78)	90.00 (71.56)	100.00 (90.00)	7.83	6.47
T20	*M.a.*+SFO + CMC	1.0 + 0.5	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.20	8.01
T21	*M.a.*+GNO + BA	0.5 + 2.0	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)	11.00	8.04
T22	*M.a.*+GNO + CMC	0.5 + 0.5	91.67 (73.26)	100.00 (90.00)	100.00 (90.00)	10.90	8.08
T23	*M.a.*+GH + BA	0.5 + 2.0	23.33 (28.86)	100.00 (90.00)	100.00 (90.00)	9.83	8.58
T24	*M.a.*+GH + CMC	0.5 + 0.5	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	8.70	8.15
T25	*M.a.*+TW + TX + GLY + SFO	0.5 + 0.03 +2+ 1	0.00 (0.00)	0.00 (0.00)	13.33 (21.39)	3.07	4.25
T26	*M.a.*+TW + TX + GLY + SFO +CNO	0.5 + 0.03 +2 + 1 + 1	0.00 (0.00)	0.00 (0.00)	16.67 (24.12)	3.13	4.05
T27	*M.a.*+TW + TX + GLY +SFO +CNO+MUO	0.5+0.03+2 +1+1+0.5	0.00 (0.00)	0.00 (0.00)	20.00 (26.56)	2.93	4.21
T28	*M.a.*+TW+TX+GLY +SFO+CNO+MUO +GNO	0.5+0.03+2 +1+1+0.5 +0.5	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	1.40	4.48

Table 22 Contd.....

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH
			3 DAI	7 DAI	10 DAI		
T29	*M.a.*+TW+TX+GLY +SFO+CNO+MUO+ GNO+SBO	0.5+0.03+2 +1+1+0.5 +0.5+o.5	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	1.50	4.30
T30	*M.a.*+TW+TX+ GLY +SFO+CNO+MUO +GNO+SBO+GH	0.5+0.03+2 +1+1+0.5 +0.5+o.5+0.5	0.00 (0.00)	0.00 (0.00)	15.00 (22.79)	2.67	4.03
T31	*M.a.*+TW+TX+GLY +SFO+CNO+MUO +GNO+SBO+GH +BA	0.5+0.03+2 +1+1+0.5 +0.5+o.5 +o.5+2	0.00 (0.00)	0.00 (0.00)	16.67 (24.12)	2.23	4.23
T32	*M.a.*+TW+TX+GLY +SFO+CNO+MUO +GNO+SBO+GH +BA+CMC	0.5+0.03+2 +1+1+0.5 +0.5+o.5 +o.5+2+0.5	0.00 (0.00)	0.00 (0.00)	13.33 (21.39)	2.43	4.20
T33	*M.a.*+TW+GLY+SFO +CMC	0.5+2+1 +0.5	10.00 (18.44)	16.67 (24.12)	100.00 (90.00)	6.70	7.00
T34	*M.a.*+TW+GLY+CNO +CMC	0.5+2+1 +0.5	18.33 (25.33)	36.67 (37.29)	100.00 (90.00)	8.70	8.63
T35	*M.a.*+TW+GLY+MUO +CMC	0.5+2+0.5 +0.5	5.00 (12.92)	50.00 (45.00)	100.00 (90.00)	7.97	8.11
T36	*M.a.*+TX+GLY+SFO +CMC	0.03+2+1 +0.5	0.00 (0.00)	0.00 (0.00)	8.33 (16.78)	2.03	7.80
T37	*M.a.*+TW+GLY+GH	0.5+2+0.5	0.00 (0.00)	50.00 (45.00)	100.00 (90.00)	7.03	7.17
T38	*M.a.*+TW+GLY+GNO +CMC	0.5+2+0.5 +0.5	0.00 (0.00)	31.67 (34.27)	100.00 (90.00)	7.10	7.00
T39	*M.a.*+TW+GLY+SBO +CMC	0.5+2+0.5 +0.5	8.33 (16.78)	68.33 (55.73)	100.00 (90.00)	6.87	7.55
T40	*M.a.*+TW	0.50	13.33 (21.39)	100.00 (90.00)	100.00 (90.00)	7.40	8.89
T41	*M.a.*+TX	0.03	0.00 (0.00)	21.67 (27.76)	100.00 (90.00)	6.70	4.42
T42	*M.a.*+GLY	2.00	75.00 (60.00)	100.00 (90.00)	100.00 (90.00)	8.30	7.40
T43	*M.a.*+BA	2.00	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	8.20	8.59
T44	*M.a.*+CMC	0.50	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	8.97	8.30
T45	*M.a.*+SFO	1.00	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	8.80	8.44
T46	*M.a.*+CNO	1.00	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.40	8.15
T47	*M.a.*+MUO	0.50	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	7.47	7.98
T48	*M.a.*+GNO	0.50	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	8.23	8.64
T49	*M.a.*+SBO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	6.70	8.20
T50	*M.a.*+GH	0.50	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	8.03	8.73
T51	Control (*M.a.*alone)	-	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)	6.60	8.10
	S.E ±		**1.21**	**2.49**	**1.40**	**0.12**	**-**
	C.D(P=0.05)		**3.40**	**6.99**	**3.94**	**0.35**	**-**

*Figures in parentheses are arcsin values. DAI = Days after inoculation *M.a. = Metarhizium anisopliae*
TW = Tween-80, TX= Triton-X-100, CMC = Carboxymethyl Cellulose GLY = Glycerol
BA = Boric acid GNO = Groundnut oil SFO = Sunflower oil SBO = Soybean oil
MUO = Mustard oil CNO = Coconut oil GH = Ghee

Effect on biomass and pH : T20- *M.a.*+SFO+CMC produced highest (11.20g) biomass. It was at par with T21- *M.a.*+GNO+BA (11.00g) and T22- *M.a.*+GNO+CMC (10.90g). The next promising treatments in their decending order for production substantial of biomass were T23- *M.a.*+GH+BA, T16- *M.a.*+GLY+GNO, T44- *M.a.*+CMC, T1- *M.a.*+TW+SFO, T15- *M.a.*+GLY+MUO, and T45- *M.a.*+SFO which produced 9.83, 9.63, 8.97, 8.90, 8.87 and 8.80g fungal biomass, respectively. The treatments with various combinations of adjuvants with triton-X-100 allowed to develop very less biomass (1.17 to 3.13g) in remaining treatments including treatments with T51- control (*M.a.*alone) 6.60g.

The pH of *M.anisopliae* cultures developed from inoculums in the formulations varied broadly from 4.03 to 8.88 as against biomass range of 1.17g to 11.20g per 40 ml medium (Table 22). It could be generalized from the pH figure that pH declined less than 5 in Triton-X-100 comprising formulations. It adversely affected the fungal development as evidenced from 1.17 to 3.13g biomass against 6.60 to 11.20g biomass with pH of 5.63 to 8.64.

Thus, among combination of chemical and edible oils as adjuvants, the advanced stage formulations of *N.rileyi* with T18-*N.r.*+GLY+GH, T6-*N.r.*+TW+GH, T33-*N.r.*+TW+GLY+SFO+CMC, T13-*N.r.*+GLY+SFO and those with *M.anisopliae* T20-*M.a.*+SF+CMC, T21-*M.a.*+GNO+BA and T22-*M.a.*+GNO+CMC were highly promising for the growth and development of the fungi. Hence, further those were included for subsequent studies.

4.1.5.3 Mixture of oils as adjuvants

The combinations of mixtures of sunflower, coconut, mustard, groundnut, soybean and ghee were added to aqua suspension of *N.rileyi* and *M.anisopliae* as test formulation bases. One ml of respective formulation was inoculated in the culture medium. The results on growth and development of the mycoagent as influenced by the treatments at 3, 7 and 10 days are presented in Table 23 and 24.

***N.rileyi* :**

Effect on growth : Data presented in Table 23 revealed that T14- *N.r.*+SFO registered significantly highest (88.33 %) surface coverage over the rest of the treatments except the treatments with T19- GH (80%).

Table 23. Effect of combinations of edible oils as adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomasss of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*N.r.*+ SFO + CNO	1.0 + 1.0	8.33 (16.78)*	100.00 (90.00)	100.00 (90.00)	10.90	7.88
T2	*N.r.*+ SFO + GNO	1.0 + 0.5	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.00	7.38
T3	*N.r.*+ SFO + GH	1.0 + 0.5	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	11.97	7.67
T4	*N.r.*+ SFO + SBO	1.0 +0.5	33.33 (35.24)	93.33 (75.00)	98.33 (82.51)	9.33	7.80
T5	*N.r.*+ SFO + MUO	1.0+0.5	11.67 (20.00)	85.00 (67.21)	95.00 (77.08)	9.87	7.60
T6	*N.r.*+ GNO+CNO	0.5+1	10.00 (18.44)	80.00 (63.44)	96.67 (79.53)	10.53	7.65
T7	*N.r.*+ GNO+GH	0.5+0.5	41.67 (40.22)	93.33 (75.00)	96.67 (79.53)	11.23	7.60
T8	*N.r.*+ GNO+SBO	0.5+0.5	31.67 (34.27)	83.33 (65.88)	95.00 (77.08)	9.17	7.90
T9	*N.r.*+ GNO+MUO	0.5+0.5	18.33 (25.33)	66.67 (54.76)	91.67 (73.26)	9.33	7.80
T10	*N.r.*+ SFO+GNO +GH	1.0+0.5 +0.5	18.33 (25.33)	66.67 (54.76)	96.67 (79.53)	9.77	7.75
T11	*N.r.*+ SFO+GNO +GH+CNO	1.0+0.5 +0.5+1.0	16.67 (24.12)	66.67 (54.76)	96.67 (79.53)	10.17	7.80
T12	*N.r.*+SFO +GNO+GH+MUO	1.0+0.5 +0.5+0.5	18.33 (25.33)	68.33 (55.73)	96.67 (79.53)	10.20	7.82
T13	*N.r.*+SFO+GNO +GH+MUO+SBO	1.0+0.5 +0.5+0.5 +0.5	18.33 (25.33)	63.33 (52.71)	98.33 (82.51)	10.27	7.85
T14	*N.r.*+ SFO	1.00	88.33 (70.00)	100.00 (90.00)	100.00 (90.00)	12.47	7.16
T15	*N.r.*+ CNO	1.00	41.67 (40.22)	90.00 (71.56)	100.00 (90.00)	9.60	7.26
T16	*N.r.*+ MUO	0.50	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	10.20	7.52
T17	*N.r.*+ GNO	0.50	75.00 (60.00)	100.00 (90.00)	100.00 (90.00)	10.90	7.63
T18	*N.r.*+ SBO	0.50	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	10.30	7.33
T19	*N.r.*+ GH	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.80	7.24
T20	Control (*N.r.*alone)	-	20.00 (26.56)	50.00 (45.00)	100.00 (90.00)	7.80	8.40
	SE ±		**1.53**	**2.14**	**1.81**	**0.11**	**-**
	C.D(P=0.05)		**4.38**	**6.13**	**5.20**	**0.32**	**-**

*Figures in parentheses are arcsin values. DAI = Days after inoculation *N.r. = Nomuraea rileyi*
SFO = Sunflower oil GH = Ghee CNO = Coconut oil
GNO = Groundnut oil SBO = Soybean oil MUO = Mustered oil

Later was followed by T17-*N.r.*+GNO (75.0%), T3-*N.r.*+SFO+GH (53.33%) at 3 DAI. Among the treatments with combination of oils, the treatment with T3-*N.r.*+SFO+GH recorded highest (53.33%) surface coverage followed by T7-*N.r.*+GNO+GH (41.67%).At 7 DAI, treatments recorded 63.33 to 100 per cent surface coverage.

Effect on biomass and pH : T14-*N.r.*+SFO alone registered significantly higher (12.47g) yield of fungal biomass than rest of the treatments (Fig.9). Among the mixtures of oils, T3-*N.r.*+SFO+GH produced maximum (11.97g) biomass. However, it was at par with T19-*N.r.*+GH alone (11.80g). The lowest (7.80g) biomass was recorded in T20 control (*N.r.*alone).

The pH of the fungal culture developed from 20 treatment formulations ranged from 7.16 in treatment T14 *N.r.*+SFO to 7.90 in T8 *N.r.*+GNO+SBO registering biomass of 12.47 and 9.17g, respectively. The pH of formulation producing maximum biomass (12.47g) was 7.16 comparing treatment adjuvants sunflower oil 1% when it was 8.40 in control producing biomass of 7.80g per 40 ml medium. These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

***M.anisopliae* :**

Effect on growth : At 3 DAI, treatments recorded 15.0 to 81.67 per cent surface coverage while at 7 DAI it ranged from 60.0 to 100.0 per cent (Table 24). At 3 DAI, T14- *M.a.*+SFO recorded significantly highest (81.67%) surface covereage in medium. T7- *M.a.*+GNO+GH recorded highest (60.0%) surface coverage. It was followed by T3- *M.a.*+SFO+GH (58.33%).

At 7 DAI, oil comprising treatments recorded cent per cent surface coverage. At 10 DAI, the treatments except T4- *M.a.*+SFO+SBO (95.00%), T5-

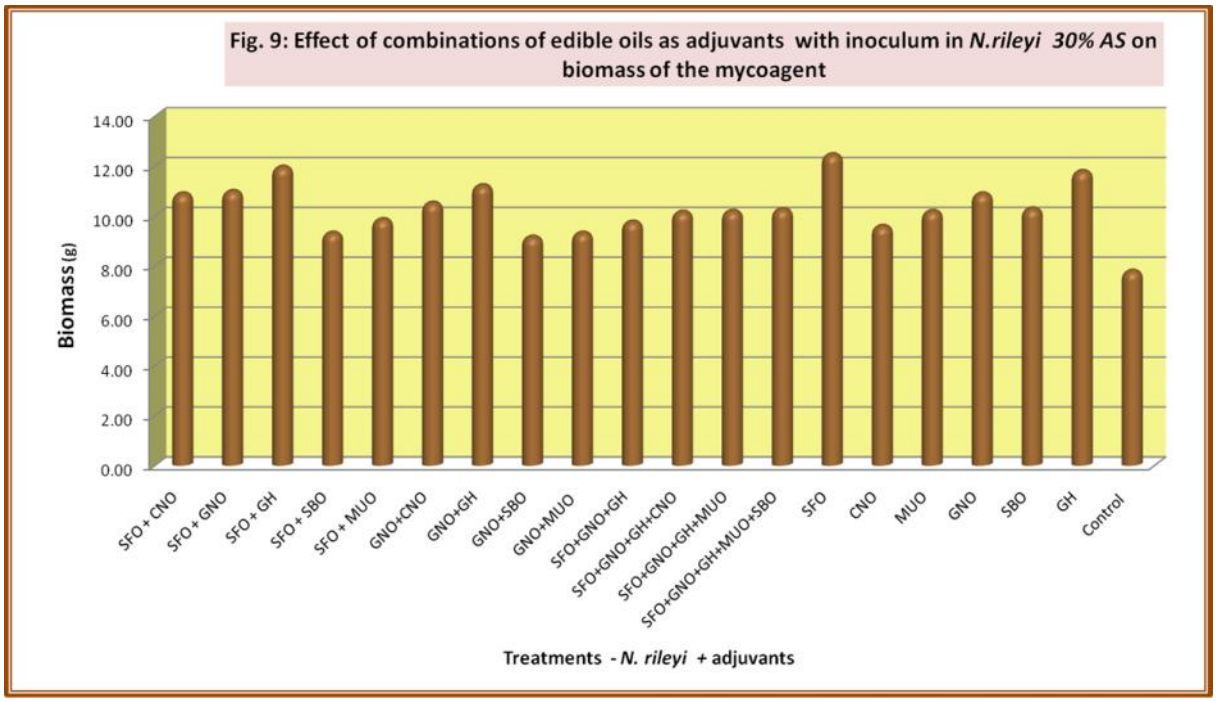


Fig. 9: Effect of combinations of edible oils as adjuvants with inoculum in N.rileyi 30% AS on biomass of the mycoagent
Biomass (g)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
SFO + CNO
SFO + GNO
SFO + GH
SFO + SBO
SFO + MUO
GNO+CNO
GNO+GH
GNO+SBO
GNO+MUO
SFO+GNO+GH
SFO+GNO+GH+CNO
SFO+GNO+GH+MUO
SFO+GNO+GH+MUO+SBO
SFO
CNO
MUO
GNO
SBO
GH
Control
Treatments - N. rileyi + adjuvants

M.a.+SFO+MUO (96.67%), T6- *M.a.*+GNO+CNO (93.33%), T8- *M.a.*+GNO+SBO (95.00%), T9- *M.a.*+GNO+MUO (93.33%), T10- *M.a.*+SFO+GNO+GH (98.33%) T11- *M.a.*+SFO+GNO+GH+CNO (95.00%), T12- *M.a.*+SFO+GNO+GH+MUO (95.00%) and T13- *M.a.*+SFO+GNO+GH+MUO+SBO (91.67%) recorded cent per cent medium surface coverage.

Table 24. Effect of combinations of edible oils as adjuvants with inoculum in *M.anisopliae* 30% AS on growth and development of in AS (30%v/v)

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*M.a.*+SFO + CNO	1.0 + 1.0	18.33 (25.33)*	96.67 (79.53)	100.00 (90.00)	8.80	8.35
T2	*M.a.*+SFO + GNO	1.0 + 0.5	18.33 (25.33)	96.67 (79.53)	100.00 (90.00)	8.60	8.10
T3	*M.a.*+SFO + GH	1.0 + 0.5	58.33 (49.79)	100.00 (90.00)	100.00 (90.00)	8.63	7.27
T4	*M.a.*+SFO + SBO	1.0 +0.5	18.33 (25.33)	90.00 (71.56)	95.00 (77.08)	8.20	8.00
T5	*M.a.*+SFO + MUO	1.0+0.5	21.67 (27.76)	78.33 (62.24)	96.67 (79.53)	8.23	8.20
T6	*M.a.*+GNO+CNO	0.5+1	15.00 (22.79)	70.00 (56.79)	93.33 (75.00)	8.13	8.30
T7	*M.a.*+GNO+GH	0.5+0.5	60.00 (50.77)	100.00 (90.00)	100.00 (90.00)	10.60	8.17
T8	*M.a.*+GNO+SBO	0.5+0.5	18.33 (25.33)	60.00 (50.77)	95.00 (77.08)	8.00	8.10
T9	*M.a.*+GNO+MUO	0.5+0.5	18.33 (25.33)	68.33 (55.73)	93.33 (75.00)	8.17	8.10
T10	*M.a.*+SFO+GNO +GH	1.0+0.5 +0.5	30.00 (33.21)	63.33 (52.71)	98.33 (82.51)	8.50	7.90
T11	*M.a.*+SFO+GNO +GH+CNO	1.0+0.5 +0.5+1.0	20.00 (26.56)	66.67 (54.76)	95.00 (77.08)	8.37	7.90
T12	*M.a.*+SFO+GNO +GH+MUO	1.0+0.5 +0.5+0.5	26.67 (31.11)	75.00 (60.00)	95.00 (77.08)	8.23	8.10
T13	*M.a.*+SFO+GNO +GH+MUO+SBO	1.0+0.5 +0.5+0.5 +0.5	18.33 (25.33)	68.33 (55.73)	91.67 (73.26)	8.23	8.30
T14	*M.a.*+SFO	1.00	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	8.90	8.44
T15	*M.a.*+CNO	1.00	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.50	8.15
T16	*M.a.*+MUO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	7.40	7.98
T17	*M.a.*+GNO	0.50	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	8.20	8.64
T18	*M.a.*+SBO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	6.80	8.20
T19	*M.a.*+GH	0.50	65.00 (53.73)	100.00 (90.00)	100.00 (90.00)	8.00	8.73
T20	Control (*M.a.*alone)	-	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)	6.70	8.10
	S.E ±		**1.24**	**2.66**	**1.16**	**0.09**	**-**
	C.D(P=0.05)		**3.55**	**7.62**	**3.32**	**0.26**	**-**

*Figures in parenthesesare arcsin values. DAI = Days after inoculation
M.a. = *Metarhizium anisopliae* SFO= Sunflower oil GH = Ghee
GNO = Groundnut oil CNO = Coconut oil MUO = Mustard oil SBO = Soybean oil

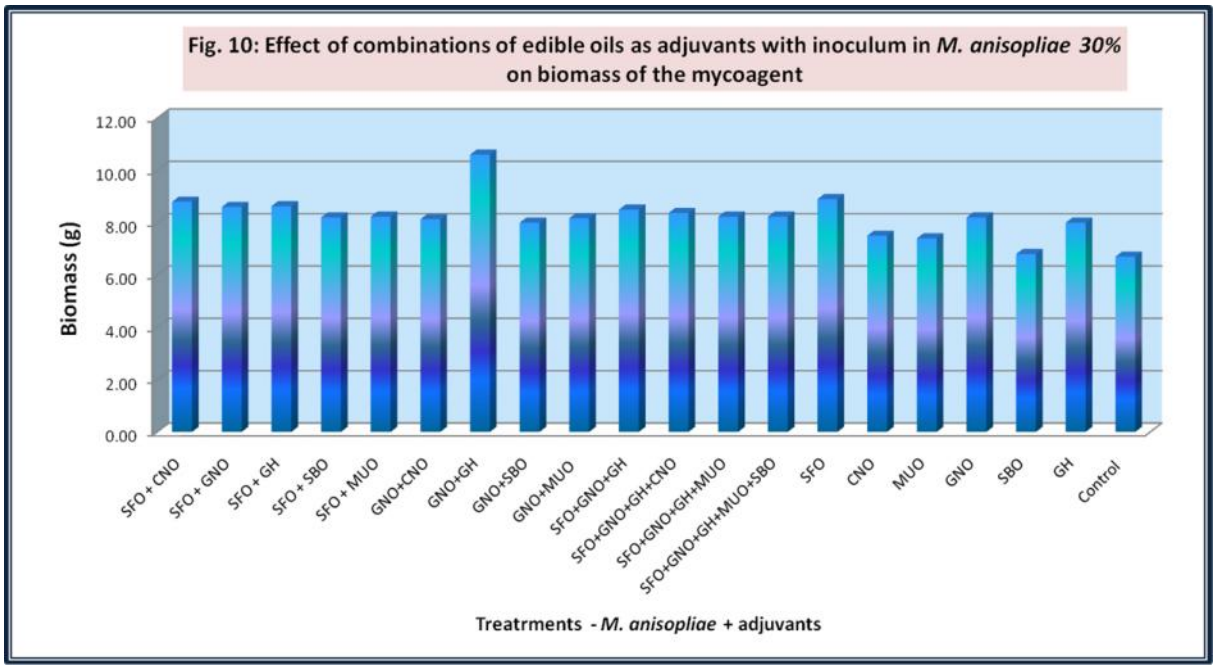


Fig. 10: Effect of combinations of edible oils as adjuvants with inoculum in M. anisopliae 30% on biomass of the mycoagent
Biomass (g)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
SFO + CNO
SFO + GNO
SFO + GH
SFO + SBO
SFO + MUO
GNO+CNO
GNO+GH
GNO+SBO
GNO+MUO
SFO+GNO+GH
SFO+GNO+GH+CNO
SFO+GNO+GH+MUO
SFO+GNO+GH+MUO+SBO
SFO
CNO
MUO
GNO
SBO
GH
Control
Treatrments - M. anisopliae + adjuvants

Effect on biomass and pH : A significant difference among the treatments for fungal biomass was observed at 10 DAI (Fig.10). The maximum fungal biomass of 10.60g in treatments T7- *M.a.*+GNO+GH was produced. The next promising at par treatments were T14-*M.a.*+SFO and T1- *M.a.*+SFO+CNO, which produced 8.90 and 8.80g of biomass, respectively.

The lowest biomass of 8.17g was recorded in treatments with T6-*M.a.*+GNO+CNO among the treatments of combination of adjuvants. However, it was significantly higher than T20-Control (6.70g) and T18-*M.a.*+SBO (6.80g). The pH of *M.anisopliae* cultures developed from inoculums in the formulations varied broadly from 7.27 to 8.64 against biomass range of 6.80g to 10.60g per 40 ml medium (Table 24).

Considering performance of the oil adjuvants based combinations at10 DAI T3-*N.r.*+SFO+GH yielded highest (11.97g) biomass. But it was at par with T19-*N.r.*+GH (11.80g). Among sole oil adjuvants T14-*N.r.*+SFO produced maximum biomass (12.47g). Hence, T3- *N.r.*+SFO+GH, T14-*N.r.*+ SFO and T19-*N.r.*+GH were selected as input for advancing *N.rileyi* and *M.anisopliae* of T7-*M.a.*+GNO+GH was selected which produced highest biomass of 10.60g. There is no published information on aspect of study.

4.1.5.4 The combinations of chemical, oils and few other edible substrates as adjuvants

***N.rilelyi* :**

Effect on growth : At 3 DAI, T6- *N.r.*+GH+HO and T23- *N.r.*+HO recorded significantly highest (90.0 % each) growth of *N.rileyi* (Table 25). However, it was at par with T24- *N.r.*+SFO (86.67%) and T29- *N.r.*+GH (85.00%), when the growth in control was 25.0 per cent.

The lowest surface coverage (6.67%) among multiple adjuvant treatments was observed in T1-*N.r.*+TW+HO, T7-*N.r.*+BA+HO, T9-*N.r.*+TW+GLY+BA+CMC+HO,T11-*N.r.*+GLY+SFO+CMC+Ho, T12-*N.r.*+TW+GLY+HO, T13-*N.r.*+TX+GLY+GH+HO and T15- *N.r.*+BA+CMC+HO (6.67% each).

At 7 DAI, the surface coverage was 21.67 to 100 per cent among the treatments with adjuvant combinations.

Table 25. Effect of combinations of chemicals, oils and other edible substrates as adjuvants with inoculum in *N.rileyi* 30% AS on growth and biomass of mycoagent

Tr. No.	Treatment details	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*N.r.*+ TW + HO	0.5 + 1.0	6.67 (15.00)*	100.00(90.00)	100.00(90.00)	11.00	7.37
T2	*N.r.*+ TX + HO	0.03 + 1.0	10.00(18.44)	26.67(31.11)	100.00(90.00)	10.37	8.29
T3	*N.r.*+ GLY + HO	2.0 + 1.0	28.33(32.14)	100.00(90.00)	100.00(90.00)	9.47	8.19
T4	*N.r.*+ SF + HO	1.0 + 1.0	36.67(37.29)	100.00(90.00)	100.00(90.00)	10.27	7.64
T5	*N.r.*+ GNO + HO	0.5 + 1.0	25.00(30.00)	100.00(90.00)	100.00(90.00)	10.30	7.77
T6	*N.r.*+ GH + HO	0.5 + 1.0	90.00(71.56)	100.00(90.00)	100.00(90.00)	9.93	8.11
T7	*N.r.*+ BA + HO	2.0 + 1.0	6.67(15.00)	100.00(90.00)	100.00(90.00)	9.90	8.75
T8	*N.r.*+ CMC + HO	0.5 + 1.0	26.67(31.11)	100.00(90.00)	100.00(90.00)	9.10	8.50
T9	*N.r.*+ TW+TX+ GLY+SFO+CNO +MUO+GNO +SBO+GH+BA +CMC+HO	0.5+0.03+2 +1+1+0.5 0.5+o.5+o.5 +2+0.5+1	6.67 (15.00)	56.67 (48.85)	100.00 (90.00)	10.57	7.34
T10	*N.r.*+ TW+GLY +BA+CMC+HO	0.5+2+2 +0.5+1	6.67(15.00)	100.00(90.00)	100.00(90.00)	11.30	7.45
T11	*N.r.*+ GLY+SFO +CMC+HO	2+1+0.5+1	6.67(15.00)	100.00(90.00)	100.00(90.00)	10.97	8.21
T12	*N.r.*+ TW+GLY+HO	0.5+2+1	6.67(15.00)	83.33(65.88)	100.00(90.00)	12.28	7.37
T13	*N.r.*+ TX+GLY +GH+HO	0.03+2 +0.5+1	6.67(15.00)	33.33(35.24)	100.00(90.00)	11.03	7.42
T14	*N.r.*+ TX+CMC+HO	0.03+0.5 +1	8.33 (16.78)	21.67 (27.76)	100.00 (90.00)	9.27	7.96
T15	*N.r.*+ BA+CMC+HO	2+0.5+1	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	9.63	7.43
T16	*N.r.*+GH+HO+GLY	0.5+1+2	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	9.03	8.94
T17	*N.r.*+ GH+HO+ GLY+CMC	0.5+1+2 +0.5	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	9.03	8.92
T18	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.70	7.78
T19	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.80	7.26
T20	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.85
T21	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.70	7.15
T22	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.80	7.71
T23	*N.r.*+ HO	1.00	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	11.90	7.70
T24	*N.r.*+ SFO	1.00	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.17	7.16
T25	*N.r.*+ CNO	1.00	46.67 (43.11)	90.00 (71.56)	100.00 (90.00)	9.50	7.26
T26	*N.r.*+ MUO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	10.35	7.52
T27	*N.r.*+ GNO	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.10	7.63
T28	*N.r.*+ SBO	0.50	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	10.00	7.33
T29	*N.r.*+ GH	0.50	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.60	7.24
T30	Control (*N.r.*alone)		25.00 (30.00)	50.00 (45.00)	100.00 (90.00)	7.50	8.40
	S.E +		**1.73**	**2.30**	**-**	**0.11**	**-**
	C.D(P=0.05)		**4.91**	**6.51**	**NS**	**0.33**	**-**

*Figures in parentheses are arcsin value DAI = Days after inoculation *N.r. = Nomuraea rileyi*
SFO = Sunflower oil TW= Tween-80 CNO = Coconut oil GLY= Glycerol GNO = Groundnut oil
GH = Ghee TX = Triton-X-100 SBO = Soybean oil BA = Boric acid MUO = Mustered oil
CMC = Carboxymethyl Cellulose

The lowest per cent surface coverage was recorded in T14- *N.r.*+TX +CMC+HO (21.67%). At 10 DAI, all the treatments showed cent per cent growth.

Effect on biomass and pH : At 10 DAI, the biomass in the treatments was 7.80 to 12.28g against 7.50g in control. T12- *N.r.*+TW+GLY+HO produced significantly highest (12.28g) fungal biomass (Fig.11). However, it was at par with T24- *N.r.*+SFO (12.17g) and T23- *N.r.*+HO (11.90g).

Next highly promising treatments were T29- *N.r.*+GH, T10- *N.r.*+TW+GLY+BA+CMC+HO and T27- *N.r.*+GNO which produced 11.60, 11.30 and 11.10g biomass, respectively.The pH of the fungal culture developed from 30 treatment formulations ranged from 7.16 in treatment T24 *N.r.*+ SFO to 8.75 in T7 *N.r.*+ *BA* + HO registering biomass of 12.17 and 9.90g respectively. The pH of formulation producing maximum biomass (12.28g) was 7.37 comparing treatment adjuvants Tween 80 0.5% + Glycerol 2% + Honey 1%, when it was 8.40 in control producing biomass of 7.50g per 40 ml medium.

***M.anisopliae* :**

Effect on growth : Significant differences among the treatments with adjuvants in combinations and adjuvants alone were registered for surface coverage by the mycoagent at 3, 7 and 10 DAI (Table 26).

At 3 DAI, surface coverage was 0 to 85.0 per cent. T5- *M.a.*+GNO+HO and T23- *M.a.*+HO gave significantly highest (85.0% each) growth of the fungus. However, it was at par with T29- *M.a.*+GH (70.0%). The growth in T2-*M.a.*+TX+HO, T9-*M.a.*+TW+TX+GLY+SFO+CNO+MUO+GNO+BA+CMC+HO, T13- *M.a.*+TX+GLY+GH+HO and T14- *M.a.*+TX+CMC+HO was inhibited at 3 and 7 DAI and that in T19- *M.a.*+TX at 3 DAI.

At 7 DAI, surface coverage ranged 40.0 to 100.0 per cent among the treatments. T4- *M.a.*+SFO+HO, T5- *M.a.*+GNO+HO, T6- *M.a.*+GH+HO, T7- *M.a.*+BA+HO, T8- *M.a.*+CMC+HO and T16- *M.a.*+GH+HO+GLY recorded cent per cent surface coverage by the fungus. At 10 DAI, all the treatments except T2-*M.a.*+TX+HO(15.0%),T9-

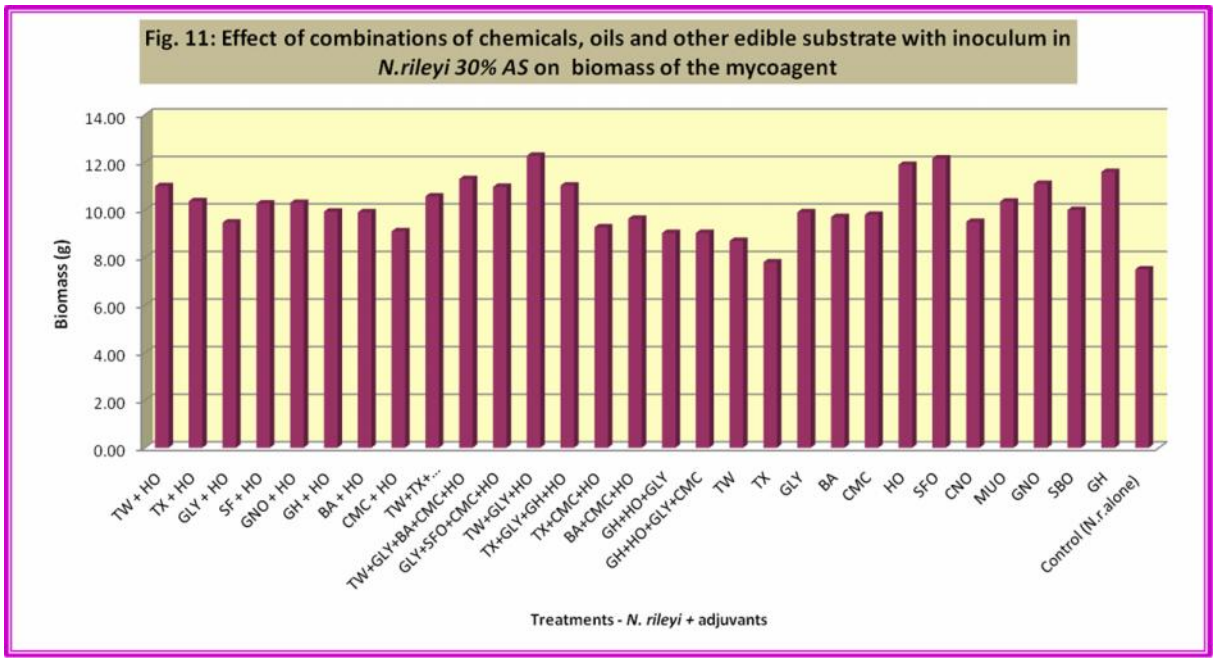


Fig. 11: Effect of combinations of chemicals, oils and other edible substrate with inoculum in N.rileyi 30% AS on biomass of the mycoagent
Biomass (g)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
TW + HO
TX + HO
GLY + HO
SF + HO
GNO + HO
GH + HO
BA + HO
CMC + HO
TW+TX+...
TW+GLY+BA+CMC+HO
GLY+SFO+CMC+HO
TW+GLY+HO
TX+GLY+GH+HO
TX+CMC+HO
BA+CMC+HO
GH+HO+GLY
GH+HO+GLY+CMC
TW
TX
GLY
BA
CMC
HO
SFO
CNO
MUO
GNO
SBO
GH
Control (N.r.alone)
Treatments - N. rileyi + adjuvants

M.a.+TW+TX+GLY+SFO+CNO+MUO+GNO+BA+CMC+HO (43.33%), T10- *M.a.*+TW+GLY+BA+CMC+HO (73.33%), T13- *M.a.*+TX+GLY+GH+HO (83.33%) and T14- *M.a.*+TX+CMC+HO (10.0%) recorded cent per cent growth.

Effect on biomass and pH : Considering the performance of oils as adjuvants at 10 DAI, T4- *M.a.*+SFO+HO yielded highest biomass (11.0g). However, it was at par with T6- *M.a.*+GH+HO (10.60g). The fungal biomass in range 1.60g to 11.00g among all treatments. The least biomass (1.60g) was observed in T14- *M.a.*+TX+CMC+HO (Fig.12).

The pH of *M.anisopliae* cultures developed from inoculums in the formulations varied broadly from 4.15 to 8.89 against biomass range of 1.70g to 11.00g per 40 ml medium (Table 26). It could be generalized from the pH figure that pH declined less than 5 in Triton-X-100 comprising formulations. It adversely affected the fungal development.

It was established that the *N.rileyi* formulations with combination of adjuvants helped in increasing production of fungal biomass at 10 DAI. T12- *N.r.*+TW+GLY+HO(12.28g) produced highest biomass and T23- *N.r.*+HO(11.90g) and T24-*N.r.*+SFO(12.17g) were at par to it. In case of *M.anisopliae* T4-*M.a.*+SFO+HO(11.0g) and T6-*M.a.*+GH+HO (10.60g) were at par. Hence, these treatments were considered for further aspects of evaluation to find out potential formulations.

Considering the overall performance of the adjuvants for growth and development of *N.rileyi* in series of lab experimentation out of 96 test formulations (4.1.5.1 to 4.1.5.4) 10 formulation comprising *N.r.*+HO (1%),*N.r*+SFO(1%),*N.r.*+GH(0.5%),*N.r.*+TW(0.5%)+GH(0.5%),*N.r.*+GLY(2%)+SFO(1%),*N.r.*+GLY(2%)+GH(0.5%),*N.r.*+SFO(1%)+GH(0.5%), *N.r.*+TW(0.5%)+GLY(2%)+SFO(1%)+CMC(0.5%), *N.r.*+TW(0.5%)+GLY(2%)+HO(1%)and *N.r.*+TW(0.5%)+GLY(2%)+CMC(0.5%) were emerged out as most promising and advanced stage formulations of *N. rileyi.*

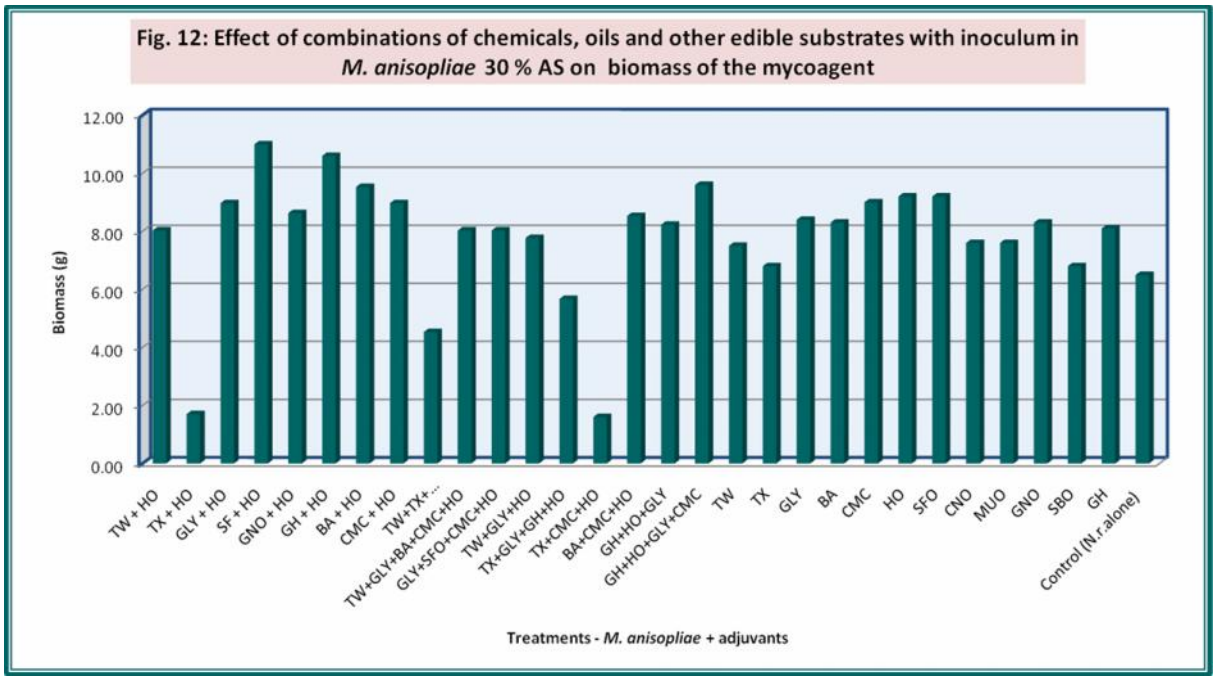


Fig. 12: Effect of combinations of chemicals, oils and other edible substrates with inoculum in M. anisopliae 30 % AS on biomass of the mycoagent
Biomass (g)
12.00
10.00
8.00
6.00
4.00
2.00
0.00
TW + HO
TX + HO
GLY + HO
SF + HO
GNO + HO
GH + HO
BA + HO
CMC + HO
TW+TX+...
TW+GLY+BA+CMC+HO
GLY+SFO+CMC+HO
TW+GLY+HO
TX+GLY+GH+HO
TX+CMC+HO
BA+CMC+HO
GH+HO+GLY
GH+HO+GLY+CMC
TW
TX
GLY
BA
CMC
HO
SFO
CNO
MUO
GNO
SBO
GH
Control (N.r.alone)
Treatments - M. anisopliae + adjuvants

Table 26. Effect of combinations of chemicals, oils and other edible substrates as adjuvants with inoculum in *M.anisopliae* 30% AS on growth and biomass of mycoagent

Tr. No.	Treatments	Conc.(%) of adjuvant	Surface coverage(%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T1	*M.a.*+TW + HO	0.5 + 1.0	5.00(12.92)*	73.33(58.89)	100.00(90.00)	8.03	7.38
T2	*M.a.*+TX + HO	0.03 + 1.0	0.00(0.00)	0.00(0.00)	15.00(22.79)	1.70	4.23
T3	*M.a.*+GLY + HO	2.0 + 1.0	28.33(32.39	76.67(61.14)	100.00(90.00)	8.97	7.35
T4	*M.a.*+SFO + HO	1.0 + 1.0	28.33 (32.39)	100.0(90.00)	100.00(90.00)	11.00	8.43
T5	*M.a.*+GNO + HO	0.5 + 1.0	85.00(67.21)	100.0(90.00)	100.00(90.0)	8.63	8.94
T6	*M.a.*+GH + HO	0.5 + 1.0	51.67(45.97)	100.00(90.00)	100.00(90.00)	10.60	8.55
T7	*M.a.*+BA + HO	2.0 + 1.0	56.67 (48.85)	100.00(90.00)	100.00(90.00)	9.53	8.78
T8	*M.a.*+CMC + HO	0.5 + 1.0	35.00(36.27)	100.00(90.00)	100.00(90.00)	8.97	8.89
T9	*M.a.*+TW+TX+ GLY +SFO+CNO+MUO +GNO+SBO+GH +BA+CMC+HO	0.5+0.03+2 +1+1+0.5+0.5 +o.5+o.5+2 +0.5+1	0.00 (0.00)	0.00 (0.00)	43.33 (41.15)	4.53	4.35
T10	*M.a.*+TW+GLY+BA +CMC+HO	0.5+2+2 +0.5+1	5.00 (12.92)	43.33 (41.15)	73.33 (58.89)	8.03	4.68
T11	*M.a.*+GLY+SFO +CMC+HO	2+1+0.5+1	11.67 (20.00)	63.33 (52.71)	100.00 (90.00)	8.03	4.10
T12	*M.a.*+TW+GLY+HO	0.5+2+1	10.00 (18.44)	40.00 (39.23)	100.00 (90.00)	7.77	7.17
T13	*M.a.*+TX+GLY +GH+HO	0.03+2+0.5+1	0.00 (0.00)	0.00 (0.00)	83.33 (65.88)	5.67	4.20
T14	*M.a.*+TX+CMC+HO	0.03+0.5+1	0.00 (0.00)	0.00 (0.00)	10.00 (18.44)	1.60	4.15
T15	*M.a.*+BA+CMC+HO	2+0.5+1	6.67 (15.00)	76.67 (61.14)	100.00 (90.00)	8.50	8.76
T16	*M.a.*+GH+HO+GLY	0.5+1+2	18.33 (25.33)	100.00 (90.00)	100.00 (90.00)	8.23	8.84
T17	*M.a.*+GH+HO +GLY+CMC	0.5+1 +2+0.5	5.00 (12.92)	50.00 (45.00)	100.00 (90.00)	9.60	8.12
T18	*M.a.*+TW	0.50	13.33 (21.39)	100.00 (90.00)	100.00 (90.00)	7.50	8.89
T19	*M.a.*+TX	0.03	0.00 (0.00)	21.67 (27.76)	100.00 (90.00)	6.80	4.42
T20	*M.a.*+GLY	2.00	65.00 (53.73)	100.00 (90.00)	100.00 (90.00)	8.40	7.40
T21	*M.a.*+BA	2.00	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	8.30	8.59
T22	*M.a.*+CMC	0.50	10.00 (18.44)	100.00 (90.00)	100.00 (90.00)	9.00	8.30
T23	*M.a.*+HO	1.00	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	9.20	8.43
T24	*M.a.*+SFO	1.00	81.67 (64.67)	100.00 (90.00)	100.00 (90.00)	9.20	8.44
T25	*M.a.*+CNO	1.00	45.00 (42.13)	100.00 (90.00)	100.00 (90.00)	7.60	8.15
T26	*M.a.*+MUO	0.50	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	7.60	7.98
T27	*M.a.*+GNO	0.50	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	8.30	8.64
T28	*M.a.*+SBO	0.50	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	6.80	8.20
T29	*M.a.*+GH	0.50	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	8.10	8.73
T30	Control (*M.a.*alone)	-	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.50	8.10
	S.E ±		**1.67**	**2.44**	**1.05**	**0.15**	-
	C.D(P=0.05)		**4.74**	**6.91**	**2.97**	**0.42**	-

*Figures in parentheses are arcsin values DAI = Days after inoculation *M.a.* = *Metarhizium anisopliae* HO = Honey TX= Triton-X-100 BA = Boric acid TW = Tween-80 GLY = Glycerol SBO = Soybean oil GH = GheeCMC = Carboxymethyl Cellulose SFO = Sunflower oil GNO = Groundnut oil CNO = Coconut oil MUO = Mustard oil

Similarly, out of another set of 96 test formulations of *M. anisopliae* 7 formulation including *M.a.*+TW(0.5%)+CMC(0.5%), *M.a.*+SFO(1.0%)+CMC(0.5%), *M.a.*+SFO(1.0%)+HO(1.0%), *M.a.*+GNO(0.5%)+BA(2.0%), *M.a.*+GNO(0.5%)+CMC(0.5%), *M.a.*+GNO(0.5%)+GH(0.5%) and *M.a.*+GH(0.5%)+HO(1.0%) were observed to be most promising advanced stage formulations of *M. anisopliae.*

4.1.6 Potential of advanced test formulations for growth, development, viability and bioefficacy of *N.rileyi and M.anisopliae*

(Period: Jan. to Apr.2011 Av.Temp.(^{0}C) :Max.-33.50 ±1, Min.-14 ±1 Av.Humidity (%) :Morn.-91, Even.-29)

The promising formulation bases, formulations comprising adjuvants honey, sunflower oil and ghee alone and formulations with combination with tweeen-80+ghee, glycerol+sunflower oil, glycerol+ghee, sunflower oil+ghee, tween-80+glycerol+sunflower oil+carboxymethyl cellulose, tween-80+glycerol+honey and tween-80+glycerol+carboxymethyl cellulose and control (*N.rileyi* alone) of *N.rileyi* and tweeen-80+ carboxymethyl cellulose, sunflower oil+ carboxymethyl cellulose, sunflower oil+honey,groundnut + boric acid, groundnut + carboxymethyl cellulose, groundnut + ghee, ghee + honey and control (*M.anisopliae* alone) of *M.anisopliae* were evaluated for their growth, development, viability and bioeffficacy against II and III instar larvae of *S. litura.*

4.1.6.1 Potential for growth, development and viability

***N.rileyi* :**

Effect on growth : The data on the growth, development and viability of advanced test formulations of *N.rileyi* are presented in Table 27 and depicted in Fig.13.

At 3 DAI, T2 *N.r.*+SFO registered significantly highest (88.33%) surface coverage. However, it was at par with T1- *N.r.*+HO(86.67%). Least (13.33%) growth of the fungus was recorded in T10- *N.r.*+ TW+GLY+CMC; when control recorded 23.33 per cent surface coverage. At 7 DAI, all the treatments recorded cent per cent surface coverage by the fungus except T8-

N.r.+TW+GLY+SFO+CMC, T9- *N.r.*+ TW+GLY+HO, T10- *N.r.*+ TW+GLY+CMC and T11- Control (Plate-IV-I).

Effect on biomass and pH : On 10 DAI, the differences for biomass production in various treatments were significant. T9- *N.r.*+ TW+GLY+HO maintained its superiority for biomass production(12.57g) over rest of the treatments (11.57 to 12.57g). However, it was at par with T2- *N.r.*+SFO (12.53g). The next promising and at par treatments were T10-*N.r.*+ TW+GLY+CMC (12.13g), T7-*N.r.*+ Sf+Gh (11.93g), T6-*N.r.*+GLY+GH (11.83g) and T4-*N.r.*+TW+GH (11.30g). Least biomass (7.67g) was produced in control.

The most of *N.rileyi* cultures developed from the inoculums in highly promising formulations was 7.16 to 7.67 except pH of 8.10 in T10-*N.r.*+TW+GLY+CMC and 8.40 in T11-Control. Although the adjuvants in the formulations are variable but ultimately at 10 DAI the fungus maintained the pH between 7.0 to 8.10 and produced the higher biomass of 11.57 to 12.53g than the 7.67g/40ml medium in control.

On the basis of results of pH, it has been established that requirement of neutral pH for all the entomopathogenic fungai is not necessary parameter as part of quality control or insisted in Insecticide Act.

There is no published information in relation of pH, growth and development of *N.rileyi* or any other mycoagent.

Effect on viability : Maximum ($2.57x10^9$) cfu count/ml was observed in T2- *N.r.*+SFO. However, it was at par with T1- *N.r.*+ HO ($2.43x10^9$cfu/ml), T7-*N.r.*+SFO+GH ($2.43x10^9$cfu/ml) and T9-*N.r.*+TW+GLY+HO ($2.50x10^9$ cfu/ml). Least ($2.0x10^9$cfu/ml) count was recorded in T11- Control. Maximum (109.68) viability index was registered in T2-*N.r.*+SFO. It was followed by T9-*N.r.*+TW+GLY+HO. The index in rest of the treatment was 60.88 to 89.22 Fig.15.

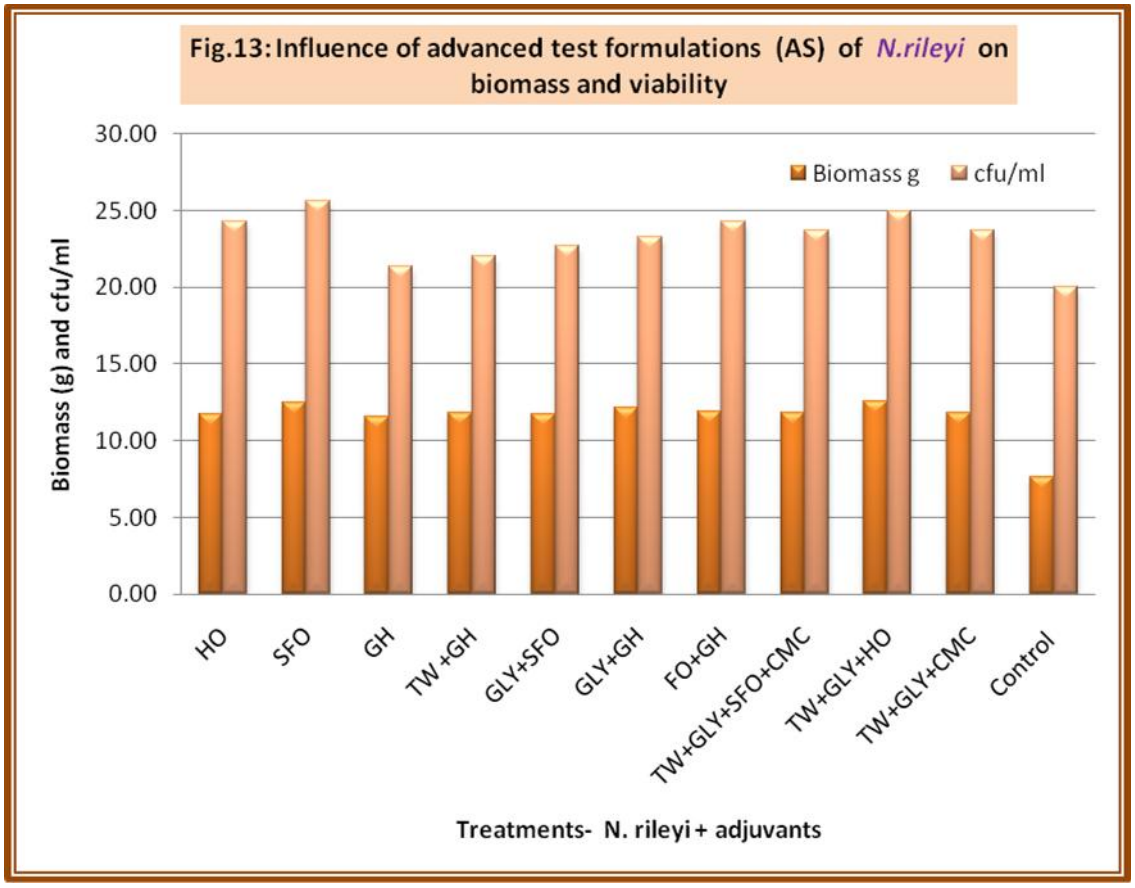


Fig.13: Influence of advanced test formulations (AS) of N.rileyi on biomass and viability
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Biomass (g) and cfu/ml
Biomass g
cfu/ml
HO
SFO
GH
TW +GH
GLY+SFO
GLY+GH
FO+GH
TW+GLY+SFO+CMC
TW+GLY+HO
TW+GLY+CMC
Control
Treatments- N. rileyi + adjuvants

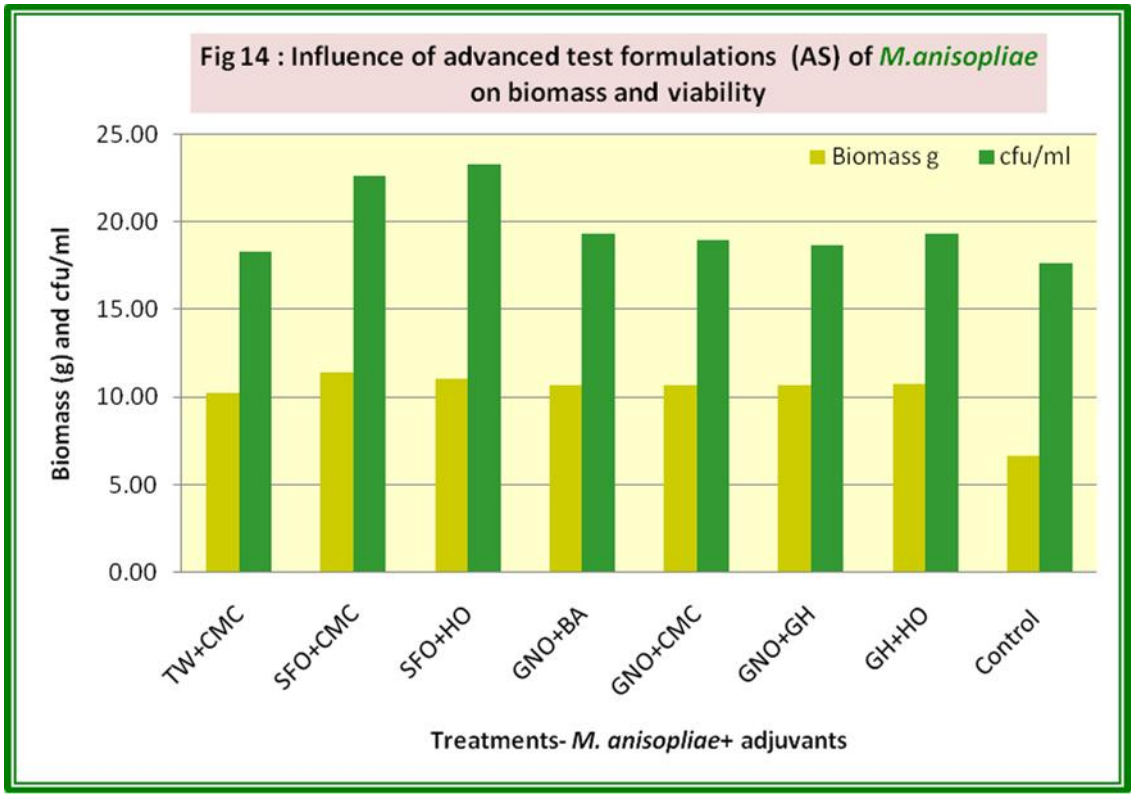


Fig 14 : Influence of advanced test formulations (AS) of M.anisopliae on biomass and viability
25.00
20.00
15.00
10.00
5.00
0.00
Biomass (g) and cfu/ml
Biomass g
cfu/ml
TW+CMC
SFO+CMC
SFO+HO
GNO+BA
GNO+CMC
GNO+GH
GH+HO
Control
Treatments- M. anisopliae+ adjuvants

Table 27. Influence of advanced test formulations of *N.rileyi* (AS) on growth, development and viability

Tr. No	Treatment	Conc. (%)of adj.	Surface area covered (%)			Biomass g/40ml medium	cfu count (x10^8 /ml)	Viability Index	pH at 10 DAI
			3 DAI	7 DAI	10 DAI				
1	*N.r.*+ HO	1.0	86.67 (68.61)*	100.00 (90.00)	100.00 (90.00)	11.70	24.33 (4.98)**	85.57	7.70
2	*N.r.*+ SFO	1.0	88.33 (70.00)	100.00 (90.00)	100.00 (90.00)	12.53	25.67 (5.12)	109.68	7.16
3	*N.r.*+ GH	0.5	83.33 (65.88)	100.00 (90.00)	100.00 (90.00)	11.57	21.33 (4.67)	60.88	7.20
4	*N.r.*+ TW +GH	0.5+ 0.5	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)	11.83	22.00 (4.74)	69.66	7.67
5	*N.r.*+ GLY+SFO	2.0 +1.0	23.33 (28.86)	100.00 (90.00)	100.00 (90.00)	11.77	22.67 (4.81)	73.94	7.44
6	*N.r.*+ GLY+GH	2.0 +0.5	75.00 (60.00)	100.00 (90.00)	100.00 (90.00)	11.83	23.33 (4.88)	84.48	7.58
7	*N.r.*+ SFO+GH	1.0 +0.5	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	11.93	24.33 (4.98)	89.22	7.67
8	*N.r.*+ TW+GLY+ SFO+CMC	0.5+2+ 1+0.5	20.00 (26.56)	88.33 (70.00)	100.00 (90.00)	11.83	23.67 (4.92)	82.54	7.21
9	*N.r.*+ TW+ GLY+HO	0.5+2.0 +1.0	23.33 (28.86)	86.67 (68.61)	100.00 (90.00)	12.57	25.00 (5.05)	104.85	7.37
10	*N.r.*+ TW+ GLY+CMC	0.5+2.0 +0.5	13.33 (21.39)	66.67 (54.76)	100.00 (90.00)	12.13	23.67 (4.92)	82.54	8.10
11	Control (*N.r.*alone)	-	23.33 (28.86)	56.67 (48.85)	100.00 (90.00)	7.67	20.00 (4.58)	-	8.40
	S.E+	-	**1.15**	**1.67**	**-**	**0.12**	**0.06**	**-**	**-**
	C.D.(P=0.05)	-	**3.39**	**4.92**	**NS**	**0.34**	**0.19**	**-**	**-**

*Figures in parentheses are arcsin values
**Figures in parentheses are √ +0.5 value
DAI = Days after inoculation
N.r. = Nomuraea rileyi
SFO = Sunflower oil
GH = Ghee
HO = Honey
GLY = Glycerol
TW = Tween-80
CMC = Carboxymethyl Cellulose

***M.anisopliae* :** The data on the growth, development and viability of *M.anisopliae* (AS) and the results are presented in Table 28 and depicted in Fig.14.

Effect on growth : At 3 DAI, T5-*M.a.*+GNO+CMC registered significantly highest (88.33%) surface coverage. The next promising formulations were T2-*M.a.*+ SFO+CMC (78.33%), T6- *M.a.*+ GNO+GH (56.67%) and T7-*M.a.*+GH+HO (51.67%). Least (8.33%) growth of the fungus was recorded in T1- TW+CMC; when, control recorded 30.0 per cent surface coverage. At 7 DAI, all the formulations, except T1- *M.a.*+TW+CMC (91.67%) recorded significantly cent per cent surface area coverage by fungus. At 10 DAI, all treatments including control covered cent per cent surface of the medium.

Fig. 15 : Influence of advanced test formulations (AS) of *N.rileyi* on viability index of the inoculum

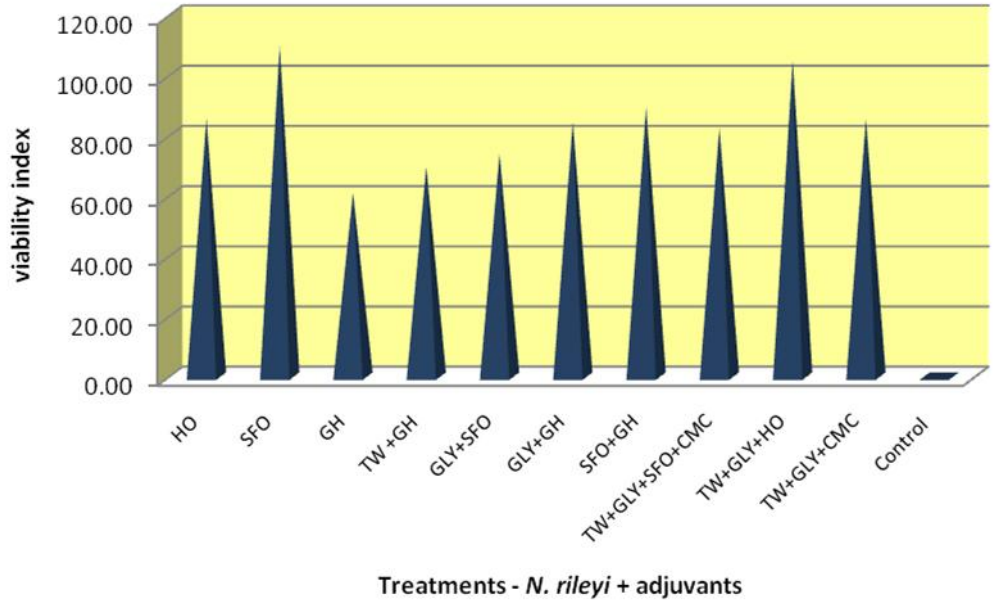
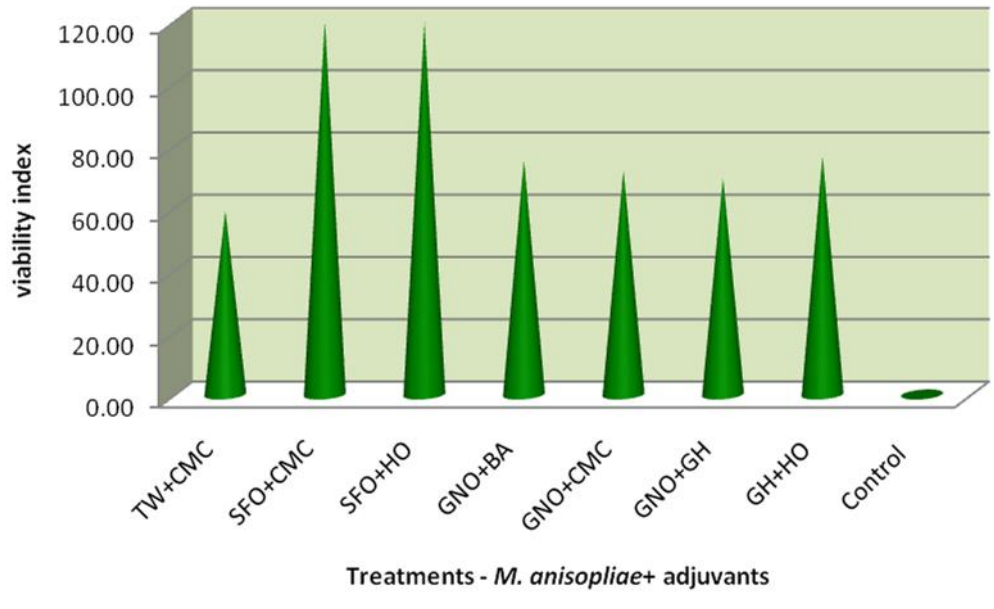


Fig. 16: Influence of advanced test formulations (AS) of *M.anisopliae* on viability index of the inoculum



Effect on biomass and pH : On 10 DAI, the differences (6.67 to 11.43g) for biomass production were significant. T2- *M.a.*+SFO+CMC maintained its superiority over rest of the formulations by producing 11.43g biomass. However, it was at par with T3- *M.a.*+SFO+HO (11.10g).

The most pH of *M.anisopliae* cultures developed from the inoculums in highly promising formulations was 7.10 to 8.17 except pH of 8.50 in T7-*M.a.*+GH+HO and 8.10 in T8-Control. Although the adjuvants in the formulations are variable but ultimately at 10 DAI the fungus maintained the pH between 7.10 to 8.50 and produced the higher biomass of 10.23 to 11.43g than the 6.67g/40 ml medium in control.

On the basis of results of pH, it has been established that requirement of neutral pH for all the entomopathogenic fungi is not necessary parameter as part of quality control or insisted in Insecticide Act. There is no published information in relation of pH, growth and development of *M.anisopliae* or any other mycoagent.

Effect on viability (cfu/ml) : The viability in terms of cfu/ml was significantly different in test formulations. Maximum cfu count was observed in T3-*M.a.*+SFO+HO ($2.33x10^9$). However, it was at par with T2-*M.a.*+SFO+CMC ($2.27x10^9$). Maximum (119.85) viability index was registered in T2-SFO+CMC. On the basis of the index next best formulation was followed by T3- *M.a.*+ SFO+HO (119.72) and T7-*M.a.*+GH+HO (76.63).

It was evident from the study that the adjuvants substantially contributed to cfu. T2-*N.r.*+SFO and T9-*N.r.*+TW+GLY+HO of *N.rileyi* and T2-*M.a.*+SFO+CMC and T3-*M.a.*+SFO+HO of *M.anisopliae* were the best formulations resulted in higher cfu than control with higher viability index than rest ones.These were considered as highly potent formulations for highest growth and biomass production of respective entomopathogenic fungi.

Table 28. Influence of advanced test formulations of *M.anisopliae* (AS) on growth, development and viability

Tr. No	Treatments	Conc. adj. (%)	Surface coverage (%)			Biomass g/40ml medium	cfu (x10^8 /ml	Viability Index	P^H at 10 DAI
			3 DAI	7 DAI	10 DAI				
1	*M.a.*+ TW+CMC	0.5+0.5	8.33 (16.78)*	91.67 (73.26)	100.00 (90.00)	10.23	18.33 (4.33)**	59.10	7.10
2	*M.a.*+ SFO+CMC	1.0+0.5	78.33 (62.24)	100.00 (90.00)	100.00 (90.00)	11.43	22.67 (4.81)	119.85	8.01
3	*M.a.*+ SFO+HO	1.0+1.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	11.10	23.33 (4.88)	119.72	8.03
4	*M.a.*+ GNO+BA	0.5+2.0	31.36 (34.27)	100.00 (90.00)	100.00 (90.00)	10.70	19.33 (4.45)	75.49	8.04
5	*M.a.*+ GNO+CMC	0.5+0.5	88.33 (70.00)	100.00 (90.00)	100.00 (90.00)	10.67	19.00 (4.42)	72.01	8.08
6	*M.a.*+ GNO+GH	0.5+0.5	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	10.70	18.67 (4.38)	69.50	8.17
7	*M.a.*+ GH+HO	0.5+1.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	10.77	19.33 (4.45)	76.63	8.50
8	Control (*M.a.*alone)	-	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.67	17.67 (4.26)	-	8.10
	S.E+		**1.42**	**0.66**	**-**	**0.04**	**0.05**	**-**	**-**
	C.D. (P=0.05)		**4.29**	**1.99**	**-**	**0.11**	**0.14**	**-**	**-**

Figures in parentheses are arc sin values.Figures in parentheses for cfu are values √n+0.5
DAI = Days after inoculation *M.a. = Metarhizium anisopliae*
TW = Tween-80 CMC = Carboxymethyl Cellulose
SFO = Sunflower oil HO = Honey
GNO = Groundnut oil BA = Boric acid GH = Ghee

Prior *et al.* (1988) reported that mixtures of oil and powder formulations stimulated the mycelial growth of *M.anisopliae.* Alves *et al.* (2001) observed that oil formulation did not cause any negative effect on conidial germination of *M.anisopliae*. Rodriguez Colorado *et al.* (2002) reported that viability was greater than 90% where maize oil adjuvant; while it was less than 85% in glycerine and neem oil. Wiwat (2004) found that carbohydrates (glucose, lactose and sucrose) were essential source for germination and conidial production of *N.rileyi.* The highest biomass production (21.88 mg/ml) of *M.anisopliae* for sunflower oil was reported by Silva *et al.* (2005). The utility of oils and carbohydrates in present findings are in corroboration with the reports as above investigators.

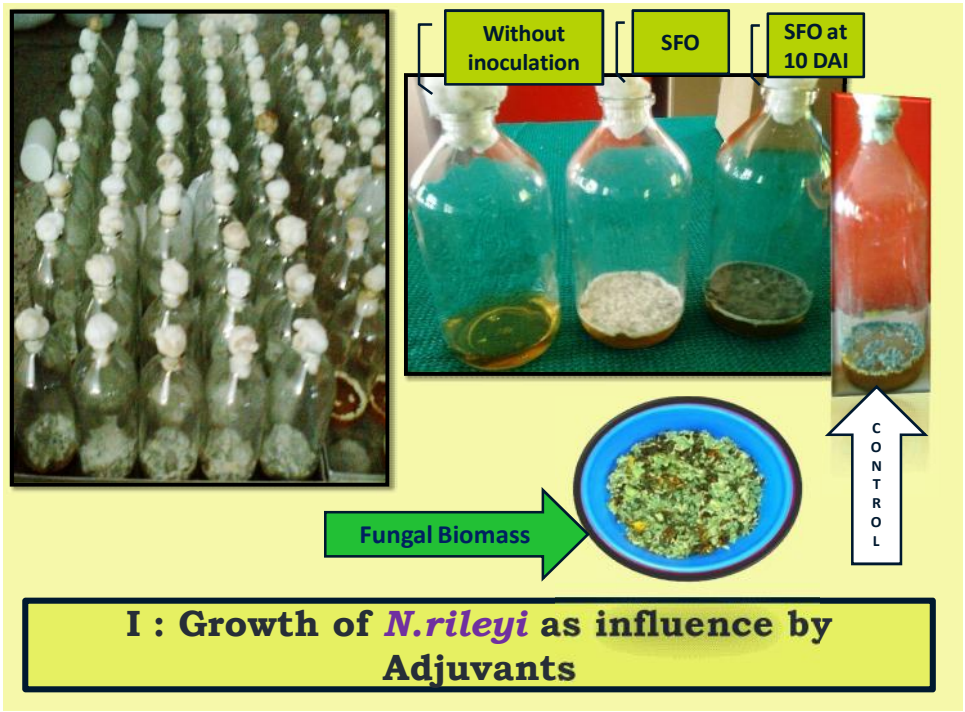


PLATE – IV

Growth of *N.rileyi* and *M.anisopliae* as influence by Adjuvants

4.1.6.2 Bioefficacy of *N.rileyi* and *M.anisopliae* against larvae of *S.litura*

The II instar larvae

N.rileyi :

Data presented in Table 29 and depicted in Fig.17 revealed that the larval mortality among the treatments ranged from 26.67 to 53.33, 46.67 to 73.33 and 66.67 to 93.33 per cent at 5,7 and 10 DAT, respectively. The mortality in control was zero.

There was significant variation in the mortality among the treatments. All the treatments were significantly superior to water spray.

The formulation with T2-*N.r.*+SFO registered significantly highest mortality of 53.33 per cent at 5 DAT. However, it was at par with T9-*N.r.*+TW+GLY+HO (50.0%) and T10-*N.r.*+TW+GLY+CMC (46.67%). The pattern of the results of the mortality at 7 DAT was more or less same.

The mortality at 10 DAT was significantly highest (93.33%) in T2-*N.r.*+SFO containing sunflower oil as adjuvant. However, it was at par with T9-*N.r.*+TW+GLY+HO (90.0%) and T10-*N.r.*+TW+GLY+CMC (86.67%). The next promising formulations for better lethal effect were T1- *N.r.*+HO (76.67%), T7- *N.r.*+SFO+GH (73.33), T3- *N.r.*+GH (73.33%), T5- *N.r.*+GLY+SFO (70.0%), T4- *N.r.*+TW+GH (70.0%), T6- *N.r.*+GLY+GH (70.0%) and T8- *N.r.*+TW+GLY+SFO+CMC (70.0%). The lowest larval kill (66.67%) was recorded in T11-control (*N.r.*alone) against zero kill in T12-water spray (Plate-VI-I).

Table 29. Effect of advanced test formulations of *N.rileyi* (AS) on mortality of II instar larvae of *S. litura*

Tr. No	Treatment	Conc. of adjuvant (%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
1	*N.r.*+ HO	1.0	40.00 (39.23)*	60.00 (50.77)	76.67 (61.14)
2	*N.r.*+ SFO	1.0	53.33 (46.89)	73.33 (58.89)	93.33 (75.00)
3	*N.r.*+ GH	0.5	33.33 (35.24)	53.33 (46.89)	73.33 (58.89)
4	*N.r.*+ TW +GH	0.5+0.5	33.33 (35.24)	53.33 (46.89)	70.00 (56.79)
5	*N.r.*+ GLY+SFO	2.0+1.0	36.67 (37.29)	53.33 (46.89)	70.00 (56.79)
6	*N.r.*+ GLY+GH	2.0+0.5	33.33 (35.24)	53.33 (46.89)	70.00 (56.79)
7	*N.r.*+ SFO+GH	1.0+0.5	36.67 (37.29)	56.67 (48.85)	73.33 (58.89)
8	*N.r.*+ TW+GLY+SFO+CMC	0.5+2+1+0.5	36.67 (37.29)	56.67 (48.85)	70.00 (56.79)
9	*N.r.*+ TW+GLY+HO	0.5+2.0+1.0	50.00 (45.00)	70.00 (56.79)	90.00 (71.56)
10	*N.r.*+ TW+GLY+CMC	0.5+2.0+0.5	46.67 (43.11)	70.00 (56.79)	86.67 (68.61)
11	Control(*N.r.*alone)	-	26.67 (31.11)	46.67 (43.11)	66.67 (54.76)
12	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±		**2.26**	**2.15**	**2.74**
	C.D.(P=0.05)		**6.60**	**6.26**	**8.02**

*Figures in parentheses indicate arcsin values DAT = Days after treatment *N.r.* = *Nomuraea rileyi*
SFO = Sunflower oil GH = Ghee HO = Honey
GLY = Glycerol TW = Tween-80 CMC= Carboxymethyl Cellulose

***M.anisopliae* :**

Data presented in Table 30 and depicted in Fig.18 revealed that the larval mortality among the treatments ranged from 16.67 to 46.67, 37.50 to 66.67 and 50.00 to 80.00 per cent at 5,7 and 10 DAT, respectively.

Table 30. Effect of advanced test formulations of *M.anisopliae* (AS) on mortality of II instar larvae of *S.liltura*

Tr. No	Treatments	Conc. adj. (%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
T1	*M.a.*+ TW+CMC	0.5+0.5	33.33 (35.24)*	53.33 (46.89)	63.33 (52.71)
T2	*M.a.*+ SFO+CMC	1.0+0.5	46.67 (43.11)	63.33 (52.71)	76.67 (61.14)
T3	*M.a.*+ SFO+HO	1.0+1.0	50.00 (45.00)	66.67 (54.76)	80.00 (63.44)
T4	*M.a.*+ GNO+BA	0.5+2.0	26.67 (31.11)	46.67 (43.11)	60.00 (50.77)
T5	*M.a.*+ GNO+CMC	0.5+0.5	36.67 (37.29)	50.00 (45.00)	60.00 (50.77)
T6	*M.a.*+ GNO+GH	0.5+0.5	43.33 (41.15)	56.67 (48.85)	66.67 (54.76)
T7	*M.a.*+ GH+HO	0.5+1.0	36.67 (37.29)	53.33 (46.89)	63.33 (52.71)
T8	Control (*M.a.* alone)	-	16.67 (24.12)	37.50 (37.76)	50.00 (45.00)
T9	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±		**2.18**	**2.40**	**2.64**
	C.D.(P=0.05)		**6.48**	**7.13**	**7.84**

***Figures in parentheses indicate arcsin values. DAI = Days after inoculation
M.a. = *Metarhizium anisopliae* TW = Tween-80 CMC = Carboxymethyl Cellulose SFO =Sunflower oil
HO = Honey GNO = Groundnut oil BA = Boric acid GH = Ghee

Fig. 17: Influence of advanced test formulations (AS) of *N.rileyi* on mortality of II and III instar larvae of *S.litura*

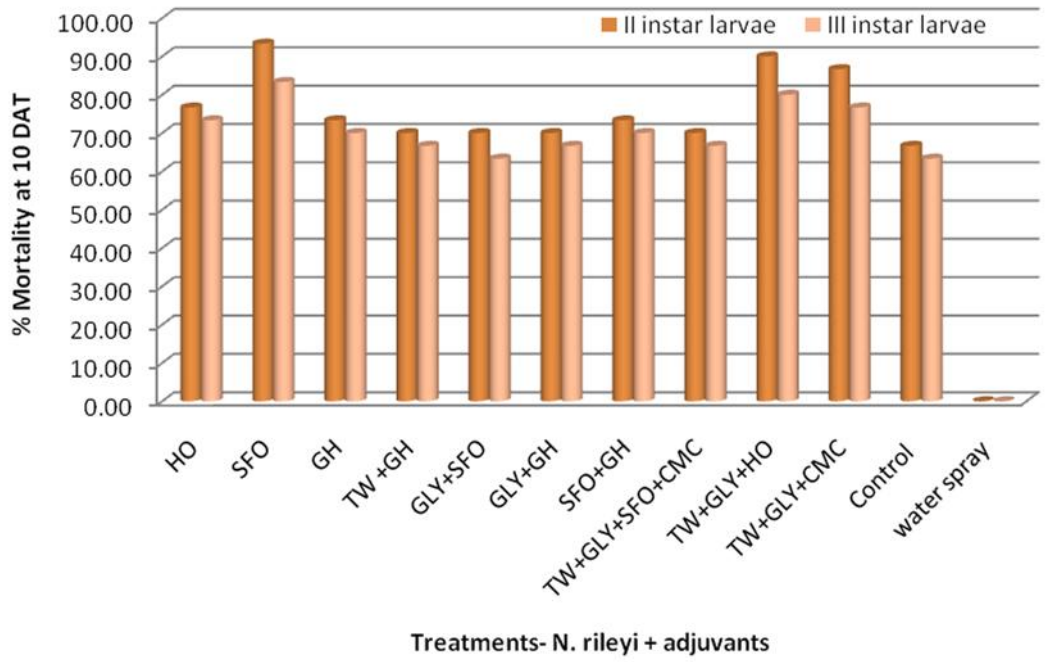
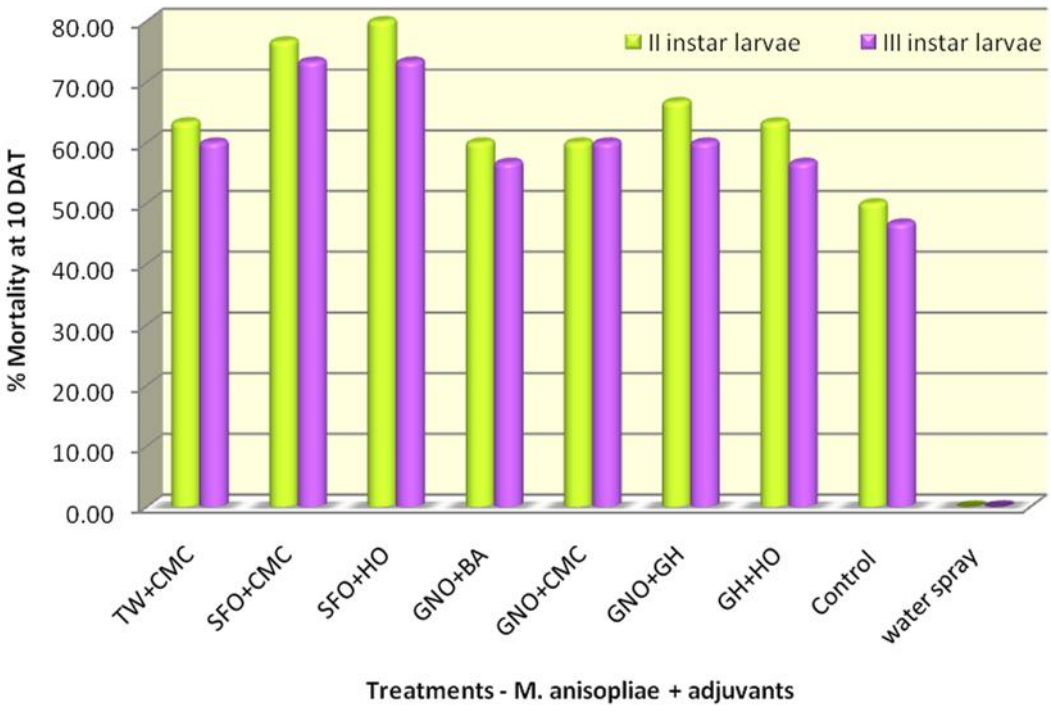


Fig. 18 :Influence of advanced test formulations (AS) of *M.anisopliae* on mortality of II and III instar larvae of *S.litura*



The trend of results was more or less similar to that was observed for *N.rileyi*. There was significant variation in mortality among the treatments; all the treatments were superior over untreated control which reported zero per cent mortality upto last 10 DAT.

T3- *M.a.*+SFO+HO registered significantly highest mortality of 50.0 per cent at 5 DAT. However, it was at par with T2- *M.a.*+SFO +CMC and T6- *M.a.*+GNO+GH which recorded 43.33 to 46.67 per cent mortality. The pattern of mortality at 7 DAT was more or less same.

At 10 DAT, the kill was again significantly highest (80.0%) in formulation with adjuvants *M.a.*+SFO+HO (T3). However, it was at par with T2- *M.a.*+SFO+CMC (76.67%). The next promising formulations for larval morality were T6- *M.a.*+GNO+GH (66.67%), T1- *M.a.*+TW+CMC (63.33), T7- *M.a.*+GH+HO (63.33%), T4- *M.a.*+GNO+BA (60.0) and T5- *M.a.*+GNO+CMC (60.0%). The lowest mortality (50.0%) was recorded in T11-control (*M.a.*alone).

The III instar larvae

***N.rileyi* :** Data presented in Table 31 and depicted in Fig.17 revealed that all the treatments showed significant variation in the larval mortality.

The per cent larval mortality was 16.67 to 43.33, 36.67 to 63.33 and 63.33 to 83.33 at 5, 7 and 10 DAT, respectively. At 5 DAT, the formulation with T2- *N.r.*+SFO recorded significantly highest larval mortality (43.33%) and control (*N.r.*alone) (16.67%). However, it was at par with T9- *N.r.*+TW+GLY+HO (43.33%) and T10- *N.r.*+TW+GLY+CMC (36.67%). The trend of larval mortality was more or less same at 7 DAT.

At 10 DAT, all the treatments with adjuvants were significantly superior (66.67 to 83.33%) than control (63.33%). T2- *N.r.*+SFO proved significantly superior in causing the highest mortality of 83.33 %. However, it was at par with T9- *N.r.*+TW+GLY+HO (80.00%), T10- *N.r.*+TW+GLY+CMC (76.67%) and T1- *N.r.*+HO (73.33%).

The treatments, T2 (*N.r.*+SFO), T9 (*N.r.*+TW+GLY+HO) and T10 (*N.r.*+TW+GLY+CMC) were found highly promising among 11 formulations.

These included combination of Tween-80, Glycerol, Honey and Carboxymethyl Cellulose and alone sunflower oil with mycoagent, *N.rileyi.* The effectiveness of all adjuvants was well documented by several research workers.

Table 31. Effect of advanced test formulations of *N.rileyi* (AS) on mortality of III instar larvae of *S.litura*

Tr. No	Treatment	Conc. of adjuvant(%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
1	*N.r.*+ HO	1.0	30.00 (33.21)*	53.33 (46.89)	73.33 (58.89)
2	*N.r.*+ SFO	1.0	43.33 (41.15)	63.33 (52.71)	83.33 (65.88)
3	*N.r.*+ GH	0.5	26.67 (31.11)	43.33 (41.15)	70.00 (56.79)
4	*N.r.*+ TW +GH	0.5+0.5	26.67 (31.11)	43.33 (41.15)	66.67 (54.76)
5	*N.r.*+ GLY+SFO	2.0+1.0	30.00 (33.21)	43.33 (41.15)	63.33 (52.71)
6	*N.r.*+ GLY+GH	2.0+0.5	30.00 (33.21)	43.33 (41.15)	66.67 (54.76)
7	*N.r.*+ SFO+GH	1.0+0.5	26.67 (31.11)	46.67 (43.11)	70.00 (56.79)
8	*N.r.*+ TW+GLY+SFO+CMC	0.5+2+1+0.5	30.00 (33.21)	46.67 (43.11)	66.67 (54.76)
9	*N.r.*+ TW+GLY+HO	0.5+2.0+1.0	43.33 (41.15)	60.00 (50.77)	80.00 (63.44)
10	*N.r.*+ TW+GLY+CMC	0.5+2.0+0.5	36.67 (37.29)	56.67 (48.85)	76.67 (61.14)
11	Control (*N.r.*alone)	-	16.67 (24.12)	36.67 (37.29)	63.33 (52.71)
12	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±		**1.67**	**2.25**	**1.99**
	C.D.(P=0.05)		**4.88**	**6.58**	**5.81**

*Figures in parentheses indicate arcsin values. DAT = Days after treatment
N.r. = *Nomuraea rileyi* SFO=Sunfloweroil GH=Ghee
HO = Honey GLY = Glycerol TW = Tween-80 CMC= Carboxymethyl Cellulose

The present investigation is in accordance with the findings of Nagraja (2005) who reported higher (95%) mortality of *S.litura* with sunflower oil 2%+tween-80 0.02% formulations of *N.rileyi* than unformulated crude formulation (77.0%). The formulations with sunflower oil registered highest mortality in present investigation as it was also reported by Burges (1998) who opinionated that higher efficacy of oil based formulation might be due preventing the desiccation of the conidia, which helps in last survival period and better penetration of peg into the integument. The mortality of larvae increased with increase in days after treatment in present study. It is in conformity with that reported by Nagraja *et al.* (2006), Patil (2000) and Vimladevi (1994). Vimladevi *et al.* (2002) reported that *N.rileyi* with sunflower oil + triton-x-100 showed 83.90% mortality of *S.litura*. This supported the

findings of present investigation where formulations with *N.r.*+SFO and *N.r.*TW+GLY+HO recorded 83.33 and 80.0% mortality of III instar larvae of *S.litura*. These results are also in conformity with those reported by Ramegowda (2005) who opinionated where in groundnut oil registered 96.0% mortality of *S.litura* was used as adjuvants followed by sunflower oil and safflower oil. There is want of literature to confirm highly promising formulation containing *N.r.*+TW+GLY+HO. Ambethgar and Loganathan (1988) observed that the mortality of *S.litura* was initiated at 3 days after treatment.

Manjula and Krishnamurthy (2005) reported that the I instar larvae of *S.litura* were found most susceptible to *N.rileyi* compared to IV instar larvae. They recorded 91.2% mortality of I instar larvae with $1x10^9$spore/ml. The considerable mortality of *S.litura* by *N.rileyi* was reported by Rao and Phadke (1977), Vimladevi (1994), Dayakar and Kanujia (2001), Kulkarni and Lingappa (2002b), Ingale *et al*. (2004) and Sonai Rajan and Muthukrishnan (2009).

M.anisopliae :

Data presented in Table 32 and depicted in Fig.18 revealed that the formulations showed significant variation in larval mortality among the treatments.

The per cent larval mortality was in the range of 13.33 to 40.0, 26.67 to 56.67 and 46.67 to 73.33 at 5, 7 and 10 DAT, respectively. At 5 DAT, T3- *M.a.*+SFO+HO recorded significantly highest larval mortality of 40.0 per cent. The rest of the formulations were significantly superior to control (13.33%). However, T3- *M.a.*+SFO+HO was at par with T2-*M.a.*+SFO+CMC (36.67%), T6- *M.a.*+GNO+GH (33.33%), T5- *M.a.*+GNO+CMC(30.00%) and T7- *M.a.*+GH+HO (30.0%). The pattern of larval mortality was more or less same at 7 DAT.

At 10 DAT, all the formulations were significantly superior (56.67 to 73.33%) than control (*M.a.*alone)(46.67%) for the larval mortality. T2-

M.a.+SFO+CMC and T3- *M.a.*+SFO+HO (73.33% each) were proved to be significantly superior to the rest of the treatments. The next promising formulations were T1- *M.a.*+TW+CMC, T5- *M.a.*+GNO+CMC and T6- *M.a.*+GNO+GH, (60.0% each). Lowest larval mortality of 46.67 per cent was recorded in control (*M.a.*alone).

The formulations, T2- *M.a.*+SFO+CMC and T3- *M.a.*+SFO+HO were most promising for *M.anisopliae* growth and development among 8 formulations. These included combination of sunflower oil, honey and carboxymethyl cellulose with mycoagent, *M.anisopliae.* Many scientist experienced effectiveness of *M.anisopliae.*

There is no literature on synergistic effect of *M.anisopliae* formulation containing sunflower oil, CMC and honey as best adjuvants in most promising formulations against *S.litura* in present study. So few relevant references in support of the results on bioefficacy have been quoted below.

Ramoska (1984) noted that *M.anisopliae* conidia in oil caused much infection at low humidity and penetration at intersegmental membrane of chinch bug. Prior *et al.* (1988) pointed out that the oil formulation of aerial conidia of *M.anisopliae* are more efficacious. Kaaya and Hassan (2000) observed that *M.anisopliae* oil formulation (10^9conidia/ml) inducted 100% mortality in larvae of *Rhipicephalus appendiarlates.* Alves *et al.* (2001) reported that the fungal activity was enhanced by 2.25% adjuvant oil and effectivity against *T.molitor*. Malsam *et al.* (2002) reported that the increase in efficacy of *M.anisopliae* for the control of white fly by addition of sublethal concentrations of oil. The sunflower oil gave higher synergistic effect reaching nearly 100% control and increasing and speed of action. The fungus at concentration of $5x10^6$conidia/ml of *M.anisopliae* recorded 66.7 to 100% mortality of white fly and also killed the nymphs and adults of red spider mite within 3 to 4 days.

Liu Binglan *et al.* (2000) reported that maltose and peptone were the best carbon and nitrogen sources for the production of destruxins. Shashi Sharma *et al.* (1998) reported that the addition of carboxy methyl cellulose to

the *M.anisopliae* reduced time to 100% mortality by more than a week in first instar larvae of *Holotrichia consanguinea.* The present investigation is in conformity with the results reported by Dayakar and Kanujia (2001 and 2003), Pandey and Kanujia (2004), Purvar and Sachan (2005), Hu *et al.* (2007), Amer *et al.* (2008), Pandey Renu and Hasan Wajid (2009) and Dayakar and Subbarao (2011) who reported the considerable mortality of *S.litura*.

Table 32. Effect of advanced test formulations of *M.anisopliae* (AS) on mortality of III instar larvae of *S.litura*

Tr. No	Treatment	Conc. Adj.	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
T1	*M.a.*+ TW+CMC	0.5+0.5	23.33 (28.86)*	43.33 (41.15)	60.00 (50.77)
T2	*M.a.*+ SFO+CMC	1.0+0.5	36.67 (37.29)	53.33 (46.89)	73.33 (58.89)
T3	*M.a.*+ SFO+HO	1.0+1.0	40.00 (39.23)	56.67 (48.85)	73.33 (58.89)
T4	*M.a.*+ GNO+BA	0.5+2.0	16.67 (24.12)	36.67 (37.29)	56.67 (48.85)
T5	*M.a.*+ GNO+CMC	0.5+0.5	30.00 (33.21)	43.33 (41.15)	60.00 (50.77)
T6	*M.a.*+ GNO+GH	0.5+0.5	33.33 (35.24)	43.33 (37.29)	60.00 (50.77)
T7	*M.a.*+ GH+HO	0.5+1.0	30.00 (33.21)	43.33 (41.15)	56.67 (48.85)
T8	Control(*M.a.*alone)	-	13.33 (21.39)	26.67 (31.11)	46.67 (43.11)
T9	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±		**2.09**	**1.87**	**2.01**
	C.D.(P=0.05)		**6.20**	**5.55**	**5.98**

*Figures in parentheses indicate arcsin values.
DAI = Days after inoculation
M.a. = Metarhizium anisopliae
TW = Tween-80
CMC = Carboxymethyl Cellulose
SFO = Sunflower oil
HO = Honey
GNO = Groundnut oil
BA = Boric acid
GH = Ghee

4.1.7 Potential of advanced test WP formulations on growth, development, viability and bioefficacy of *N.rileyi* and *M.anisopliae*

(Period: Jan. to May 2011 Av.Temp.(^{0}C) :Max.-35 ±1, Min.-18 ±1 Av.Humidity (%) :Morn.-91, Even.-29)

The promising WP formulations comprising adjuvants honey, sunflower oil and ghee alone and formulations with combination with tweeen-80+ghee, glycerol+sunflower oil, glycerol+ghee, sunflower oil+ghee, tween-80+glycerol+sunflower oil+carboxymethyl cellulose, tween-80+glycerol+honey and tween-80+glycerol+carboxymethyl cellulose and

control (*N.rileyi* alone) and tweeen-80+ carboxymethyl cellulose, sunflower oil+ carboxymethyl cellulose, sunflower oil+honey,groundnut + boric acid, groundnut + carboxymethyl cellulose, groundnut + ghee and ghee + honey and and control (*M.anisopliae* alone) of *M.anisopliae* were tested for their growth, development, viability and bioeffficacy against II and III instar larvae of *S. litura.*

4.1.7.1 The potential for growth, development and viability

***N.rileyi* :**

Effect on growth : The data on the growth, development and viability of *N.rileyi* WP formulations and the results are presented in Table 33 and depicted in Fig.19.

At 3 DAI, T2-*N.r.*+SFO covered significantly highest (70.0%) surface of medium. However, it was at par with T9- *N.r.*+TW+GLY+HO (66.67%). Least (5.00%) growth of the fungus was recorded in T11- control. At 7 DAI T1-*N.r.*+TW+CMC, T2-*N.r.*+SFO, T3-*N.r.*+GH, T6- *N.r.*+GLY+GH and T9-*N.r.*+TW+GLY+HO recorded cent per cent surface coverage by the fungus (Plate-VIII-I).

Effect on biomass : At 10 DAI, the differences for biomass production in various formulations were significant. T2-*N.r.*+SFO maintained its superiority over rest of the treatments (11.17 to 12.57g) by producing 12.57g biomass. However, it was at par with T9-*N.r.*+ TW+GLY+HO (12.50g). The next promising and at par treatments were T1-*N.r.*+TW+CMC (11.53g), T3-*N.r.*+GH (11.50g), T4- *N.r.*+TW+GH (11.43g) and T5-*N.r.*+GLY+SFO (11.40g), T7-*N.r.*+SFO+GH (11.37g), T10-*N.r.*+TW+GLY+CMC (11.33g), T6-*N.r.*+GLY+GH (11.20g) and T8- *N.r.*+TW+GLY+SFO+CMC (11.17g). Least (6.10g) biomass was produced in control. The pH of the formulation was almost same (7.6 to 7.9).

Effect on viability (cfu/ml) : Maximum ($2.23x10^9$) cfu count was observed in T2-*N.r.*+SFO. However, it was at par with T9- *N.r.*+TW+GLY+HO (2.23

x10^9) and T1- *N.r.*+HO (2.20 x10^9). Least viability (1.47 x10^9) was recorded in T11- Control. Maximum (234.85) viability index was registered in T2- *N.r.*+SFO+CMC (Fig.21). It was followed by T9- *N.r.*+TW+GLY+HO (viability index of 218.71).

Table 33. Influence of advanced test WP formulations of *N.rileyi* on growth, development and viability

Tr. No	Treatment	Conc. of adj. (%)	Surface coverage (%)			Biomass g/40ml medium	cfu count (x10^8 cfu/ml)	Viability Index	pH at 10 DAI
			3 DAI	7 DAI	10 DAI				
1	*N.r.*+ HO	1.0	56.67 (48.85)*	100.00 (90.00)	100.00 (90.00)	11.53	22.00 (4.74)**	189.63	7.7
2	*N.r.*+ SFO	1.0	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	12.57	23.33 (4.88)	234.85	7.7
3	*N.r.*+ GH	0.5	53.33 (46.72)	100.00 (90.00)	100.00 (90.00)	11.50	18.67 (4.38)	145.16	7.7
4	*N.r.*+ TW +GH	0.5+0.5	26.67 (31.11)	83.33 (65.88)	100.00 (90.00)	11.43	18.00 (4.30)	134.92	7.8
5	*N.r.*+ GLY +SFO	2.0+1.0	18.33 (25.33)	56.67 (48.85)	96.67 (79.53)	11.40	18.67 (4.38)	143.02	7.8
6	*N.r.*+ GLY +GH	2.0+0.5	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	11.20	20.33 (4.56)	159.99	7.8
7	*N.r.*+ SFO +GH	1.0+0.5	18.33 (25.33)	43.33 (41.15)	81.67 (64.67)	11.37	19.67 (4.49)	155.37	7.8
8	*N.r.*+ TW+GLY +SFO+CMC	0.5+2+1 +0.5	18.33 (25.33)	38.33 (38.23)	78.33 (62.24)	11.17	18.67 (4.38)	138.11	7.9
9	*N.r.*+ TW +GLY+HO	0.5+2.0 +1.0	66.67 (54.76)	100.00 (90.00)	100.00 (90.00)	12.50	22.33 (4.78)	218.71	7.6
10	*N.r.*+ TW+GLY +CMC	0.5+2.0 +0.5	11.67 (20.00)	38.33 (38.23)	85.00 (67.21)	11.33	19.33 (4.45)	150.06	7.7
11	Control (*N.r.*alone)	-	5.00 (12.92)	33.33 (35.24)	55.00 (47.87)	6.10	14.67 (3.89)	-	7.9
	S.E+		**2.12**	**1.32**	**2.10**	**0.18**	**0.06**	**-**	**-**
	C.D. (P=0.05)		**6.23**	**3.89**	**6.18**	**0.52**	**0.19**	**-**	**-**

*Figures in parentheses are arcsin values. **Figures in parentheses are √ n+0.5 transformed values
DAI = Days after Inoculation *N.r. = Nomuraea rileyi*
SFO = Sunflower oil HO = Honey GH = Ghee
GLY = Glycerol TW = Tween-80 CMC= Carboxymethyl Cellulose

It was established from the study that adjuvants substantially contributed to cfu. T2-*N.r.*+SFO and T9- *N.r.*+TW+GLY+HO resulted in higher cfu (22.33 to 23.33x10^8) and viability index (218.71 to 234.85) than control (14.67x10^8cfu/ml) were considered as highly promising potent WP formulations for growth and biomass production. These are coded as **A-$N_{30}S_{1/1}$ and B-$N_{30}T_{1/2}G_{2/1}H_{1/1}$** (Plate-IX-I)

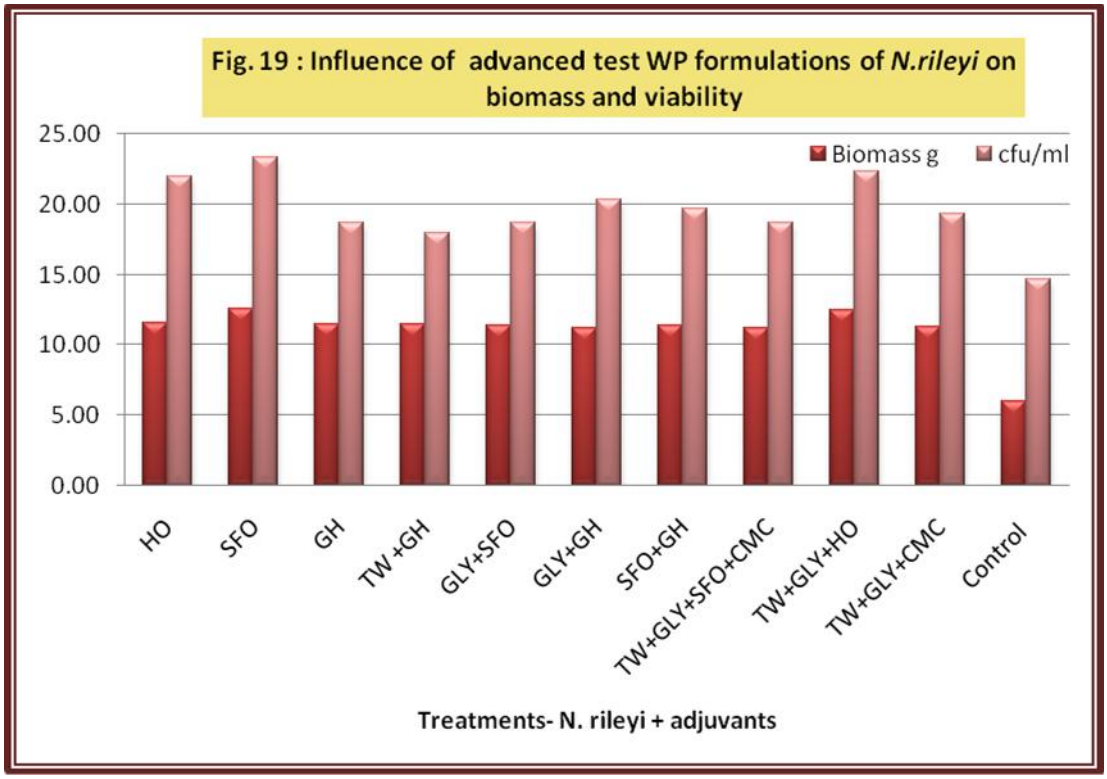


Fig. 19 : Influence of advanced test WP formulations of N.rileyi on biomass and viability
25.00
20.00
15.00
10.00
5.00
0.00
Biomass g
cfu/ml
HO
SFO
GH
TW+GH
GLY+SFO
GLY+GH
SFO+GH
TW+GLY+SFO+CMC
TW+GLY+HO
TW+GLY+CMC
Control
Treatments- N. rileyi + adjuvants

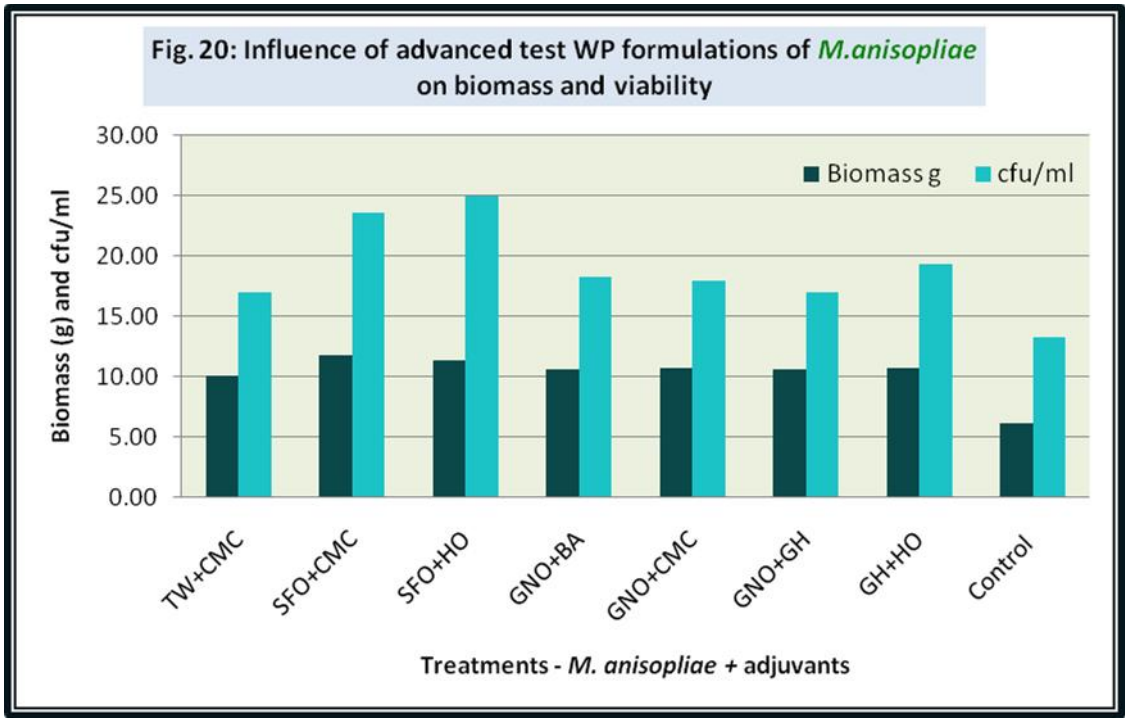


Fig. 20: Influence of advanced test WP formulations of M.anisopliae on biomass and viability
Biomass (g) and cfu/ml
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Biomass g
cfu/ml
TW+CMC
SFO+CMC
SFO+HO
GNO+BA
GNO+CMC
GNO+GH
GH+HO
Control
Treatments - M. anisopliae + adjuvants

***M.anisopliae* : Effect on growth :** The data on the growth, development and viability of *M.anisopliae* liquid culture are presented in Table 34 and depicted in Fig.20.

At 3 DAI, T2-*M.a.*+SFO+CMC covered significantly highest (66.67%) surface of medium. However, it was at par with T3- *M.a.*+SFO+HO (63.33%). The next promising formulations were T5- *M.a.*+GNO+CMC (51.67%), T7- *M.a.*+GH+HO (46.67%) and T1- *M.a.*+TW+CMC (36.67%). Least (10.0%) growth of the fungus was recorded in T8- control. At 7 DAI T2- *M.a.*+SFO+CMC, T3- *M.a.*+SFO+HO and T7- *M.a.*+GH+HO recorded cent per cent surface coverage by the fungus. At 10 DAI, all treatments including control covered cent per cent surface of the medium (Plate-VII-II).

Effect on biomass : At 10 DAI, the differences (6.20 to 11.83g) for biomass production were significant. T2- *M.a.*+SFO+CMC maintained its superiority over rest of the treatments by producing 11.83g biomass. However, it was at par with T3- *M.a.*+SFO+HO (11.43g).

Effect on viability (cfu/ml) : The differences in cfu count was significant in the test formulations. Maximum ($2.50x10^9$) cfu count was observed in T3- *M.a.*+SFO+HO. However, it was at par with T2- *M.a.*+SFO+CMC ($2.37x10^9$). Maximum (245.74) viability index was registered in T3- *M.a.*+SFO+HO (Fig.22). It was followed by T2- *M.a.*+SFO+CMC (238.80).

It was evident from the study that the adjuvants substantially contributed to cfu. T2-*M.a.*+SFO+CMC and T3- *M.a.*+SFO+HO resulted in higher cfu than control and viability index were considered as highly promising potent WP formulations for highest growth and biomass production these were coded as **A1-$M_{30}S_{1/1}C_{1/2}$** and **B1-$M_{30}S_{1/1}H_{1/1}$** (Plate-IX-II). There is no published information on aspect of the study.

Fig. 21: Influence of advanced test WP formulations of *N.rileyi* on viability index of inoculum

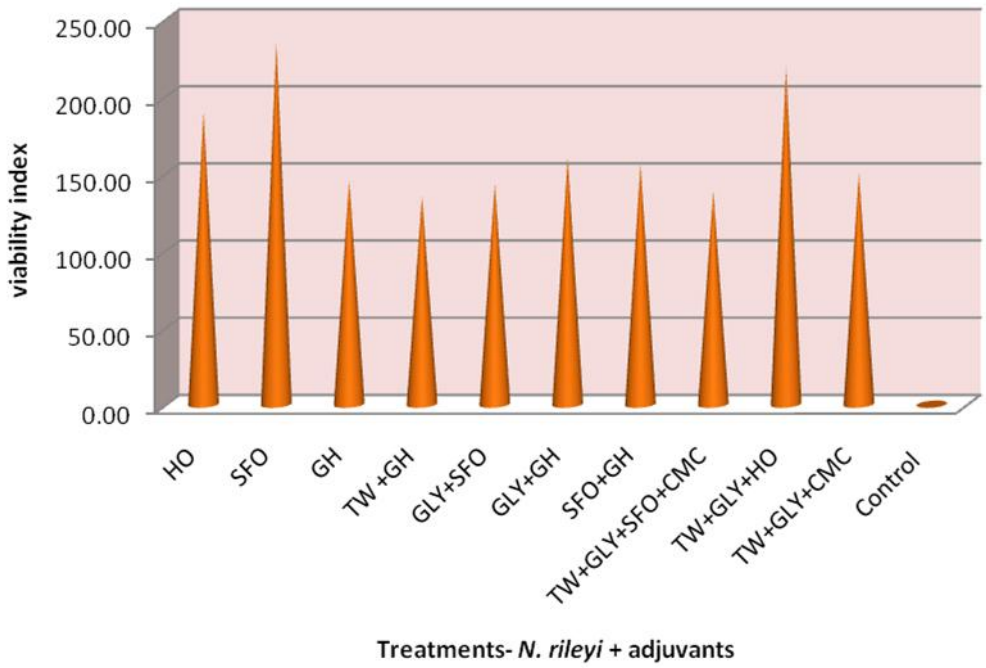
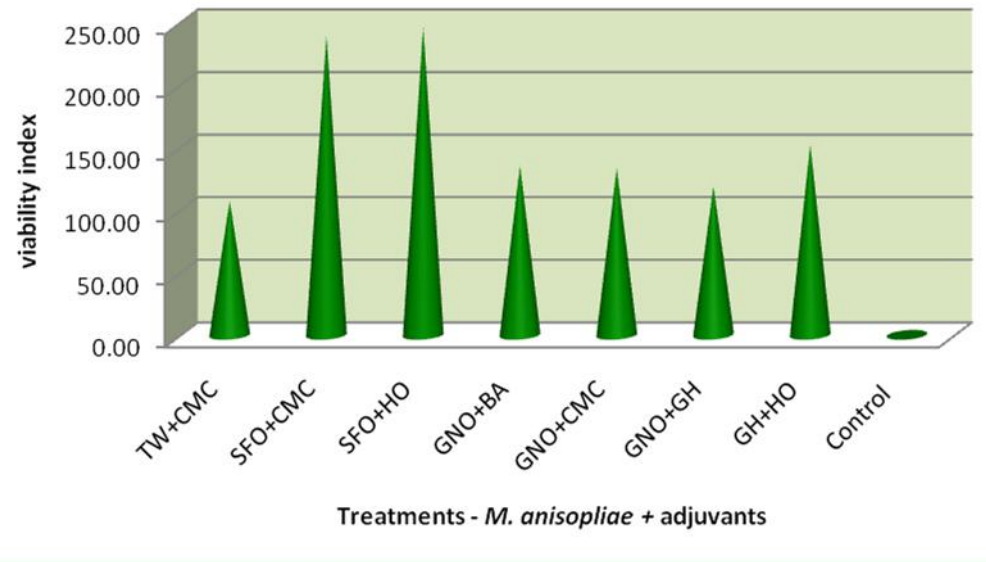
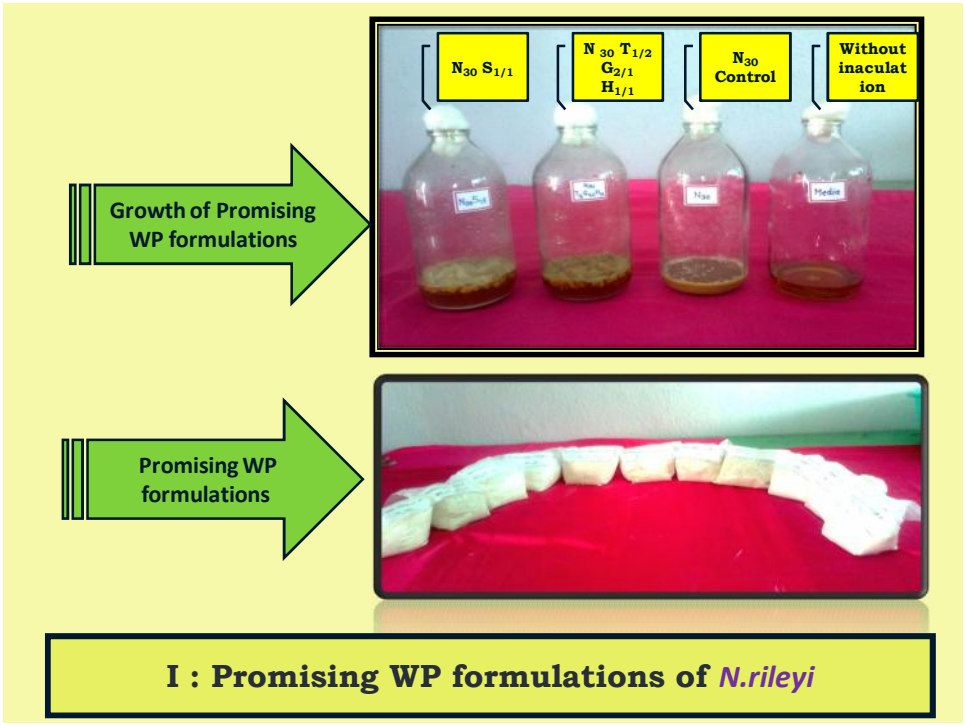


Fig. 22: Influence of advanced test WP formulations of *M.anisopliae* on viability index of inoculum





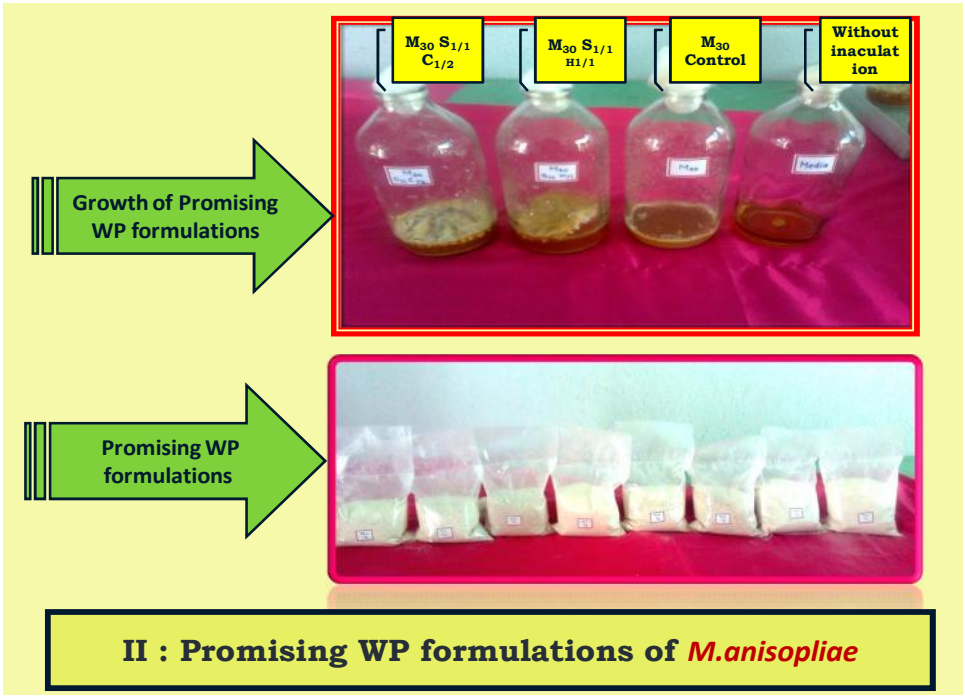


PLATE – VIII
Advanced test WP formulations of *N.rileyi* and *M.anisopliae*

Table 34. Influence of advanced test WP formulations of *M.anisopliae* on growth, development and viability

Tr. No	Treatment	Conc. adj. (%)	Surface coverage (%)			Biomass g/40ml medium	cfu (x108 cfu /ml)	Viability Index	PH at 10 DAI
			3 DAI	7 DAI	10DAI				
1	*M.a.*+ TW +CMC	0.5+0.5	36.67 (37.29)*	83.33 (65.88)	100.00 (90.00)	10.07	17.00 (4.18)**	107.13	7.7
2	*M.a.*+SFO +CMC	1.0+0.5	66.67 (54.76)	100.00 (90.00)	100.00 (90.00)	11.83	23.67 (4.92)	238.80	7.6
3	*M.a.*+SFO +HO	1.0+1.0	63.33 (52.71)	100.00 (90.00)	100.00 (90.00)	11.43	25.00 (5.05)	245.74	7.6
4	*M.a.*+GNO +BA	0.5+2.0	31.67 (34.27)	76.67 (61.14)	100.00 (90.00)	10.60	18.33 (4.34)	135.09	7.6
5	*M.a.*+GNO +CMC	0.5+0.5	51.67 (45.97)	86.67 (68.61)	100.00 (90.00)	10.70	18.00 (4.30)	133.02	7.6
6	*M.a.*+GNO +GH	0.5+0.5	26.67 (31.11)	76.67 (61.14)	100.00 (90.00)	10.63	17.00 (4.18)	118.64	7.7
7	*M.a.*+GH +HO	0.5+1.0	46.67 (43.11)	100.00 (90.00)	100.00 (90.00)	10.77	19.33 (4.45)	151.88	7.7
8	Control (*M.a.* alone)	-	10.00 (18.44)	61.67 (51.77)	100.00 (90.00)	6.20	13.33 (3.72)	-	7.7
	S.E+		**1.46**	**1.59**	**-**	**0.14**	**0.06**	**-**	**-**
	C.D. (P=0.05)		**4.40**	**4.80**	**-**	**0.42**	**0.18**	**-**	**-**

*Figures in parentheses are arcsin values.**Figures in parentheses are √ n+0.5 transformed values
DAI = Days after inoculation *M.a. = Metarhizium anisopliae*
TW = Tween-80 CMC = Carboxymethyl Cellulose
SFO = Sunflower oil HO = Honey
GNO = Groundnut oil BA = Boric acid GH = Ghee

4.1.7.2 Bioefficacy of *N.rileyi* and *M.anisopliae* WP formulations against larvae of *S.litura*

The II instar larvae

***N.rileyi* :**

Data presented in Table 35 and depicted in Fig.23 and Plate-VII-II revealed that the larval mortality among the treatments ranged from 23.33 to 56.67, 40.00 to 76.67 and 60.00 to 93.33 per cent at 5, 7 and 10 DAT, respectively. The mortality at 10 DAT was significantly highest (93.33%) in formulations with adjuvant sunflower oil. However, it was at par with formulation T9- *N.r.*+TW+GLY+HO (90.0%) and T10- *N.r.*+TW+GLY+CMC (86.67%). The lowest larval mortality of 60.0 per cent was recorded in control (*N.r.*alone).

Table 35 : Bioefficacy of advanced test WP formulations of *N.rileyi* against II instar larvae of *S.litura*

Tr. No	Treatment	Conc. of adjuvant (%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
1	*N.r.*+ HO	1.0	43.33 (41.15)*	63.33 (52.71)	76.67 (61.14)
2	*N.r.*+ SFO	1.0	56.67 (48.85)	76.67 (61.14)	93.33 (75.00)
3	*N.r.*+ GH	0.5	36.67 (37.29)	56.67 (48.85)	73.33 (58.89)
4	*N.r.*+ TW +GH	0.5+0.5	33.33 (35.24)	56.67 (48.85)	73.33 (58.89)
5	*N.r.*+ GLY+SFO	2.0+1.0	36.67 (37.29)	53.33 (46.89)	70.00 (56.79)
6	*N.r.*+ GLY+GH	2.0+0.5	33.33 (35.24)	53.33 (46.89)	70.00 (56.79)
7	*N.r.*+ SFO+GH	1.0+0.5	36.67 (37.29)	56.67 (48.27)	73.33 (58.89)
8	*N.r.*+TW+GLY+SFO+CMC	0.5+2+1+0.5	36.67 (37.29)	53.33 (46.89)	70.00 (56.79)
9	*N.r.*+ TW+GLY+HO	0.5+2.0+1.0	46.67 (41.96)	70.00 (56.79)	90.00 (71.56)
10	*N.r.*+ TW+GLY+CMC	0.5+2.0+0.5	43.33 (41.15)	66.67 (54.76)	86.67 (68.61)
11	Control (*N.r.*alone)	-	23.33 (28.86)	40.00 (39.23)	60.00 (50.77)
12	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E+		**2.03**	**1.57**	**2.41**
	C.D.(P=0.05)		**5.97**	**4.62**	**7.09**

*Figures in parentheses are arcsin values DAT = Days after Treatment *N.r. = Nomuraea rileyi*
SFO = Sunflower oil GH = Ghee GLY = Glycerol
TW = Tween-80 CMC=Carboxymethyl Cellulose HO = Honey

***M.anisopliae* :** Data presented in Table 36 and depicted in Fig.24 and Plate-VII-II revealed that the larval mortality among the treatments ranged from 13.33 to 43.33, 36.67 to 66.67 and 56.67 to 83.33 per cent at 5,7 and 10 DAT, respectively.

Table 36. Bioefficacy of advanced test WP formulations of *M.anisopliae* against II instar larvae of *S.litura*

Tr. No	Treatment	Conc. adj.(%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
T1	*M.a.*+ TW+CMC	0.5+0.5	30.00 (33.21)*	46.67 (43.11)	63.33 (52.71)
T2	*M.a.*+SFO+CMC	1.0+0.5	40.00 (39.23)	63.33 (52.71)	80.00 (63.44)
T3	*M.a.*+SFO+HO	1.0+1.0	43.33 (41.15)	66.67 (54.76)	83.33 (65.88)
T4	*M.a.*+GNO+BA	0.5+2.0	26.67 (31.11)	46.67 (43.11)	56.67 (48.85)
T5	*M.a.*+GNO+CMC	0.5+0.5	30.00 (33.21)	46.67 (43.11)	63.33 (52.71)
T6	*M.a.*+GNO+GH	0.5+0.5	33.33 (35.24)	56.67 (48.85)	63.33 (52.71)
T7	*M.a.*+GH+HO	0.5+1.0	26.67 (31.11)	53.33 (46.89)	56.67 (48.85)
T8	Control (*M.a.* alone)	-	13.33 (21.39)	36.67 (37.29)	56.67 (48.85)
T9	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E +		**2.11**	**2.08**	**1.98**
	C.D.(P=0.05)		**6.39**	**6.28**	**6.02**

*Figures in parentheses are arcsin values DAI = Days after inoculation
M.a. = Metarhizium anisopliae TW = Tween-80 CMC = Carboxymethyl Cellulose
SFO = Sunflower oil HO = Honey GNO =Groundnut oil BA =Boric acid GH = Ghee

Fig .23:Influence of advanced test WP formulations of *N.rileyi* on mortality of II and III instar larvae of *S.litura*

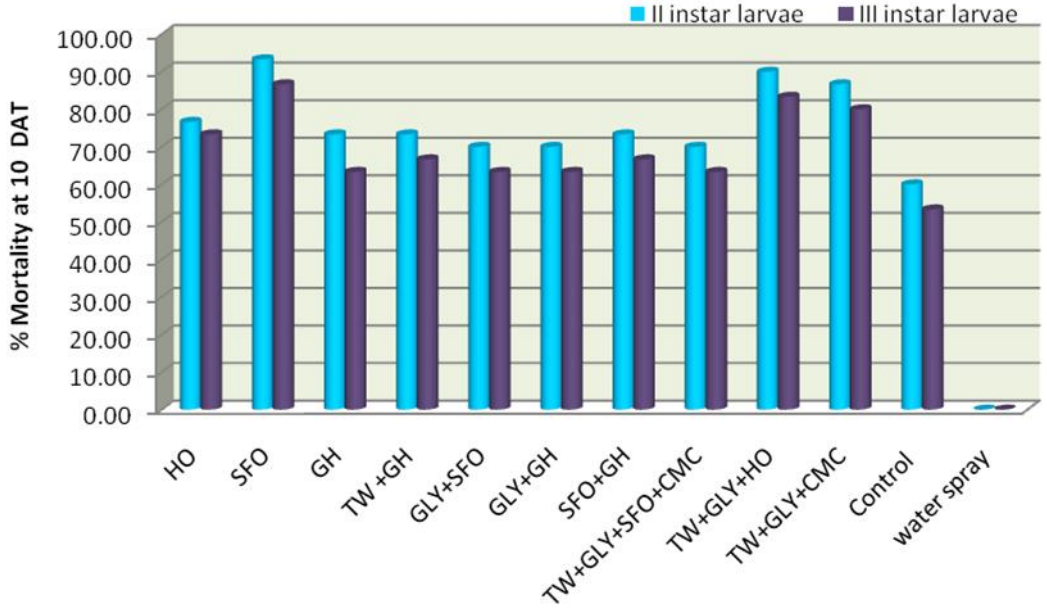
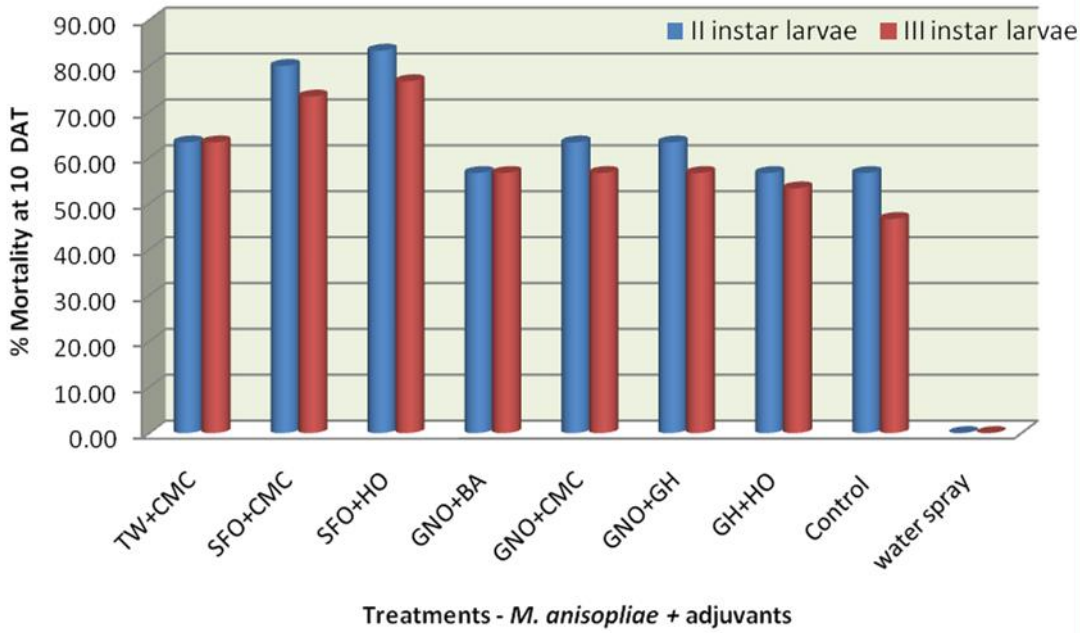


Fig. 24: Influence of advanced test WP formulations of *M.anisopliae* on mortality of II and III instar larvae of *S.litura*



The mortality at 10 DAT was significantly highest (83.33%) in formulations with adjuvants *M.a.*+SFO+HO. However, it was at par with formulations *M.a.*+SFO+CMC (80.0%). The next promising formulations for larval morality were TW+CMC (63.33), and *M.a.*+GNO+CMC (63.33%). The lowest larval mortality of 56.67 per cent was recorded in control (*M.a.*alone).

The III instar larvae

***N.rileyi* :**

The per cent larval mortality was in the range of 16.67 to 46.67, 33.33 to 70.0 and 53.33 to 86.67 at 5, 7 and 10 DAT, respectively (Table 37).

At 10 DAT, all the formulations containing adjuvants were significantly superior (63.33 to 86.67%) than the control (53.33%). The treatment T2-*N.r.*+SFO proved significantly superior in causing highest (86.67%) kill of larvae.

Table 37 : Bioefficacy of advanced test WP formulations of *N.rileyi* against III instar larvae of *S.litura*

Tr. No	Treatment	Conc. of adjuvant(%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
1	*N.r.*+ HO	1.0	30.00 (33.21)*	53.33 (46.89)	73.33 (58.89)
2	*N.r.*+ SFO	1.0	46.67 (43.11)	70.00 (56.79)	86.67 (68.61)
3	*N.r.*+ GH	0.5	30.00 (33.21)	50.00 (45.00)	63.33 (52.71)
4	*N.r.*+ TW +GH	0.5+0.5	26.67 (31.11)	53.33 (46.89)	66.67 (54.76)
5	*N.r.*+ GLY+SFO	2.0+1.0	30.00 (33.21)	46.67 (43.11)	63.33 (52.71)
6	*N.r.*+ GLY+GH	2.0+0.5	26.67 (31.11)	43.33 (41.15)	63.33 (52.71)
7	*N.r.*+ SFO+GH	1.0+0.5	33.33 (35.24)	50.00 (45.00)	66.67 (54.76)
8	*N.r.*+TW+GLY+SFO+CMC	0.5+2+1+0.5	33.33 (35.24)	46.67 (43.11)	63.33 (52.71)
9	*N.r.*+ TW+GLY+HO	0.5+2.0+1.0	40.00 (39.23)	63.33 (52.71)	83.33 (65.88)
10	*N.r.*+ TW+GLY+CMC	0.5+2.0+0.5	36.67 (37.29)	60.00 (50.77)	80.00 (63.44)
11	Control (*N.r.*alone)	-	16.67 (24.12)	33.33 (35.24)	53.33 (46.89)
12	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±		**2.08**	**1.55**	**2.05**
	C.D.(P=0.05)		**6.12**	**4.56**	**6.04**

*Figures in parentheses are arcsin values DAT = Days after Treatment *N.r. = Nomuraea rileyi*
SFO = Sunflower oil GH = Ghee HO = Honey
GLY = Glycerol TW = Tween-80 CMC=Carboxymethyl Cellulose

However, it was at par with T9- *N.r.*+TW+GLY+HO (83.33%), T10- *N.r.*+TW+GLY+CMC (80.0%). On the basic study on bioefficacy of the promising formulations T2- *N.r.*+SFO 1.0 and T9- *N.r.*+TW+GLY+HO were emerged as potencial advanced promising formulations.

***M.anisopliae* :**

At 10 DAT, all the formulations were significantly superior (46.67 to 76.67%) to fungus culture alone (46.67%) for the larval mortality. T3- *M.a.*+SFO+HO (76.67%) was significantly superiority over the rest of the treatments. However, it was at par with T2- *M.a.*+SFO+CMC (73.33%). The next promising formulations were T1- *M.a.*+TW+CMC (63.33%), T4, T5 and T6 (56.67% each). The control recorded lowest larval mortality of 46.67 per cent (Table 38).

Out of 11 *N.rileyi* formulations T2- *N.r.*+SFO and T9- *N.r.*+TW+GLY+HO and out of 8 formulations of *M.anisopliae* T2- *M.a.*+SFO+CMC and T3- *M.a.*+SFO+HO were highly promising. These included combination of Tween 80, Glycerol, Honey, CMC and alone sunflower oil with mycoagent, *N.rileyi* and *M.anisopliae.*

The present investigation is in conformity with the results reported by following research workers for bioefficacy of *N.rileyi*. Vimladevi (1994) reported that application of 10^8, 10^9, 10^{10} and 10^{11} spores/litre of spray solution resulted in 100% mortality of first instar larvae of *S.litura*. Zang *et al*. (1999) reported 85.5% mortality of *S.litura*. Wiwat (2004) observed that *N.rileyi* conidia with bentonite and sucrose powder recorded lower LC_{50} values. Nagaraja (2005) reported that oil formulation (sunflower+tween-80), wettable powder and crude formulation recorded 95.0, 83.10 and 77.0 per cent mortality of *S.litura* at concentration of $2x10^8$conidia/ml. Ramegowda (2005) reported that the cumulative per cent mortality after nine days was relatively higher with oil formulation compared to wettable powder formulation. The groundnut oil registered highest (96.0%) mortality followed by sunflower oil.

The finding of Paulina Vega Aquino *et al*. (2010) was in support of the result on bioefficacy of *N.rileyi* with oils caused cent per cent mortality. Sood *et al*. (2001) found that the wettable powder formulation of *B.bassiana* @ $2x10^7$cfu/ml against third instar larvae of *Plutella xylostella* was more effective.

The present investigation is in conformity with the results reported by Dayakar and Kanujia (2001 and 2003), Pandey and Kanujia (2004), Purwar and Sachan (2005), Rajesh Anand *et al*. (2009) and Dayakar and Subbarao (2011) who reported that morality of larvae of *S.litura* increased with increase in concentration of *M.anisopliae* and decreased with age of larvae as it was observed in present study.

This finding of effectiveness of *M.anisopliae* against *S.litura* in the study is in agreement with those reported by Gopalakrishnan and Narayanan (1987 and 1988) who reported 80 to 100% mortality of *H.armigera* at $1.8x10^9$spores/ml and Saxena *et al*. (1990).

Table 38. : Bioefficacy of advanced test WP formulations of *M.anisopliae* against III instar larvae of *S.litura*

Tr. No	Treatment	Conc. adj. (%)	Larval mortalitly (%)		
			5 DAT	7 DAT	10 DAT
T1	*M.a.*+ TW+CMC	0.5+0.5	23.33 (28.86)*	36.67 (37.29)	63.33 (52.71)
T2	*M.a.*+SFO+CMC	1.0+0.5	33.33 (35.24)	53.33 (46.89)	73.33 (58.89)
T3	*M.a.*+SFO+HO	1.0+1.0	40.00 (39.23)	56.67 (48.85)	76.67 (61.14)
T4	*M.a.*+GNO+BA	0.5+2.0	26.67 (31.11)	36.67 (37.29)	56.67 (48.85)
T5	*M.a.*+GNO+CMC	0.5+0.5	30.00 (33.21)	43.33 (41.15)	56.67 (48.85)
T6	*M.a.*+GNO+GH	0.5+0.5	33.33 (35.24)	40.00 (39.23)	56.67 (48.85)
T7	*M.a.*+GH+HO	0.5+1.0	30.00 (33.21)	43.33 (41.15)	53.33 (46.89)
T8	Control (*M.a.*alone)	-	16.67 (24.12)	30.00 (33.21)	46.67 (43.11)
T9	Water spray	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E +		**1.86**	**1.84**	**2.04**
	C.D.(P=0.05)		**5.63**	**5.55**	**6.18**

*Figures in parentheses are arcsin values
DAI = Days after inoculation
M.a. = Metarhizium anisopliae
TW = Tween-80
SFO = Sunflower oil
CMC = Carboxymethyl Cellulose
HO = Honey
BA = Boric acid
GNO = Groundnut oil
GH = Ghee

4.1.9 Effect of UVC rays on growth and development of inoculum in advanced formulations of *N.rileyi* and *M.anisopliae*

4.1.9.1 Effect on growth after exposure to UVC rays

***N.rileyi* :** The data on the advanced promising formulations of *N.rileyi* exposed to UVC rays for 10 to 50 minutes and 2, 3 and 5 hours are presented in Table 39 to 41.

UVC exposure- 10 to 50 minutes : After 10 minutes exposure to UVC rays adjuvants in T1- *N.r.*+HO recorded highest (83.33%) growth of fungus at 3 DAI. However, it was at par with T2- *N.r.*+SFO (78.33%), followed by T3- *N.r.*+GH (70.00%) and T6- *N.r.*+GLY+GH (66.67%). Least (16.67%) surface coverage was registered in T11-control.. Similar trend of growth and development was observed at 7 DAI. T1- *N.r.*+HO, T2- *N.r.*+SFO, T3- *N.r.*+GH, T8- *N.r.*+TW+GLY+SFO+CMC, T9-*N.r.*+TW+GLY+HO and T10- *N.r.*+TW+GLY+CMC registered cent per cent growth and development of fungus in culture medium.

The per cent surface coverage after 20, 30, 40 and 50 minutes to UVC exposure, the treatments at 3 DAI was in the range of 13.33 to 76.67, 13.33 to 76.67, 13.33 to 75.00 and 13.33 to 68.33 against corresponding figures of 58.33 to 100.0, 51.67 to 100.0, 50.00 to 100.0 and 43.33 to 100.0 at 7 DAI, resepectively. At 10 DAI, all the treatments registered cent per cent surface coverage after 10, 20 and 30 minutes.

UVC exposure- 2, 3 and 5 hours : Data presented in Table 41 revealed that all the aqua suspension formulations exposed to UVC rays prevented the growth of fungus at 3 DAI. The surface coverage by the fungus on medium was in the range 33.33 to 100.0, 28.33 to 98.33 and 23.33 to 96.67 at 7 DAI against 75.00 to 100.0, 73.33 to 100.0 and 65.00 to 100.0 at 10 DAI at 2, 3 and 5 hours exposure, respectively.

Table 39. Influence of advanced test AS formulations of *N.rileyi* exposed to UVC rays for 10 and 20 minutes on growth of inoculum

Tr. No.	Treatment	Conc. of adj. (%)	Surface coverage (%)					
			10 min. UVC exposure			20 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*N.r.*+ HO	1.0	83.33 (65.88)*	100.00 (90.00)	100.00 (90.00)	73.33 (58.89)	100.00 (90.00)	100.00 (90.00)
T2	*N.r.*+ SFO	1.0	78.33 (62.24)	100.00 (90.00)	100.00 (90.00)	76.67 (61.14)	100.00 (90.00)	100.00 (90.00)
T3	*N.r.*+ GH	0.5	70.00 (56.79)	100.00 (90.00)	100.00 (90.00)	66.67 (54.76)	100.00 (90.00)	100.00 (90.00)
T4	*N.r.*+TW +GH	0.5+0.5	28.33 (32.14)	71.67 (57.76)	100.00 (90.00)	26.67 (31.11)	68.33 (55.73)	100.00 (90.00)
T5	*N.r.*+ GLY+SFO	2.0+1.0	26.67 (31.11)	93.33 (75.00)	100.00 (90.00)	26.67 (31.11)	91.67 (73.26)	100.00 (90.00)
T6	*N.r.*+ GLY+GH	2.0+0.5	66.67 (54.51)	91.67 (73.26)	100.00 (90.00)	63.33 (52.71)	90.00 (84.26)	100.00 (90.00)
T7	*N.r.*+ SFO +GH	1.0+0.5	28.33 (32.14)	71.67 (57.76)	100.00 (90.00)	26.67 (31.11)	71.67 (57.86)	100.00 (90.00)
T8	*N.r.*+ TW+GLY +SFO+CMC	0.5+2 +1+0.5	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)	21.67 (27.76)	100.00 (90.00)	100.00 (90.00)
T9	*N.r.*+ TW +GLY+HO	0.5+2 +1	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)
T10	*N.r.*+ TW +GLY+CMC	0.5+2 +0.5	28.33 (32.14)	100.00 (90.00)	100.00 (90.00)	25.00 (30.00)	100.00 (90.00)	100.00 (90.00)
T11	Control (*N.r.*alone)	-	16.67 (24.12)	61.67 (51.77)	100.00 (90.00)	13.33 (21.39)	58.33 (49.79)	100.00 (90.00)
T12	Control (W.UVC)	-	28.33 (32.14)	65.00 (53.73)	100.00 (90.00)	30.00 (33.21)	61.67 (51.77)	100.00 (90.00)
	S.E±		**1.67**	**1.57**	**-**	**1.37**	**1.71**	**-**
	C.D (P=0.05)		**4.87**	**4.59**	**-**	**4.00**	**5.00**	**-**

*Figures in parentheses are arcsin values W.UVC=without UVCDAI = Days ferinoculation
N.r. = Nomuraea rileyi
SFO = Sunflower oil GH = Ghee HO = Honey
GLY = Glycerol TW = Tween-80 CMC = Carboxymethyl Cellulose

Table 40. Influence of advanced test AS formulations of *N.rileyi* exposed to UVC rays for 30, 40 and 50 minutes on growth of inoculum

Tr. No	Treatment	Conc .of adj. (%)	Surface area covered (%)								
			30 min. UVC exposure			40 min. UVC exposure			50 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*N.r.*+ HO	1.0	71.67 (57.86)	98.33 (82.51)	100.0 (90.0)	66.67 (54.76)	100.00 (90.00)	100.0 (90.0)	60.00 (50.77)	98.33 (82.51)	100.0 (90.00)
T2	*N.r.*+ SFO	1.0	76.67 (61.14)	100.00 (90.00)	100.0 (90.0)	75.0 (60.0)	100.00 (90.00)	100.0 (90.0)	68.33 (55.73)	100.00 (90.00)	100.0 (90.00)
T3	*N.r.*+ GH	0.5	65.00 (53.73)	100.00 (90.00)	100.0 (90.0)	61.67 (51.77)	100.0 (90.0)	100.0 (90.0)	55.00 (47.87)	100.00 (90.00)	100.0 (90.00)
T4	*N.r.*+ TW + GH	0.5 +0.5	25.00 (30.00)	63.33 (52.71)	100.0 (90.0)	23.33 (28.86)	58.33 (49.79)	96.67 (79.53)	18.33 (25.33)	51.67 (45.97)	93.33 (75.00)
T5	*N.r.*+ GLY+ SFO	2.0 +1.0	25.00 (30.00)	86.67 (68.61)	100.0 (90.0)	23.33 (28.86)	80.0 (63.44)	100.0 (90.0)	20.00 (26.56)	76.67 (61.14)	98.33 (82.51)
T6	*N.r.*+ GLY+ GH	2.0 +0.5	51.67 (45.97)	86.67 (68.61)	100.0 (90.0)	48.33 (44.03)	85.0 (67.21)	98.33 (82.51)	43.33 (41.15)	81.67 (64.67)	98.33 (82.51)
T7	*N.r.*+ SFO +GH	1.0 +0.5	25.00 (30.00)	66.67 (54.76)	100.0 (90.0)	23.33 (28.86)	61.67 (51.77	93.33 (75.0)	20.00 (26.56)	58.33 (49.79)	91.67 (73.26)
T8	*N.r.*+ TW+GLY +SFO+ CMC	0.5+2 +1 +0.5	20.00 (26.56)	93.33 (75.0)	100.0 (90.0)	20.0 (26.56)	91.67 (73.26)	96.67 (79.53)	20.00 (26.56)	90.00 (71.56)	93.33 (75.00)
T9	*N.r.*+ TW +GLY+H O	0.5 +2+1	26.67 (31.11)	96.67 (79.53)	100.0 (90.0)	25.0 (30.0)	93.33 (75.00)	100.0 (90.0)	23.33 (28.86)	91.67 (73.26)	100.0 (90.00)
T10	*N.r.*+ TW +GLY+C MC	0.5+2 +0.5	25.00 (30.00)	95.00 (77.08)	100.0 (90.0)	23.33 (28.86)	93.33 (75.00)	96.67 (79.53)	20.00 (26.56)	86.67 (68.61)	95.00 (77.08)
T11	Control (*N.r* alone)	-	13.33 (21.39)	51.67 (45.97)	100.0 (90.0)	13.33 (21.39)	50.0 (45.0)	91.67 (73.26)	13.33 (21.39)	43.33 (41.15)	90.00 (71.56)
T12	Control (W.UVC)	-	30.00 (33.21)	65.00 (53.73)	100.0 (90.0)	30.00 (3321)	65.00 (53.73)	100.0 (90.0)	28.33 (32.14)	61.67 (51.77)	100.0 (90.0)
	S.E+		**1.19**	**1.88**	**-**	**1.17**	**1.04**	**1.86**	**1.56**	**1.37**	**1.57**
	C.D (P=0.05)		**3.47**	**5.49**	**-**	**3.40**	**3.03**	**5.44**	**4.55**	**4.01**	**4.60**

*Figures in parentheses are arc sin values
DAI = Days after inoculation
SFO = Sunflower oil
CMC = Carboxymethyl Cellulose
W.UVC= without UVC
TW = Tween-80
GH = Ghee
N.r. = Nomuraea rileyi
HO = Honey
GLY = Glycerol

Table 41. Influence of advanced test AS formulations of *N.rileyi* exposed to UVC rays for 2, 3 and 5 hours on growth of inoculum

Tr. No	Treatment	Conc. of adj. (%)	Surface coverage (%)								
			2 hrs. UVC exposure			3 hrs. UVC exposure			5 hrs. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*N.r.*+ HO	1.0	0.00 (0.00)*	98.33 (82.51)	100.00 (90.0)	0.00 (0.00)	95.00 (77.08)	100.00 (90.00)	0.00 (0.00)	90.00 (71.56)	98.33 (82.51)
T2	*N.r.*+ SFO	1.0	0.00 (0.00)	100.00 (90.00)	100.00 (90.0)	0.00 (0.00)	98.33 (82.51)	100.00 (90.00)	0.00 (0.00)	96.67 (79.53)	100.00 (90.00)
T3	*N.r.*+ GH	0.5	0.00 (0.00)	96.67 (79.53)	100.00 (90.0)	0.00 (0.00)	93.33 (75.00)	100.00 (90.00)	0.00 (0.00)	86.67 (68.61)	98.33 (82.51)
T4	*N.r.*+ TW + GH	0.5 +0.5	0.00 (0.00)	41.67 (40.22)	81.67 (64.67)	0.00 (0.00)	38.33 (38.23)	76.67 (61.14)	0.00 (0.00)	28.33 (32.14)	68.33 (55.73)
T5	*N.r.*+ GLY+ SFO	2.0 +1.0	0.00 (0.00)	66.67 (54.76)	90.00 (71.56)	0.00 (0.00)	66.67 (54.76)	86.67 (68.61)	0.00 (0.00)	58.33 (49.79)	81.67 (64.67)
T6	*N.r.*+ GLY+ GH	2.0 +0.5	0.00 (0.00)	65.00 (53.73)	93.33 (75.00)	0.00 (0.00)	53.33 (46.89)	88.33 (70.00)	0.00 (0.00)	48.33 (44.03)	78.33 (62.24)
T7	*N.r.*+ SFO +GH	1.0 +0.5	0.00 (0.00)	51.67 (45.97)	83.33 (65.88)	0.00 (0.00)	46.67 (43.11)	80.00 (63.44)	0.00 (0.00)	36.67 (37.29)	70.00 (56.79)
T8	*N.r.*+ TW+GLY +SFO+ CMC	0.5+ 2 +1+ 0.5	0.00 (0.00)	83.33 (65.88)	88.33 (70.00)	0.00 (0.00)	78.33 (62.24)	85.00 (67.21)	0.00 (0.00)	68.33 (55.73)	78.33 (62.24)
T9	*N.r.*+ TW +GLY+ HO	0.5 +2+ 1	0.00 (0.00)	81.67 (64.67)	96.67 (79.53)	0.00 (0.00)	66.67 (54.76)	86.67 (68.61)	0.00 (0.00)	63.33 (52.71)	81.67 (64.67)
T10	*N.r.*+ TW +GLY +CMC	0.5+ 2 +0.5	0.00 (0.00)	76.67 (61.14)	85.00 (67.21)	0.00 (0.00)	63.33 (52.71)	81.67 (64.67)	0.00 (0.00)	56.67 (48.85)	70.00 (56.79)
T11	Control (*N.r.* alone)	-	0.00 (0.00)	33.33 (35.24)	75.00 (60.0)	0.00 (0.00)	28.33 (32.14)	73.33 (58.89)	0.00 (0.00)	23.33 (28.86)	65.00 (53.73)
T12	Control (W.UVC)	-	30.00 (33.21)	65.00 (53.73)	100.0 (90.0)	30.00 (33.21)	65.00 (53.73)	100.0 (90.0)	30.00 (33.21	61.67 (51.77)	100.0 (90.0)
	S.E+		-	**1.55**	**1.64**	-	**1.36**	**1.47**	-	**1.42**	**1.57**
	C.D (P=0.05)		-	**4.53**	**4.79**	-	**3.98**	**4.30**	-	**4.15**	**4.58**

*Figures in parentheses are arcsin values
DAI = Days after inoculation
SFO = Sunflower oil
GLY = Glycerol
GH = Ghee
TW = Tween-80
W.UVC= without UVC
N.r. = Nomuraea rileyi
HO = Honey
CMC = Carboxymethyl Cellulose

***M.anisopliae* :**

The data on the advanced promising AS formulations of *M.anisopliae* exposed to UVC rays for 10 to 50 minutes and 2, 3 and 5 hours are presented in Table 42 to 44.

UVC exposure- 10 to 50 minutes : After 10 minutes exposure to UVC rays, T3- SFO + HO recorded highest (28.33%) growth of fungus at 3 DAI. However, it was at par with T2- *M.a.*+SFO+CMC (26.67%), it was followed by T4-*M.a.*+GNO+ BA (25.0%), T5- *M.a.*+GNO+CMC (23.33%), T6- *M.a.*+GNO+GH and T7- *M.a.*+GH+HO (21.67%each). Least (3.33%) surface coverage by fungus was registered in T11-control. Similar trend of growth was observed at 7 DAI. At 10 DAI, formulations except T1- *M.a.*+TW+CMC (83.33%) and T8- Control (46.67%) registered cent per cent surface coverage. The coverage in the 20, 30, 40 and 50 minutes treatments at 3 DAI was in the range of 3.33 to 25.00, 3.33 to 21.67, 1.67 to 20.00 and 1.67 to 16.67 against corresponding figures of 30.0 to 91.67, 26.67 to 86.67, 25.0 to 83.33 and 18.33 to 80.00 at 7 DAI and at 10 DAI, it was 40.0 to 100, 36.67 to 100, 31.67 to 100 and 26.67 to 100, respectively.

UVC exposure- 2, 3 and 5 hours : Data presented in Table 44 revealed that all the aqua suspension formulations exposed to UVC rays prevented the growth of fungus at 3 DAI after exposure to UVC rays for 3 and 5 hours. The surface coverage in the 2, 3 and 5 hours exposure was in the range 13.33 to 73.33, 11.67 to 65.0 and 8.33 to 58.33 at 7 DAI against 20.0 to 93.33, 13.33 to 83.33 and 10.0 to 75.0 at 10 DAI, respectively.

Table 42. Influence of advanced test AS formulations of *M.anisopliae* exposed to UVC rays for 10 and 20 minutes on growth of inoculum

Tr. No.	Treatment	Conc. of adj. (%)	Surface coverage (%)					
			10 min. UVC exposure			20 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+ TW+CMC	0.5+0.5	18.33 (25.33)*	38.33 (38.23)	83.33 (65.88)	15.00 (22.79)	35.00 (36.27)	80.00 (63.44)
T2	*M.a.*+ SFO+CMC	1.0+0.5	26.67 (31.11)	86.67 (68.61)	100.00 (90.00)	23.33 (28.86)	83.33 (65.88)	100.00 (90.00)
T3	*M.a.*+ SFO+HO	1.0+1.0	28.33 (32.14)	95.00 (77.08)	100.00 (90.00)	25.00 (30.00)	91.67 (73.26)	100.00 (90.00)
T4	*M.a.*+ GNO+BA	0.5+2.0	25.00 (30.00)	95.00 (77.08)	100.00 (90.00)	21.67 (27.76)	90.00 (71.56)	100.00 (90.00)
T5	*M.a.*+ GNO+CMC	0.5+0.5	23.33 (28.86)	86.67 (68.61)	100.00 (90.00)	18.33 (25.33)	83.33 (65.88)	100.00 (90.00)
T6	*M.a.*+ GNO+GH	0.5+0.5	21.67 (27.76)	83.33 (65.88)	100.00 (90.00)	20.00 (26.56)	78.33 (62.24)	100.00 (90.00)
T7	*M.a.*+ GH+HO	0.5+1.0	21.67 (27.76)	68.33 (55.73)	100.00 (90.00)	20.00 (26.56)	66.67 (54.76)	100.00 (90.00)
T8	Control (*M.a.*alone)	-	3.33 (10.47)	33.33 (35.24)	46.67 (43.11)	3.33 (10.47)	30.00 (33.21)	40.00 (39.23)
T9	Control (*M.a.*alone) (wt.UVC)	-	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)
	S.E ±		**1.54**	**1.96**	**1.94**	**1.24**	**1.41**	**1.08**
	C.D (P=0.05)		**4.57**	**5.82**	**5.78**	**3.69**	**4.20**	**3.22**

*Figures in parentheses are arcsin values DAI = Days after inoculation
M.a.. = Metarhizium anisopliae
SFO = Sunflower oil GNO = Groundnut oil GH = Ghee
HO = Honey TW = Tween-80 BA = Boric acid
CMC = Carboxymethyl Cellulose

Table 43. Influence of advanced test AS formulations of *M.anisopliae* exposed to UVC rays for 30, 40 and 50 minutes on growth of inoculum

Tr No	Treatment	Conc. of adj. (%)	Surface coverage (%)								
			30 min. UVC exposure			40 min. UVC exposure			50 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+ TW+ CMC	0.5+ 0.5	13.33 (21.39)*	31.67 (34.27)	76.67 (61.14)	10.00 (18.44)	28.33 (32.14)	71.67 (57.86)	8.33 (16.78)	25.00 (30.00)	66.67 (54.76)
T2	*M.a.*+ SFO+ CMC	1.0+ 0.5	18.33 (25.33)	81.67 (64.67)	100.00 (90.00)	15.00 (22.79)	78.33 (62.24)	100.00 (90.00)	11.67 (20.00)	73.33 (58.89)	100.00 (90.00)
T3	*M.a.*+ SFO+ HO	1.0+ 1.0	21.67 (27.76)	86.67 (68.61)	100.00 (90.00)	18.33 (25.33)	83.33 (65.88)	100.00 (90.00)	13.33 (21.39)	80.00 (63.44)	100.00 (90.00)
T4	*M.a.*+ GNO+ BA	0.5+ 2.0	20.00 (26.56)	86.67 (68.61)	100.00 (90.00)	20.00 (26.56)	81.67 (64.67)	100.00 (90.00)	16.67 (24.12)	76.67 (61.14)	100.00 (90.00)
T5	*M.a.*+ GNO+ CMC	0.5+ 0.5	18.33 (25.33)	78.33 (65.24)	100.00 (90.00)	15.00 (22.79)	73.33 (58.89)	100.00 (90.00)	13.33 (21.39)	70.00 (56.79)	100.00 (90.00)
T6	*M.a.*+ GNO+ GH	0.5+ 0.5	16.67 (24.12)	73.33 (58.89)	100.00 (90.00)	15.00 (22.79)	70.00 (56.79)	100.00 (90.00)	11.67 (20.00)	65.00 (53.73)	100.00 (90.00)
T7	*M.a.*+ GH+HO	0.5+ 1.0	16.67 (24.12)	63.33 (52.71)	100.00 (90.00)	13.33 (21.39)	58.33 (49.79)	100.00 (90.00)	11.67 (20.00)	53.33 (46.89)	100.00 (90.00)
T8	Control (*M.a.* alone)	-	3.33 (10.47)	26.67 (31.11)	36.67 (37.29)	1.67 (7.49)	25.00 (30.00)	31.67 (34.27)	1.67 (7.49)	18.33 (25.33)	26.67 (31.11)
T9	Control (*M.a.* alone) (W. UVC)	-	35.00 (36.27)	100.0 (90.0)	100.0 (90.0)	38.33 (38.23)	100.0 (90.0)	100.0 (90.0)	36.67 (37.29)	100.0 (90.0)	100.0 (90.0)
	S.E +		**1.55**	**1.48**	**1.30**	**1.65**	**1.24**	**1.51**	**1.77**	**1.47**	**1.16**
	C.D (P=0.05)		**4.59**	**4.41**	**3.85**	**4.90**	**3.69**	**4.48**	**5.26**	**4.38**	**3.45**

*Figures in parentheses are arcsin values
DAI = Days after inoculation
M.a.. = Metarhizium anisopliae
W.UVC=without UVC
SFO = Sunflower oil
GNO = Groundnut oil
GH = Ghee
HO = Honey
TW = Tween-80
BA = Boric acid
CMC = Carboxymethyl Cellulose

Table 44. Influence of advanced test AS formulations of *M.anisopliae* Exposed to UVC rays for 2, 3 and 5 hours on growth of inoculum

Tr. No	Treatment	Conc of adj. (%)	Surface coverage (%)								
			2 hrs. UVC exposure			3 hrs. UVC exposure			5 hrs. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+ TW+ CMC	0.5+ 0.5	6.67 (15.00)*	21.67 (27.76)	58.33 (49.79)	0.00 (0.00)	16.67 (24.12)	51.67 (45.97)	0.00 (0.00)	11.67 (20.00)	41.67 (39.82)
T2	*M.a.*+ SFO+ CMC	1.0+ 0.5	8.33 (16.78)	66.67 (54.76)	91.67 (73.26)	0.00 (0.00)	61.67 (51.77)	81.67 (64.67)	0.00 (0.00)	53.33 (46.89)	75.00 (60.00)
T3	*M.a.*+ SFO+ HO	1.0+ 1.0	10.00 (18.44)	73.33 (58.89)	93.33 (75.00)	0.00 (0.00)	65.00 (53.73)	83.33 (65.88)	0.00 (0.00)	58.33 (49.79)	75.00 (60.00)
T4	*M.a.*+ GNO+ BA	0.5+ 2.0	11.67 (20.00)	70.00 (56.79)	90.00 (71.56)	0.00 (0.00)	60.00 (50.77)	81.67 (64.67)	0.00 (0.00)	53.33 (46.89)	71.67 (57.86)
T5	*M.a.*+ GNO+ CMC	0.5+ 0.5	10.00 (18.44)	63.33 (52.71)	91.67 (73.26)	0.00 (0.00)	58.33 (49.79)	83.33 (65.88)	0.00 (0.00)	48.33 (44.03)	75.00 (60.00)
T6	*M.a.*+ GNO+ GH	0.5+ 0.5	8.33 (16.78)	58.33 (49.79)	90.00 (71.56)	0.00 (0.00)	51.67 (45.97)	80.00 (63.44)	0.00 (0.00)	46.67 (43.11)	68.33 (55.73)
T7	*M.a.*+ GH+ HO	0.5+ 1.0	8.33 (16.78)	46.67 (43.11)	88.33 (70.00)	0.00 (0.00)	41.67 (40.22)	81.67 (64.67)	0.00 (0.00)	33.33 (35.24)	68.33 (55.73)
T8	Control (*M.a.*alone)	-	0.00 (0.00)	13.33 (21.39)	20.00 (26.56)	0.00 (0.00)	11.67 (20.00)	13.33 (21.39)	0.00 (0.00)	8.33 (16.78)	10.00 (18.44)
T9	Control (*M.a* alone) (W.UVC)	-	35.00 (36.27)	100.0 (90.0)	100.0 (90.0)	36.67 (37.29)	100.0 (90.0)	100.0 (90.0)	38.33 (38.23	100.0 (90.0)	100.0 (90.0)
	S.E ±		**1.44**	**1.22**	**2.38**	**-**	**1.16**	**1.81**	**-**	**1.43**	**1.74**
	C.D (P=0.05)		**4.28**	**3.61**	**7.08**	**-**	**3.44**	**5.37**	**-**	**2.26**	**5.16**

*Figures in parentheses are arcsin values DAI = Days after inoculation
M.a.. = Metarhizium anisopliae W.UVC=without UVC
SFO = Sunflower oil GNO = Groundnut oil GH = Ghee
HO = Honey TW = Tween-80 BA = Boric acid
CMC = Carboxymethyl Cellulose

4.1.9.2 Effect of UVC rays on biomass production of *N.rileyi* and *M.anisopliae*

The biomass produced by the mycoagent in formulation treatments with various adjuvants in SDY medium after UVC irradiation for 10 to 50 minutes and 2,3 and 5 hours are presented in Table 45 and depicted in Fig.25. The differences in biomass production in the treatments were significant and trend of performance of formulations were more or less similar to that observed for medium surface coverage at 3 and 7 days.

***N.rileyi* :** After 10 minutes UVC exposure, *N.rileyi* with adjuvant in T2- *N.r.*+SFO produced significantly highest (5.60g) biomass. The next best and at par formulations in their desending order for potential to produce the biomass were T9- *N.r.*+TW+GLY+HO (5.07g), T1- *N.r.*+HO (4.93g), T3- *N.r.*+GH (4.87g) and T10- *N.r.*+TW+GLY+CMC (4.87g).

After the 20 minutes exposure, significantly maximum biomass (5.53g) in T2- *N.r.*+SFO was registered. The next promising formulations were T9- *N.r.*+TW+GLY+HO (5.03g), T10- *N.r.*+TW+GLY+CMC (4.83g), T1- *N.r.*+HO (4.83g) and T3- *N.r.*+GH (4.55g) which were on par with each other.

After 30 minutes exposure, T2- *N.r.*+SFO produced 5.37g biomass which was significantly highest than other formulations (4.27 to 4.98g) and control (3.20g). The next promising formulation for production of biomass was T9- *N.r.*+TW+GLY+HO (4.98g).

The trend of results of 40 and 50 minutes, 2, 3 and 5 hours exposure were more or less similar to that of 30 minutes UVC rays exposure.

It was indicated that surface coverage in medium and biomass produced *N.rileyi* with or without adjuvants in culture medium after exposure to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours decreased with increased exposure period. The adjuvants reacted variably for their UVC ray protectant capacity for *N.rileyi.* Among the various formulations tested T2- *N.r.*+SFO, T9- *N.r.*+TW+GLY+HO and T10- *N.r.*+TW+GLY+CMC act as promising appreciable UVC protectant.

Table 45. Effect of UVC treatment on biomass production by advanced test *N.rileyi* AS formulations

Tr. No.	Treatments	Conc. (%)of adj.	Biomass (g) at 10 DAI after indicated exposure							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*N.r.*+ HO	1.0	4.93	4.83	4.67	4.55	4.45	4.22	4.05	3.92
T2	*N.r.*+ SFO	1.0	5.60	5.53	5.37	5.28	5.22	5.13	5.02	4.95
T3	*N.r.*+ GH	0.5	4.87	4.78	4.60	4.45	4.35	4.13	3.72	3.90
T4	*N.r.*+TW + GH	0.5+ 0.5	4.60	4.55	4.42	4.25	4.15	3.98	3.82	3.65
T5	*N.r.*+ GLY+SFO	2.0+ 1.0	4.53	4.50	4.38	4.25	4.15	3.97	3.80	3.62
T6	*N.r.*+ GLY +GH	2.0+ 0.5	4.57	4.52	4.35	4.25	4.15	3.93	3.73	3.58
T7	*N.r.*+ SFO +GH	1.0+ 0.5	4.47	4.43	4.27	4.10	4.00	3.80	3.60	3.50
T8	*N.r.*+ TW+GLY +SFO+CMC	0.5+2 +1+0.5	4.67	4.60	4.43	4.23	4.13	3.90	3.70	3.60
T9	*N.r.*+ TW +GLY+HO	0.5+2 +1	5.07	5.03	4.98	4.85	4.80	4.65	4.50	4.40
T10	*N.r.*+ TW +GLY+CMC	0.5+2 +0.5	4.87	4.83	4.75	4.63	4.57	4.37	4.18	4.08
T11	Control (*N.r.*alone)	-	3.43	3.35	3.20	3.03	2.95	2.58	2.45	2.23
T12	Control (*N.r.*alnoe) (W.UVC)	-	6.70	7.00	6.80	6.90	6.90	7.00	6.90	6.90
	S.E +		**0.06**	**0.07**	**0.06**	**0.05**	**0.05**	**0.05**	**0.05**	**0.04**
	C.D(P=0.05)		**0.17**	**0.19**	**0.17**	**0.15**	**0.16**	**0.15**	**0.14**	**0.13**

W.UVC = without UVC
DAI = Days after inoculation *N.r. = Nomuraea rileyi*
SFO = Sunflower oil GH = Ghee HO = Honey
GLY = Glycerol TW = Tween-80 CMC = Carboxymethyl Cellulose

***M.anisopliae* :** The biomass produced by *M.anisopliae* with various adjuvants in medium after UVC irradiation for 10 to 50 minutes and 2, 3 and 5 hours are presented in Table 46 and depicted in Fig.26. The differences in biomass production in various promising formularies were significant and trend of performance of formulations were more or less similar to that was observed for surface coverage at 3 and 7 DAI.

After 10 minutes, UVC rays exposure the biomass produced by various promising formulations and control was in the range of 2.98 to 5.33g. T3-*M.a.*+SFO+HO produced significantly highest (5.33g) biomass. However, it

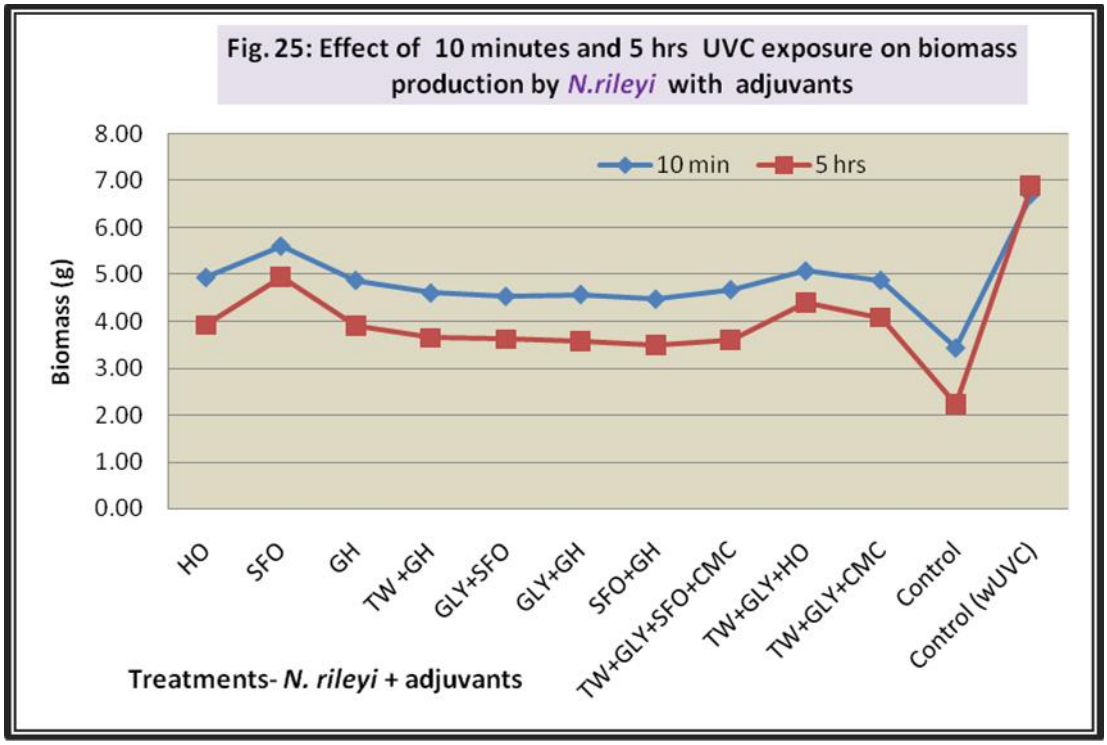


Fig. 25: Effect of 10 minutes and 5 hrs UVC exposure on biomass production by N.rileyi with adjuvants
Biomass (g)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
10 min
5 hrs
HO
SFO
GH
TW +GH
GLY+SFO
GLY+GH
SFO+GH
TW+GLY+SFO+CMC
TW+GLY+HO
TW+GLY+CMC
Control
Control (wUVC)
Treatments- N. rileyi + adjuvants

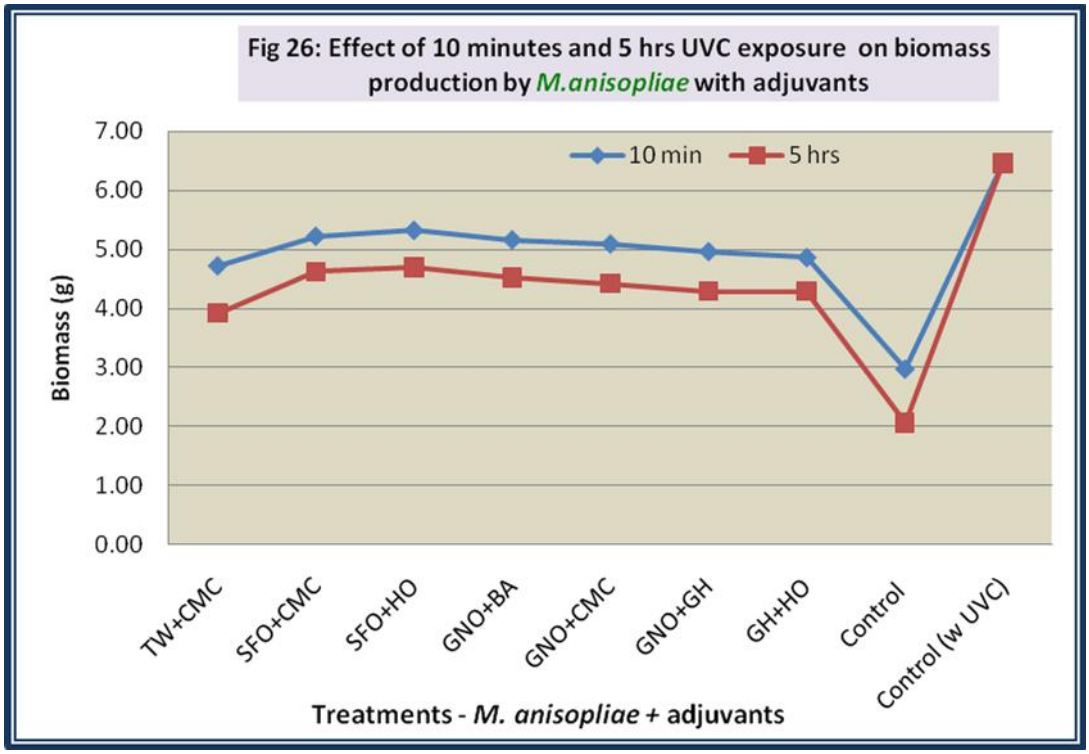


Fig 26: Effect of 10 minutes and 5 hrs UVC exposure on biomass production by M.anisopliae with adjuvants
Biomass (g)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
10 min
5 hrs
TW+CMC
SFO+CMC
SFO+HO
GNO+BA
GNO+CMC
GNO+GH
GH+HO
Control
Control (w UVC)
Treatments - M. anisopliae + adjuvants

was at par with T2- *M.a.*+SFO+CMC (5.23g). The next best formularies were T4- *M.a.*+GNO+BA (5.17g) and T5- *M.a.*+GNO+CMC (5.10g).

T3- *M.a.*+SFO+HO recorded highest (5.27g) biomass. However, it was at par with T2- *M.a.*+SFO+CMC (5.18g). The next promising formulations were T4- *M.a.*+GNO+BA (5.12g) and T5- *M.a.*+GNO+CMC (5.07g) when exposed for 20 minutes to UVC rays. The least biomass (2.93g) was in control.

After 30 minutes exposure, T3- *M.a.*+SFO+HO produced highest (5.22g) biomass. However, it was at par with T2- *M.a.*+SFO+CMC (5.13g).

The trend of results of 40, 50 minutes, 2, 3 and 5 hours exposure on biomass development was more or less similar to that of 30 minutes UVC rays exposure. The biomass production decreased with increase in time of exposure to UVC rays. The formulations T3- *M.a.*+SFO+HO produced significantly highest biomass of 5.13, 5.10, 5.00, 4.90 and 4.70g; however, it was at par with T2- *M.a.*+SFO+CMC which produced 5.10, 5.03, 4.93, 4.83 and 4.63g of biomass after 40, 50 minutes, 2, 3 and 5 hours UVC rays exposure, respectively. The next promising formulations for better production of biomass on exposure of 40 minutes to 5 hrs were T4- *M.a.*+GNO+BA (5.13 to 4.78g) and T5- *M.a.*+GNO+CMC (4.70 to 4.43g).

The lowest biomass of 2.82, 2.77, 2.57, 2.47 and 2.07g developed in adjuvants devoid in T8- control (*M.a.*alone) was registered on the exposure for 40,50 min, 2,3 and 5 hrs, respectively.

It is indicated that medium surface coverage and biomass development of *M.anisopliae* in culture medium on inoculation of formulations either with or without adjuvants after exposure to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours decreased with increase in exposure period. The UVC protecting ability of *N.rileyi* and *M.anisopliae* by adding various adjuvants have been discussed under 4.1.4.

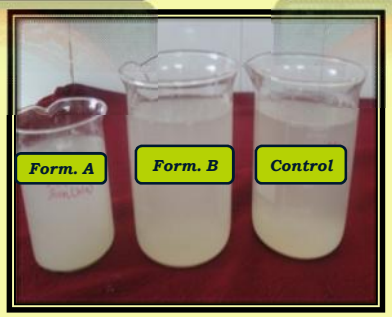
I :Bioassay study of *N.rileyi* and *M.anisopliae*

II : Study on UV irradiation protectability of *N.rileyi* and *M.anisopliae*

PLATE – VI

Bioassay and UVC rays study of *N.rileyi* and *M.anisopliae*

I : Developed WP formulations of *N.rileyi*



II : Developed WP formulations of *M.anisopliae*

PLATE – IX
Developed WP formulations of *N.rileyi* and *M.anisopliae*

Table 46. Effect of UVC treatment on biomass production by advanced test *M.anisopliae* AS formulations

Tr. No.	Treat ments	Conc. (%) of adj.	Biomass (g) at 10 DAI after indicated exposure							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*M.a.*+ TW+CMC	0.5+0.5	4.73	4.63	4.57	4.52	4.47	4.30	4.20	3.93
T2	*M.a.*+ SFO+CMC	1.0+0.5	5.23	5.18	5.13	5.10	5.03	4.93	4.83	4.63
T3	*M.a.*+ SFO+HO	1.0+1.0	5.33	5.27	5.22	5.13	5.10	5.00	4.90	4.70
T4	*M.a.*+ GNO+BA	0.5+2.0	5.17	5.12	5.07	4.77	4.90	4.80	4.70	4.53
T5	*M.a.*+ GNO+CMC	0.5+0.5	5.10	5.07	5.02	4.70	4.83	4.73	4.63	4.43
T6	*M.a.*+ GNO+GH	0.5+0.5	4.97	4.93	4.88	4.80	4.72	4.60	4.50	4.30
T7	*M.a.*+ GH+ HO	0.5+1.0	4.87	4.83	4.78	4.72	4.63	4.50	4.37	4.30
T8	Control (*M.a.*alone)	-	2.98	2.93	2.87	2.82	2.77	2.57	2.47	2.07
T9	Control (*M.a.*alone) (W.UVC)	-	6.47	6.50	6.50	6.60	6.63	6.60	6.50	6.47
	S.E ±		**0.04**	**0.03**	**0.03**	**0.04**	**0.05**	**0.04**	**0.04**	**0.05**
	C.D (P=0.05)		**0.11**	**0.09**	**0.09**	**0.11**	**0.14**	**0.13**	**0.13**	**0.14**

DAI = Days after inoculation
M.a.. = Metarhizium anisopliae
GH = Ghee
TW = Tween-80
W.UVC = without UVC
SFO = Sunflower oil
CMC = Carboxymethyl Cellulose
BA = Boric acid
GNO = Groundnut oil
HO = Honey

4.2 Pathogenicity of each of two most promising final stage WP formulations of *N.rileyi* and *M.anisopliae*

4.2.1 LC_{50} and LC_{90} values of *S.litura*

***N.rileyi* :** The LC_{50} values for *S.litura* of II and III instar larvae were determined through bioassay and probit analysis. The results are presented in Table 47.

The results revealed that the LC_{50} values of formulation A ($N_{30}S_{1/1}$) on the basis of product (BAI) were 0.0116% and 0.0157% for II and III instar larvae of *S.litura*, respectively. The LC_{50} values of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0120% and 0.0176% against II and III instar larvae of *S.litura.* LC_{90} values of formulation A on the basis of product (BAI)

were 0.0710% and 0.0820% for II and III instar larvae of *S.litura*, respectively. The LC_{90} values of formulation B were 0.0748% and 0.0920% against II and III instar larvae of *S.litura.*

It indicated that among two larval instars of *S.litura* tested, II instar larvae found to be most susceptible to the *N.rileyi* WP formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$).

Table 47. LC_{50} and LC_{90} values of final stage WP formulations of *N.rileyi* of II and III instar larvae of *S.litura*

Sr. No	Formulations *N.rileyi* 5% WP	Host tested (*S.litura* larvae)	Chi - square	Regression equation	LC_{50} on BAI (%)	Fiducial limit		LC_{90} on BAI (%)	Fiducial limit	
						Lower	Upper		Lower	Upper
1	A(N_{30} $S_{1/1}$)	II instar	2.30	Y= 3.2547 + 1.6339 X	0.0116	0.0079	0.0172	0.0710	0.0301	0.1671
2	A(N_{30} $S_{1/1}$)	III instar	1.38	Y= 1.8647 + 1.7850 X	0.0157	0.0122	0.0201	0.0820	0.0400	0.1684
3	B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)	II instar	1.43	Y= 3.2532 + 1.6960 X	0.0120	0.0083	0.0174	0.0748	0.0325	0.1722
4	B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)	III instar	0.39	Y= 2.7725 + 1.7867 X	0.0176	0.0140	0.0220	0.0920	0.0438	0.1933

***M.anisopliae* :**

The LC_{50} values of *M.anisopliae* for II and III instar larvae of *S.litura* were determined through bioassay and probit analysis. The results are presented in Table 48.

The results revealed that the LC_{50} values of formulation A1($M_{30}S_{1/1}C_{1/2}$) were 0.0174% and 0.0180% for II and III instar larvae of *S.litura*, respectively. The LC_{50} values of formulation B1($M_{30}S_{1/1}H_{1/1}$) were 0.0149% and 0.0163% against II and III instar larvae of *S.litura*, respectively. LC_{90} values of formulation A1 were 0.0940% and 0.1088% for II and III

instar larvae of *S.litura*, respectively. The LC_{90} values of formulation B1 were 0.0928% and 0.1006% against II and III instar larvae of *S.litura*, respectively.

It indicated that among two larval instar of *S.litura* tested, II instar of larvae was most susceptible to *M.anisopliae* WP formulation A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$).

The LC_{50} value of *N.rileyi* WP formulations was lower in formulation A(0.0116) than B(0.0120) while *M.anisopliae* formulation B1(0.0149) showed the lesser value than A1(0.0174) for II instar larvae. The formulation A($N_{30}S_{1/1}$) of *N.rileyi* and B1($M_{30}S_{1/1}H_{1/1}$) of *M.anisopliae* was the most virulent formulations as evidenced from lowest LC_{50} values. The chi-square test showed homogeneity of test population in all bioassays which indicated the good fit of the observed and expected responses.

It is established from the results that as the larval instar of *S.litura* advanced, it required higher doses of *N.rileyi* and *M.anisopliae* AS and WP formulations to kill it. These results are in conformity with the results reported by Patil (2000), Dayakar and Kanujia (2001), Dayakar and Kanujia (2003), Wiwat (2004), Ramegowda (2005) and Sonai Rajan and Muthukrishnan (2009) for *N.rileyi* and Dayakar and Kanujia (2001), Dayakar and Kanujia (2003), Pandey and Kanujia (2003 and 2004), Purvar and Sachan (2005), Hu *et al.*, (2007), Amer *et.al.*(2008), Panday and Hasan Wajid (2009) and Dayakar and Subbarao (2011) for *M.anisopliae*. The results of the bioassays indicated that susceptibility of the pest decreased with the age of the larvae interms of both LC_{50} and LT_{50}. The present investigation on relative virulence demarcated that II instar larvae of *S.litura* were more susceptible to *N.rileyi* and *M.anisopliae* as compared to III instar larvae. However, all the researchers determined the LC_{50} values for *S.litura*. These were $16.11x10^5$conidia/ml for II instar larvae (Dayakar and Kanujia, 2001). Wiwat (2004) reported that *N.rileyi* conidia along with bentonite and sucrose powder (1:7:7) and aluminium silicate (1:1:8), bentonite soil (1:7:7) and bentonite (1:1:8) recorded lower LC_{50} values of 168, 311, 416 and 586 conidia/larvae whereas that for fresh conidia was 797conidia/larvae. Ramegowda (2005) recorded LC_{50} values of $80.09x10^3$conidia/ml of wettable

powder formulation. Dayakar and Kanujia (2001) worked out LC_{50} values of *M.anisopliae* for II instar larvae of *S.litura* was 12.52x10^5conidia/ml. Dayakar and Subbarao (2011) reported the LC_{50} values of *M.anisopliae* isolate MUCL8237 was 21.32x10^5conidia/ml.

Table 48. LC_{50} and LC_{90} values of final stage WP formulations of *M.anisopliae* of II and III instar larvae of *S.litura*

Sr. No.	Formulations *M.anisopliae* 3% WP	Host tested (*S.litura* larvae)	Chi-square	Regression equation	LC_{50} on BAI (%)	Fiducial limit		LC_{90} on BAI (%)	Fiducial limit	
						Lower	Upper		Lower	Upper
1	**A1($M_{30}S_{1/1}C_{1/2}$)**	II instar	0.80	Y= 2.8218 + 1.7530 X	0.0174	0.0138	0.0220	0.0940	0.0418	0.2117
2	A1(**$M_{30}S_{1/1}C_{1/2}$**)	III instar	0.51	Y= 2.9360 + 1.6227 X	0.0180	0.0141	0.0229	0.1088	0.0446	0.2649
3	B1(**$M_{30}S_{1/1}H_{1/1}$**)	II instar	1.21	Y= 3.1012 + 1.6162 X	0.0149	0.0112	0.0199	0.0928	0.0413	0.2086
4	B1(**$M_{30}S_{1/1}H_{1/1}$**)	III instar	0.80	Y= 3.0297 + 1.6238 X	0.0163	0.0125	0.0212	0.1006	0.0423	0.2388

4.2.2 LT_{50} values of WP formulations of *N.rileyi* and *M.anisopliae* against II and III instar larvae of *S. litura*

***N.rileyi* :** The LT_{50} values were estimated from the data of bioassays of two formulation of *N.rileyi* and results are given in Table 49. The data indicated that LT_{50} values of formulation A($N_{30}S_{1/1}$) at concentration 0.02 per cent was 6.34 days and it was the lowest time registered for 50 per cent kill of II instar larvae compared to formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$). The formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) recorded in 6.38 days for 50 per cent kill of II instar larvae of *S.litura.*

In case of III instar larvae of *S.litura* formulation A registered 7.12 days while formulation B recorded 7.29 days for 50 per cent mortality of larvae of *S.litura.* Thus, it was established from the results that formulation A($N_{30}S_{1/1}$) taken minimum time to kill 50 per cent population and was most virulent. It

was noticed from the comparative performance of two formulations A($N_{30}S_{1/1}$) and B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) that all caused mortality to II and III instar larvae of *S.litura* but there was significant variation on mortality at all intervals of observation. Their efficiency was found proportionate to bioactive ingredient concentrations. The formulation A($N_{30}S_{1/1}$) was superior against *S.litura* which was evidenced from LC_{50} and LT_{50} value compared to other formulation.

Table 49. LT_{50} values of final stage WP formulations of *N.rileyi* of II and III instar larvae of *S.litura*

Sr. No.	Formulations *N.rileyi* 3% WP	Host tested (larvae of *S.litura*)	Chi-square	Regression equation	LT_{50} (days)	Fiducial limit	
						Lower	Upper
1	A($\mathbf{N_{30}S_{1/1}}$)	II instar	0.114	Y= 1.147 +4.805 X	6.337	5.654	7.027
2	A($\mathbf{N_{30}S_{1/1}}$)	III instar	1.415	Y= 1.384+ 4.243 X	7.115	6.333	8.082
3	B($\mathbf{N_{30}T_{1/2}\ G_{2/1}H_{1/1}}$)	II instar	1.764	Y= 1.379+ 4.499 X	6.381	5.657	7.123
4	B ($\mathbf{N_{30}T_{1/2}\ G_{2/1}\ H_{1/1}}$)	III instar	2.119	Y= 1.631+ 3.905 X	7.289	6.438	8.425

M.anisopliae : The LT_{50} values were estimated from the data of bioassays of two formulation of *M.anisopliae* and results are given in Table 50. The data indicated that LT_{50} values of formulation B1($M_{30}S_{1/1}H_{1/1}$) at concentration 0.02 per cent was 7.78 days and it was the lowest time registered for 50 per cent kill of II instar larvae compared to formulation A1($M_{30}S_{1/1}C_{1/2}$. The formulation A1 recorded 8.12 days for 50 per cent kill of second instar larvae of *S.litura.*

In case of III instar larvae of *S.litura* formulation B1 registered 8.68 days while formulation A1 recorded 9.22 days for 50 per cent mortality of larvae of *S.litura.* Thus, it was established from the results that formulation B1 taken minimum time to kill 50 per cent population and was most virulent.

It was noticed from the comparative performance of two formulation *viz.* formulation A1($M_{30}S_{1/1}C_{1/2}$) and formulation B1($M_{30}S_{1/1}H_{1/1}$) that all caused mortality to II and III instar larvae of *S.litura* but there was

significant variation on mortality at all intervals of observation. Their efficiency was found to be proportionate to BAI concentrations. The formulation B1($M_{30}S_{1/1}H_{1/1}$) found superior in causing mortality which is evidenced from LC_{50} and LT_{50} value compared to other formulation.

Patil (2000) reported LT_{50} values for first to fifth instar larvae of *S.litura* of *N.rileyi* were 130.71, 137.77, 148.04, 235.55 and 263.10h respectively. Paulina VegaAquino *et al*. (2010) observed the highest activity of *N.rileyi* in oil against *Spodoptera* spp. with LT_{50} values of 2.5 days. Amer *et al*. (2008) reported that *M.anisopliae* gave the highest mortality of the II instar larvae and IV instar larvae of *S.litua* with lethal time 7 and 10 days, respectively. The LT_{50} values that were calculated at highest concentrations of the *M.anisopliae* were 101.16, 116.51 and 149.75h for 4-5, 10-11 and 15-16 days old larvae of *S.litura* (Pandey Renu and Hasan Wajid, 2009) and Dayakar and Subbarao (2011).

Table 50. LT_{50} values of final stage WP formulations of *M.anisopliae* of II and III instar larvae of *S.litura*

Sr. No.	Formulations *M.anisopliae* 3% WP	Host tested (*S.litura* larvae)	Chi-square	Regression equation	LT_{50} (days)	Fiducial limit	
						Lower	Upper
1	A1($\mathbf{M_{30}S_{1/1}C_{1/2}}$)	II instar	0.341	Y= 2.489+ 2.762X	8.117	6.859	10.856
2	A1($\mathbf{M_{30}S_{1/1}C_{1/2}}$)	III instar	0.714	Y= 1.436+ 3.694X	9.221	8.006	11.896
3	B1($\mathbf{M_{30}S_{1/1}H_{1/1}}$)	II instar	1.019	Y= 1.929+ 3.447 X	7.780	6.783	9.413
4	B1($\mathbf{M_{30}S_{1/1}H_{1/1}}$)	III instar	0.364	Y= 1.311 + 3.931 X	8.679	7.641	10.628

4.3 Bioefficacy of final stage WP formulations of *N.rileyi* and *M.anisopliae* against *S.litura*

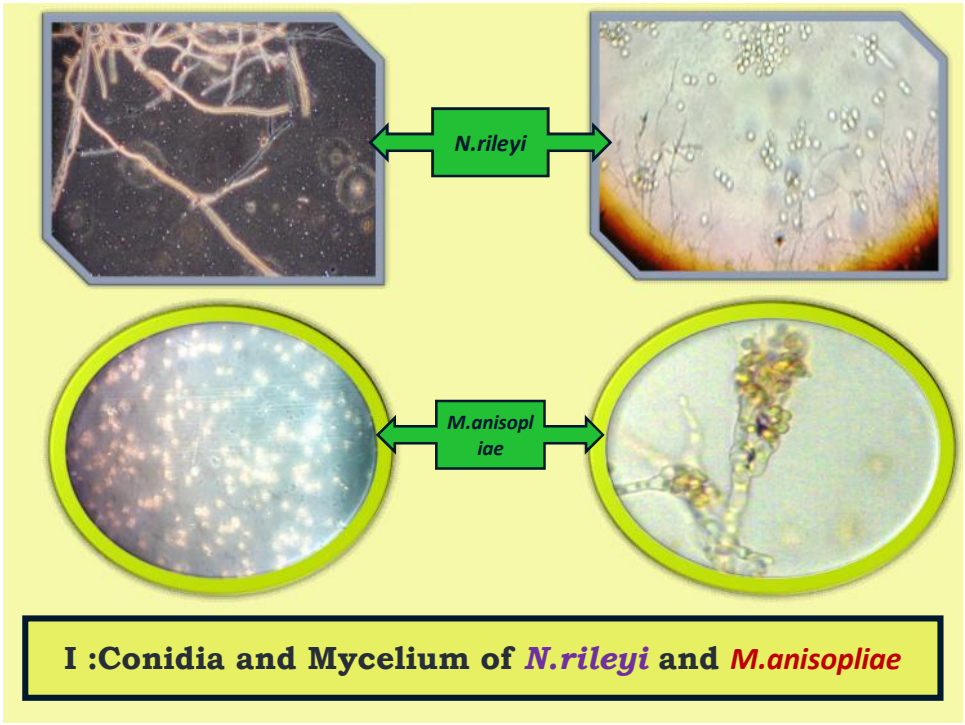
4.3.1 II instar larvae

N.rileyi **:**

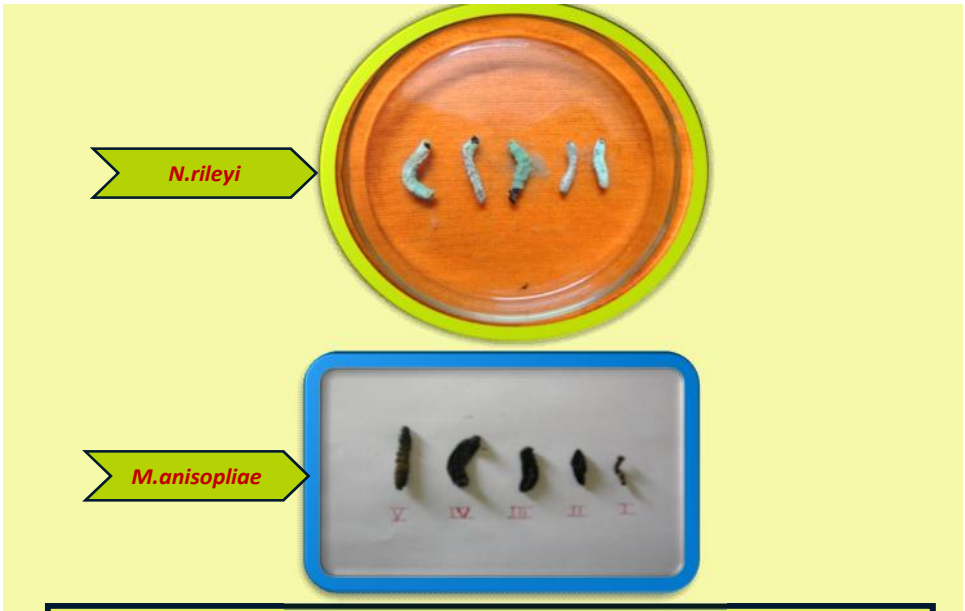
The final stage WP formulation A ($N_{30}S_{1/1}$) and formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) (Plate-IX-I and X) having outstanding performance for the fungal growth, development and viability were evaluated at various doses (Table 51 and Fig.27) ranged from 0.01 to 0.04% against II instar larvae of *S.litura* to decide the optimum dose to be used to suppress the pest in the field. The mortality was in the range of 27.50 to 65.0 per cent at 5 DAT. Formulation A ($N_{30}S_{1/1}$) 0.03% and 0.04% caused highest mortality of 65.0 per cent at 5 DAT. The concentrations 0.04% and 0.03% of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were on par to the concentration at 0.03% and 0.04% of formulation A ($N_{30}S_{1/1}$) for the effect.

The minimum (22.50%) mortality was noticed in treatment with formulation A and B (27.50%) 0.01% at 5 DAT. The treatment with formulation (*N.r.*alone*)* 0.02% recorded 27.50 per cent mortality. However, it was 55.00 and 52.50 per cent in treatment with formulation A and B 0.02%. The pattern of the lethal effect at 7 DAT was more or less same. The kill in formulation A ranged from 42.50 to 82.50 per cent while it was 42.50 to 80.0 per cent in formulation B. The formulation A 0.04% showed highest (82.50%) mortality which was on par to formulation A 0.03% (80.0%), B 0.04% (80.0%), A 0.03% (77.50%) and A 0.025% (77.50%).

The effectiveness at 10 DAT was again highest (92.50%) in formulation A 0.04% and 0.03% and B 0.04%. However, it was at par with formulation B 0.03% (90.0%), A 0.025% (87.50%) and B 0.025% (85.0%). These were followed by formulation A 0.02% (77.50%) and B 0.02% (75.0%). The concentrations at 0.01% and 0.015% of both formulations showed at par 52.50 and 60.0 to 65.0% mortality of the pest.



I :Conidia and Mycelium of *N.rileyi* and *M.anisopliae*



II :Infected cadaver of *S.litura* by *N.rileyi* and *M.anisopliae*

PLATE – VII

Conidia, Mycelium and infected cadaver

Table 51. Bioefficacy of final stage WP formulations of *N.rileyi* against II instar larvae of *S.litura*

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortalitly (%)		
				5 DAT	7 DAT	10 DAT
T1	*N.rileyi* 5%WP- A	0.01	2.0	27.50 (31.63)*	42.50 (40.69)	52.50 (46.43)
T2	*N.rileyi* 5%WP- A	0.015	3.0	37.50 (37.76)	57.50 (49.31)	65.00 (53.73)
T3	*N.rileyi* 5%WP- A	0.02	4.0	55.00 (47.87)	72.50 (58.37)	77.50 (61.68)
T4	*N.rileyi* 5%WP- A	0.025	5.0	57.50 (49.31)	77.50 (61.68)	87.50 (69.30)
T5	*N.rileyi* 5%WP- A	0.03	6.0	65.00 (53.73)	80.00 (63.44)	92.50 (74.11)
T6	*N.rileyi* 5%WP- A	0.04	8.0	65.00 (53.73)	82.50 (65.27)	92.50 (74.11)
T7	*N.rileyi* 5%WP- B	0.01	2.0	22.50 (28.32)	42.50 (40.69)	52.50 (46.43)
T8	*N.rileyi* 5%WP- B	0.015	3.0	35.00 (36.27)	55.00 (47.87)	60.00 (50.77)
T9	*N.rileyi* 5%WP- B	0.02	4.0	52.50 (46.43)	70.00 (56.79)	75.00 (60.00)
T10	*N.rileyi* 5%WP- B	0.025	5.0	55.00 (47.87)	75.00 (60.00)	85.00 (67.21)
T11	*N.rileyi* 5%WP- B	0.03	6.0	60.00 (50.77)	77.50 (61.68)	90.00 (71.56)
T12	*N.rileyi* 5%WP- B	0.04	8.0	62.50 (52.24)	80.00 (63.44)	92.50 (74.11)
T13	*N.rileyi* alone 5%WP	0.02	4.0	27.50 (31.63)	42.50 (40.69)	57.50 (49.31)
T14	Control (water spray)	-	-	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	S.E ±			**1.49**	**1.39**	**2.59**
	C.D.(P=0.05)			**4.26**	**3.98**	**7.40**

*Figures in parentheses are arcsin values
N.r. = Nomuraea rileyi
A= ($N_{30}S_{1/1}$)
DAT = Days after treatment
BAI= Bioactive ingredient
B= ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)

M.anisopliae **:** The formulation A1($M_{30}S_{1/1}C_{1/2}$) and formulation B1($M_{30}S_{1/1}H_{1/1}$) (Plate-X-II and Plate-XI) evaluated at concentrations (Table 52 and Fig.28) of 0.01 to 0.04% against II instar larvae of *S.litura* to find out the suitable dose to be used in the field. The mortality among treatments was in the range of 22.50 to 65.0 per cent at 5 DAT.

Table 52. Bioefficacy of final stage WP formulations of *M.anisopliae* against II instar larvae of *S.litura*

Tr. No.	Treatment Formulation	BAI Conc.(%)	Dose g/l	Larval mortality (%)		
				5 DAT	7 DAT	10 DAT
T1	*M.anisopliae* 5%WP- A1	0.01	2.0	22.50 (28.32)*	32.50 (34.76)	50.00 (45.00)
T2	*M.anisopliae* 5%WP- A1	0.015	3.0	27.50 (31.63)	42.50 (40.69)	52.50 (46.43)
T3	*M.anisopliae* 5%WP- A1	0.02	4.0	45.00 (42.13)	60.00 (50.77)	67.50 (55.24)
T4	*M.anisopliae* 5%WP- A1	0.025	5.0	52.50 (46.43)	60.00 (50.77)	70.00 (56.79)
T5	*M.anisopliae* 5%WP- A1	0.03	6.0	57.50 (49.31)	72.50 (58.37)	77.50 (61.68)
T6	*M.anisopliae* 5%WP- A1	0.04	8.0	60.00 (50.77)	72.50 (58.37)	82.50 (65.27)
T7	*M.anisopliae* 5%WP- B1	0.01	2.0	22.50 (28.32)	42.50 (40.69)	52.50 (46.43)
T8	*M.anisopliae* 5%WP- B1	0.015	3.0	27.50 (31.63)	42.50 (40.69)	52.50 (46.43)
T9	*M.anisopliae* 5%WP- B1	0.02	4.0	42.50 (40.69)	60.00 (50.77)	65.00 (53.73)
T10	*M.anisopliae* 5%WP- B1	0.025	5.0	52.50 (46.43)	65.00 (53.73)	72.50 (58.37)
T11	*M.anisopliae* 5%WP- B1	0.03	6.0	62.50 (52.24)	70.00 (56.79)	82.50 (65.27)
T12	*M.anisopliae* 5%WP- B1	0.04	8.0	65.00 (53.73)	75.00 (60.00)	85.00 (67.21)
T13	*M.anisopliae* alone 5%WP	0.02	4.0	22.50 (28.32)	37.50 (37.76)	46.67 (43.11)
T14	Control (water spray)	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E ±			**1.73**	**2.12**	**2.56**
	C.D.(P=0.05)			**4.96**	**6.06**	**7.32**

*Figures in parentheses are arcsin values. DAT = Days after treatment
M.a.= Metarhizium anisopliae BAI = Bioactive ingredient
A1=($M_{30}S_{1/1}C_{1/2}$) B1=($M_{30}S_{1/1}H_{1/1}$)

Formulation B1 0.03% and 0.04% registered highest (62.50 and 65.0%) mortality at 5 DAT. The concentrations at 0.04% and 0.03% of formulation B1 were on par to the concentration 0.04% and 0.03% of formulation A1 recording 60.0 and 57.50 per cent mortality,respectively. The minimum (22.50%) mortality was observed in treatment with formulation A1 and B1 0.01%. The treatment with control (*M.a.*alone) 0.02% recorded 22.50 per cent kill, when it was 45.0 and 42.50 per cent in treatment with 0.02% each of formulation A1 and B1. The trend of mortality at 7 DAT was more or less same. The mortality in formulation A1 ranged 32.50 to 72.50 per cent; while, it was 42.50 to 75.0 per cent in formulation B1.

The mortality at 10 DAT was higher in the formulation B1 registered highest (85.0%) mortality of II instar larvae. However, it was at par with

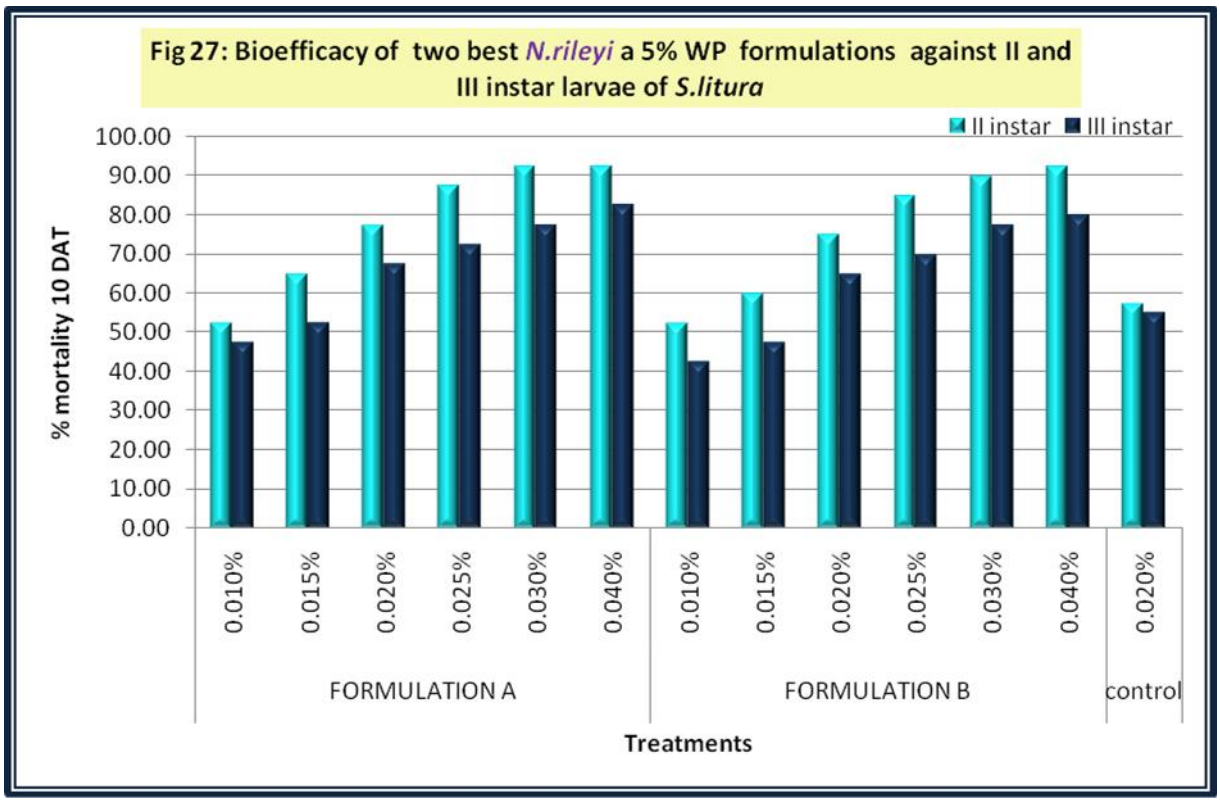


Fig 27: Bioefficacy of two best *N.rileyi* a 5% WP formulations against II and III instar larvae of *S.litura*

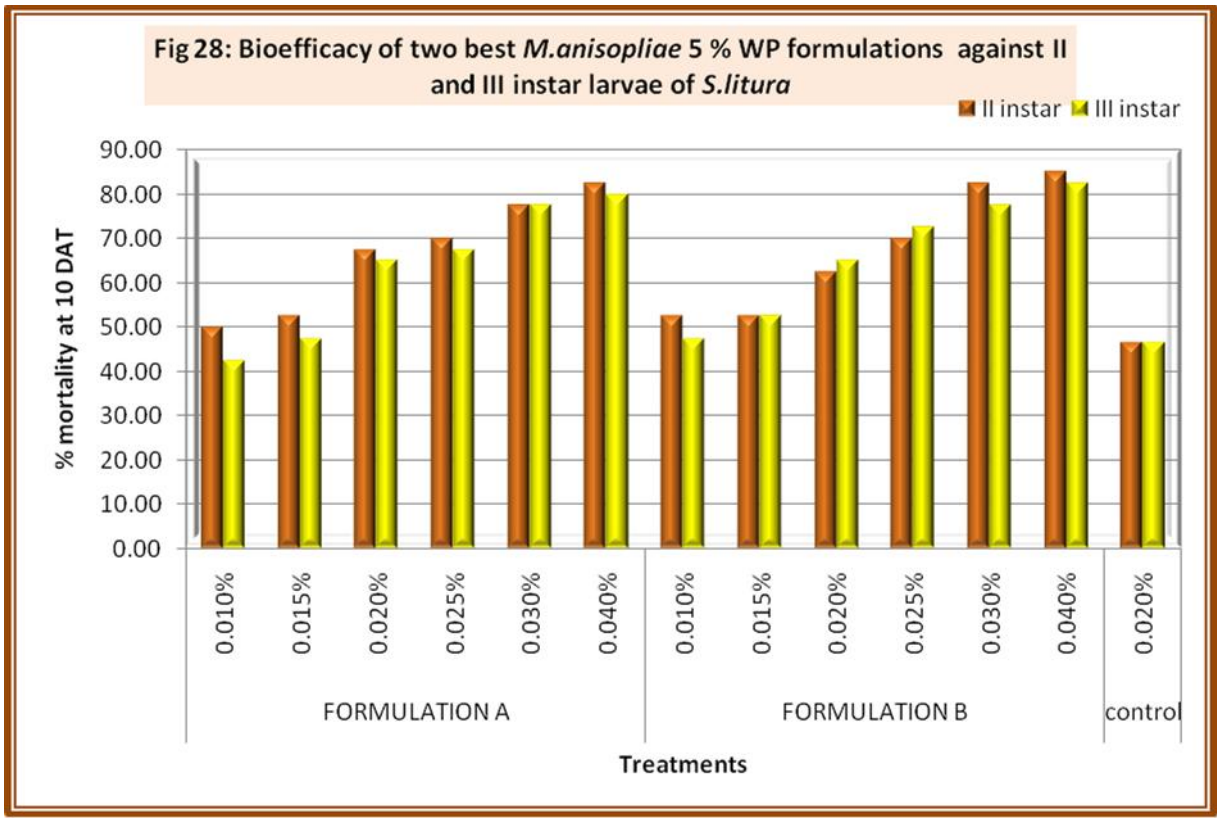


Fig 28: Bioefficacy of two best *M.anisopliae* 5 % WP formulations against II and III instar larvae of *S.litura*

formulation B1 0.03% (82.50%), A1 0.04% (82.50%) and A1 0.03% (77.50%). The next promising treatments were formulation B1 and A1 both 0.025% (72.50 and 70.0%). The concentration 0.01% of both formulations showed up to 50.0 per cent mortality.

4.3.2 III instar larvae

***N.rileyi* :**

The per cent mortality (Table 53) in the formulation treatments was 15.0 to 55.0 and 42.50 to 82.50 per cent at 5 and 10 DAT, respectively.

Table 53. Bioefficacy of final stage WP formulations of *N. rileyi* against III instar larvae of *S.litura*

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortalitly (%)		
				5 DAT	7 DAT	10 DAT
T1	*N.rileyi* 5%WP- A	0.01	2	15.00 (22.79)*	37.50 (37.76)	47.50 (43.57)
T2	*N.rileyi* 5%WP- A	0.015	3	25.00 (30.00)	42.50 (40.69)	52.50 (46.43)
T3	*N.rileyi* 5%WP- A	0.02	4	45.00 (42.13)	62.50 (52.24)	67.50 (55.24)
T4	*N.rileyi* 5%WP- A	0.025	5	47.50 (43.57)	65.00 (53.73)	72.50 (58.37)
T5	*N.rileyi* 5%WP- A	0.03	6	52.50 (46.43)	72.50 (58.37)	77.50 (61.68)
T6	*N.rileyi* 5%WP- A	0.04	8	55.00 (47.87)	75.00 (60.00)	82.50 (65.27)
T7	*N.rileyi* 5%WP- B	0.01	2	17.50 (24.73)	32.50 (34.76)	42.50 (40.69)
T8	*N.rileyi* 5%WP- B	0.015	3	30.00 (33.21)	42.50 (40.69)	47.50 (43.57)
T9	*N.rileyi* 5%WP- B	0.02	4	47.50 (43.57)	57.50 (49.31)	65.00 (53.73)
T10	*N.rileyi* 5%WP- B	0.025	5	47.50 (43.57)	60.00 (50.77)	70.00 (58.79)
T11	*N.rileyi* 5%WP- B	0.03	6	52.50 (46.43)	67.50 (55.24)	77.50 (61.68)
T12	*N.rileyi* 5%WP- B	0.04	8	55.00 (47.87)	72.50 (58.37)	80.00 (63.44)
T13	*N.rileyi* alone 5%WP	0.02	4	16.67 (24.12)	36.67 (37.29)	55.00 (47.87)
T14	Control (water spray)	-	-	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	S.E ±			**1.57**	**1.86**	**2.71**
	C.D.(P=0.05)			**4.49**	**5.32**	**7.74**

Figures in parentheses are arc sin values
N.r. = Nomuraea rileyi
A= ($N_{30}S_{1/1}$)
DAT = Days after treatment
BAI= Bioactive ingredient
B= ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)

At 10 DAT, the mortality was highest (82.50%) in the treatment of formulation A 0.04%, which was on par to formulation B 0.04% (80.0%), A 0.03% (77.50%), B 0.03% (77.50%) and formulation A 0.025% (72.50%) and B 0.025% (70.0%). The next best treatments were formulation A 0.02% (65.0%), A 0.015% (52.50%) and B 0.015% (47.50%).

***M.anisopliae* :**

The per cent mortality (Table 54) in the treatments was 15.0 to 55.0, 32.50 to 70.0 and 42.50 to 82.50 per cent at 5, 7 and 10 DAT, respectively. It was highest (82.50%) in formulation B1($M_{30}S_{1/1}H_{1/1}$) 0.04%; which was on par to formulation A1($M_{30}S_{1/1}C_{1/2}$) 0.04% (80.0%), B1 0.03% (77.50%), and A1 0.03% (77.50%). The next best treatments were formulation B1 0.025% (70.0%), A1 0.025% (67.50%), B1 and A1 0.02% (65.0%).

It was established from the study on bioefficacy of final stage WP formulation of *N.rileyi* and *M.anisopliae* against II and III instar larvae of *S.litura* (Table 47 to 50) that there was increase in mortality with increase in dose and decrease in duration for lethal effect. The cumulative performance of formulations at test concentrations proved that the dose of 8, 6 and 5g/l of formulation both fungi were equality (at par) effective. The dose of 4g/l of the formulations was found next best. Hence, the dosages of 4, 5 and 6g/l of water were selected for evaluation of the bioefficacy of entomopathogenic fungi formulations under field conditions.

The results pertaining to bioefficacy of *N.rileyi* and *M.anisopliae* has been discussed under 4.1.6 and 4.1.7.

Table 54. Bioefficacy of final stage WP formulations of *M.anisopliae* against III instar larvae of *S.litura*

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortalitly (%)		
				5 DAT	7 DAT	10 DAT
T1	*M.anisopliae* 5%WP- A1	0.01	2.0	15.00 (22.79)*	32.50 (34.76)	42.50 (40.69)
T2	*M.anisopliae* 5%WP- A1	0.015	3.0	25.00 (30.00)	40.00 (39.23)	47.50 (43.57)
T3	*M.anisopliae* 5%WP- A1	0.02	4.0	40.00 (39.23)	57.50 (49.31)	65.00 (53.73)
T4	*M.anisopliae* 5%WP- A1	0.025	5.0	47.50 (43.57)	60.00 (50.77)	67.50 (55.24)
T5	*M.anisopliae* 5%WP- A1	0.03	6.0	52.50 (46.43)	65.00 (53.73)	77.50 (61.68)
T6	*M.anisopliae* 5%WP- A1	0.04	8.0	55.00 (47.87)	67.50 (55.24)	80.00 (63.44)
T7	*M.anisopliae* 5%WP- B1	0.01	2.0	17.50 (24.73)	37.50 (37.76)	47.50 (43.57)
T8	*M.anisopliae* 5%WP- B1	0.015	3.0	25.00 (30.00)	42.50 (40.69)	52.50 (46.43)
T9	*M.anisopliae* 5%WP- B1	0.02	4.0	40.00 (39.23)	57.50 (49.31)	65.00 (53.73)
T10	*M.anisopliae* 5%WP- B1	0.025	5.0	47.50 (43.57)	62.50 (52.24)	70.00 (56.79)
T11	*M.anisopliae* 5%WP- B1	0.03	6.0	52.50 (46.43)	67.50 (55.24)	77.50 (61.68)
T12	*M.anisopliae* 5%WP- B1	0.04	8.0	55.00 (47.87)	70.00 (56.79)	82.50 (65.27)
T13	*M.anisopliae* alone 5%WP	0.02	4.0	16.67 (24.12)	33.33 (35.24)	46.67 (43.11)
T14	Control (water spray)	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E±			**2.55**	**1.64**	**1.67**
	C.D.(P=0.05)			**7.30**	**4.69**	**4.77**

*Figures in parentheses are arcsin values. DAT = Days after treatment
M.a.= Metarhizium anisopliae BAI = Bioactive ingredient
A1=($M_{30}S_{1/1}C_{1/2}$) B1=($M_{30}S_{1/1}H_{1/1}$)

4.4 Shelf life assessment of 5% WP formulations of *N.rileyi* and *M.anisopliae*

(Period: April to Dec.2011 and Jan.,Feb.2012 Av.Temp.(^{0}C) :Max.-33 ±1,Min.-17 ±1 Av.Humidity (%) :Morn.-91, Even.-38)

***N.rileyi* :**

Data on effect of storage of the newly developed *N.rileyi* 5% WP formulation A($N_{30}S_{1/1}$) and B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *N.rileyi* alone in kaolinite (control) on viability of the fungus are presented in Table 55. At 10 DAI, surface coverage varied from 100 to 36.67, 100 to 33.33 and 100 to 0.0% in formulation A, B and control, respectively from 0 to 300 days storage samples. The formulations A and B

stored upto 150 days showed cent per cent surface coverage against the 71.70 per cent in control. Significantly higher biomass (10.40 to 11.10g/40ml medium) was produced by the inoculums in formulation A and B (9.67 to 10.67g) stored upto 180 days as compared to that (1.90g) in formulation A and B (1.77g) stored for 300 days. The biomass produced in control was 5.47g in fresh sample against no biomass production after 270 and 300 days storage (Plate-XII).

Cfu count varied from 24.33 to $1.33x10^8$, 23.67 to $1.33x10^8$ and 23.33 to $0x10^8$ cfu/ml in formulation A, B and control, respectively from 0 to 300 days storage (Fig.29). At 0 day age, formulation A recorded highest ($24.33x10^8$ ml) cfu count. It was at par with formulation stored at 30 days. In case of formulation B samples stored at 0 days recorded maximum ($23.67x10^8$cfu/ml) viability. However, it was at par with the viability (23.10^8cfu/ml) at 30 days of storage. Formulation A and B maintained their superiority over the control viability of the inoculums, while formulation without adjuvants recorded decline in viability. The reduction in cfu was rapid from 270 to 300 days. There was complete loss of viability of the inoculums in control at 270 days of storage.

Considering surface coverage (%), biomass produced and viability (cfu/g) the *N.rileyi* 5%WP formulation A, B and control could be stored upto 10, 10 and 7 months, respectively for maximum cfu count of $1x10^8$/g for WP formulations as per norms of Central Insecticide Board and Registration Committee, Faridabad, Haryana.

The present findings are in conformity with those reported by Chaudhari (2010) that *N.rileyi* formulated as wettable powder was better in shelf life with $5.50x10^6$cfu/ml after 180 days of storage at 27^0C. The formulation of *N.rileyi* inoculated after 15 months resulted in growth and development of the mycoagent. Nahar *et al.* (2004) recorded greater than 80% conidial germination of *N.rileyi* after 36 hrs of storage in sunflower oil, diesel and sunflower oil mixture 7:3 and tween-80. Ramegowda (2005) reported that the rice flour, talc and sorghum flour was the best carrier for *N.rileyi*. The vability of conidia after one year of storage was 22.21 per cent in

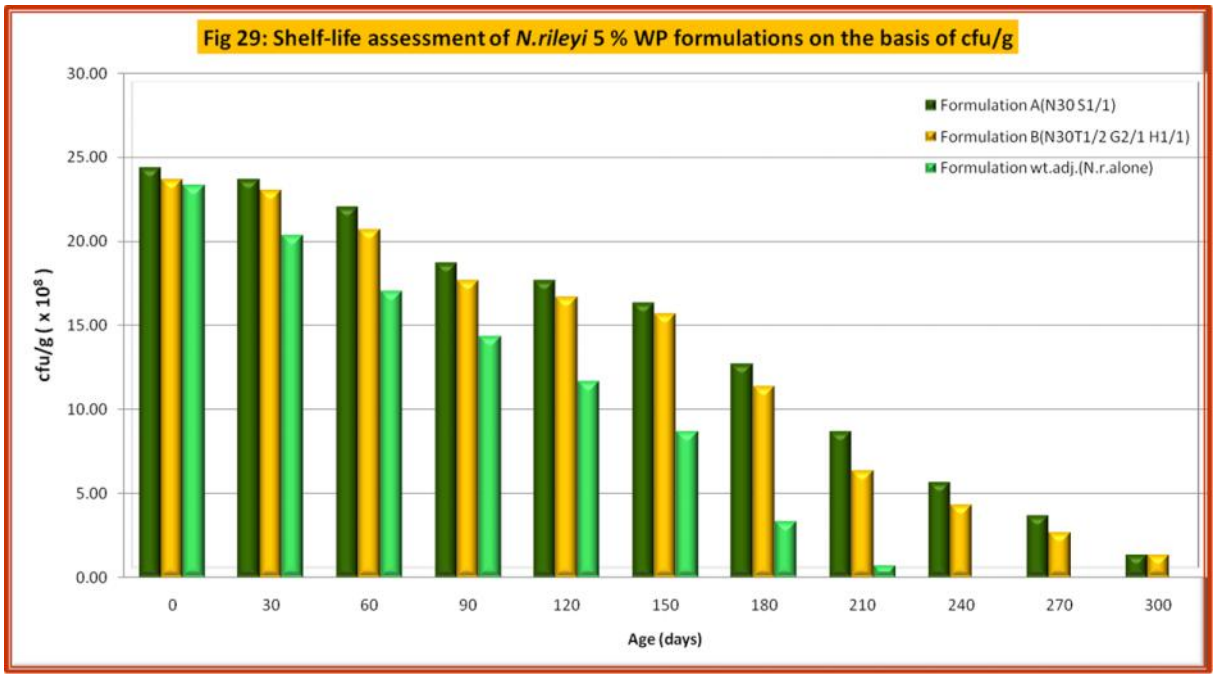


Fig 29: Shelf-life assessment of N.rileyi 5 % WP formulations on the basis of cfu/g
30.00
25.00
20.00
15.00
10.00
5.00
0.00
cfu/g (x 10⁸)
0
30
60
90
120
150
180
210
240
270
300
Age (days)
Formulation A(N30 S1/1)
Formulation B(N30T1/2 G2/1 H1/1)
Formulation wt.adj.(N.r.alone)

refrigerated condition while it was 15.64 per cnet at ambient condition. But the literature on shelf-life of wettable powder and sprayable formulation was not available.

Silva *et al*. (1993) found that the dead larvae of velvet bean caterpillar *Anticarsia gemmatalis* covered with sopres of *N.rileyi* were kept their viability and pathogenicity for upto 6 years of storage. The highest conidial viability after 45 days was recorded by Hidalgo *et al.*(1998) with the conidia in fat formulation. It was (84.70% at 25^{0}C and 91.30% at 4^{0}C). Faria *et al*. (1999) experienced decrease in germination of conidia in 16 to 84 months old of *N.rileyi*, *M.anisopliae* and *B.bassiana* culture stored in liquid nitrogen.

Table 55. Effect of storage of *N.rileyi* 5% WP formulations on growth, biomass and viability under ambient conditions

Tr. No.	Treatment Age in days	Surface coverage (%) at 10 DAI			Biomass g/40ml medium			Cfu/g (x 10^8)		
		Form. A**	Form. B***	Form. Control (*N.r.* alone)	Form. A	Form. B	Form. Control (*N.r.* alone)	Form. A	Form. B	Form. Control (*N.r.* alone)
1	00	100.0 (90.00)*	100.0 (90.00)	100.0 (90.00)	11.10	10.67	5.47	24.33	23.67	23.33
2	30	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.90	10.57	5.43	23.67	23.00	21.67
3	60	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.90	10.37	5.27	22.00	20.67	20.33
4	90	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	10.70	10.17	5.27	18.67	17.67	17.00
5	120	100.0 (90.00)	100.0 (90.00)	90.0 (71.56)	10.60	10.10	5.07	17.67	16.67	14.33
6	150	100.0 (90.00)	100.0 (90.00)	71.70 (57.86)	10.40	9.68	3.43	16.33	15.67	11.67
7	180	85.00 (67.21)	88.30 (70.00)	55.00 (47.87)	10.40	9.67	2.23	12.67	11.33	8.67
8	210	70.00 (56.79)	70.00 (56.79)	45.00 (42.13)	7.20	7.10	1.33	8.67	6.67	3.67
9	240	55.00 (47.87)	56.67 (48.79)	30.00 (33.21)	5.90	5.77	0.30	5.67	4.33	0.67
10	270	43.33 (41.15)	50.00 (45.00)	15.00 (22.79)	5.20	5.10	0.0	3.67	2.33	0.00
11	300	36.67 (37.29)	33.33 (35.24(	0.00 (0.0)	1.90	1.77	0.0	1.33	1.33	0.00
	S.E ±	**1.78**	**1.14**	**1.41**	**0.14**	**0.08**	**0.07**	**0.33**	**0.33**	**0.60**
	C.D.(P=0. 05)	**5.34**	**3.41**	**4.23**	**0.42**	**0.24**	**0.21**	**0.99**	**0.99**	**1.79**

*Figures in parentheses are arcsin values DAI= Days after inoculation

**A= (N_{30} $S_{1/1}$) ***B = ($T_{1/2}$ $G_{2/1}$ $H_{1/1}$)

Greater conidial viability and better survivability of talc based formulation of *B.bassiana* was obtained in upto 11 months where average temperature ranged from 5 to 25^{0}C. Alves *et al.*(2001) reported the better

viability of *M.anisopliae* in medium term storage of 40 weeks at 10^0C and 27^0C for oil based formulation. Peanut oil maintained viability of conidia greater than 90%.

***M.anisopliae*:** Data on effect of storage on viability of the newly developed *M.anisopliae* 5% WP formulation A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$) (comprising adjuvants, fungus and kaolinite) and *M.anisopliae* alone in kaolinite (control) on viability of the fungus are presented in Table 56. At 10 DAI, surface coverage by the fungus varied from 100 to 45.0, 100 to 46.67 and 100 to 0.0% in formulation A1, B1 and control, respectively, when stored for 0 to 300 days. The samples stored upto 150 days showed cent per cent surface coverage in formulation A1 and B1 except control. Significantly higher biomass (9.10 to10.03 g/40ml medium) was produced in samples of formulation A1 and B1 (8.77 to 10.07g) stored upto 210 days as compared to that (3.40g) in formulation A1 and B1 (3.70g) stored for 300 days. The biomass in control was 6.27g in fresh sample against no biomass in sample stored for 300 days
(Plate-XII).

The viability varied from 31.33 to $5.33x10^8$, 30.67 to $6.33x10^8$ and 30.67 to $0.0x10^8$ cfu/ml in formulation A1, B1 and control, respectively, from 0 to 300 days storage (Fig.30). The formulation A1 registered substantially highest ($31.33x10^8$/ml) cfu count. Formulation A1 and B1 maintained its superiority for the viability (5.67 to $6.67x10^8$ cfu/ml) upto 300 days, while it declined substantially (6.33 to $0x10^7$cfu/ml) in control formulation after 180 days of storage.

Considering surface coverage (%), biomass produced and viability (cfu/g) the *M.anisopliae* 5%WP formulation A1, B1 and control could be stored upto 10, 10 and 6 months, respectively for the minimum cfu count of $1x10^8$/g for WP formulations as per norms of Central Insecticide Board and Registration Committee, Faridabad, Haryana.

The present findings are in corroboration with those reported by Daust *et al*. (1983) the conidia stored in 20% dust retained high viabilities over 12

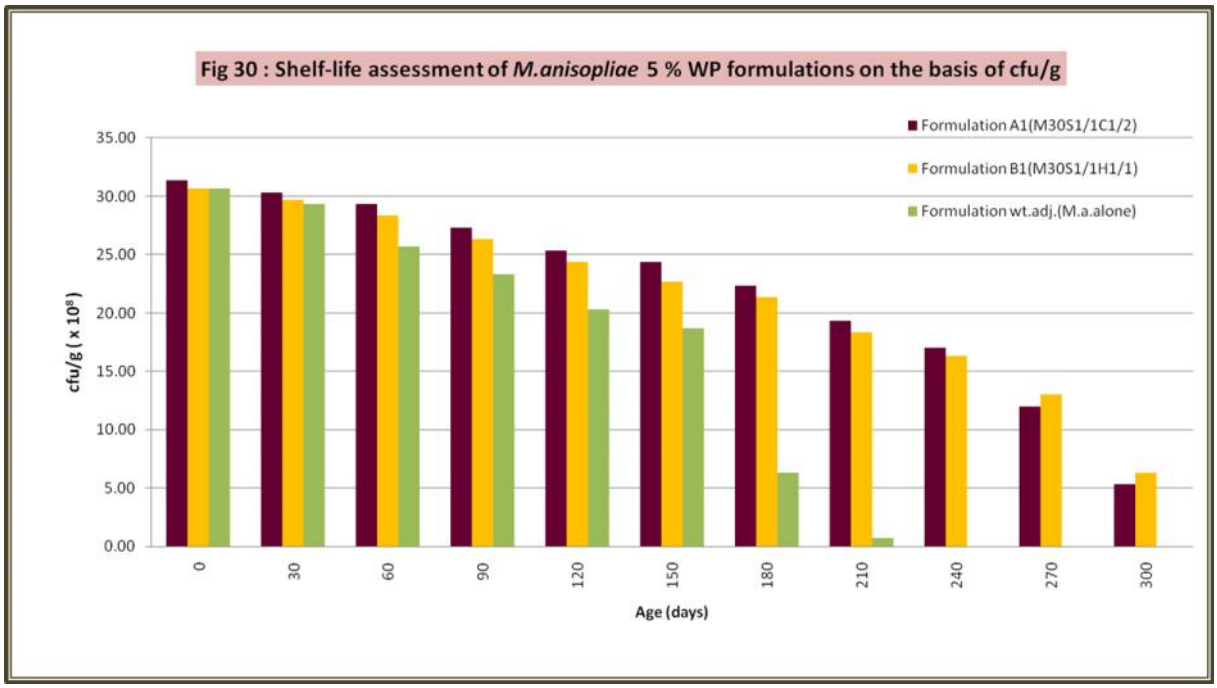
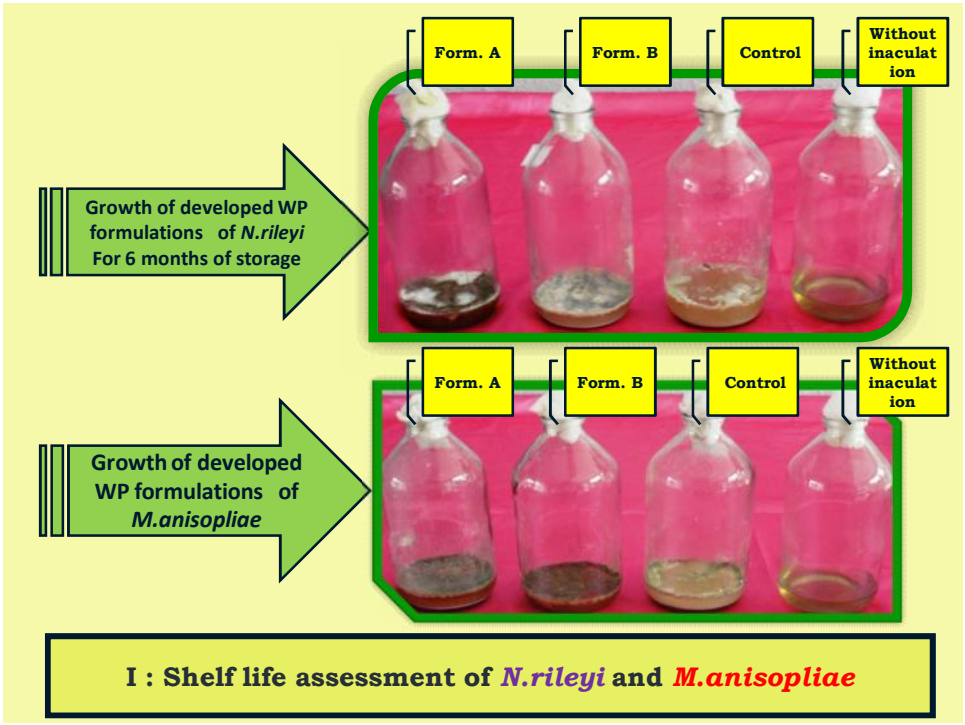


Fig 30 : Shelf-life assessment of M.anisopliae 5 % WP formulations on the basis of cfu/g
Formulation A1(M30S1/1C1/2)
Formulation B1(M30S1/1H1/1)
Formulation wt.adj.(M.a.alone)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
cfu/g (x 10⁸)
0
30
60
90
120
150
180
210
240
270
300
Age (days)



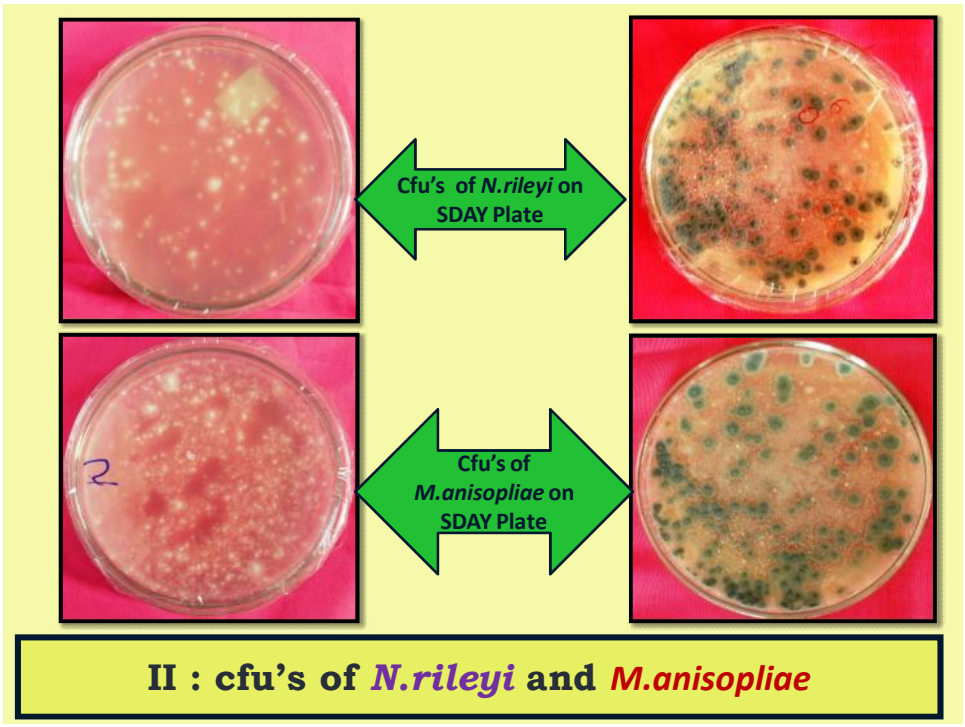


PLATE – XII

Growth and cfu's of *N.rileyi* and *M.anisopliae*

months. Moore *et al*. (1993) reported that dried conidia stored as powder retained germination level of 95% at 10-14^{0}C but the germination was upto 27% at 28 to 32^{0}C. Batt (2003) concluded that the fungal conidia of *Metarhizium anisopliae* formulated in invert emulsion (water in oil formulation) remained viable for 30.8 months with 50% reduction in conidial viability after 4 to 6 months at 20 $\pm$ 1^{0}C.

Rachappa (2007) observed that storing the conidia of *M.anisopliae* under refrigeration provided longer life compared to ambient temperature. After 180 days of the storage, the cfu reduced from 250 to 176.50x10^6/g. The cfu count was least affected by kaolinite. The wettable powder formulation, attapulgite and kaolinite retained viability of conidia (33.5 and 31.9%), respectively, after one year. It was followed by sorghum flour (27.9%) and talc (26.90%).

Table 56. Effect of storage of *M.anisopliae* 5% WP formulations on growth, biomass and viability under ambient conditions

Tr. No.	Treatment Age in days	Surface coverage (%) at 10 DAI			Biomass g/40ml medium			Cfu/g (x 10^8)		
		Form. A1**	Form. B1***	Form. Control (*M.a.* alone)	Form. A1**	Form. B1***	Form. Control (*M.a.* alone)	Form. A1**	Form. B1***	Form. Control (*M.a.* alone)
1	00	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	10.03	10.07	6.27	31.33	30.67	30.67
2	30	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.87	9.97	6.20	30.33	29.67	29.33
3	60	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.67	9.77	6.00	29.33	28..33	25.67
4	90	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	9.70	9.80	5.90	27.33	26.33	23.33
5	120	100.00 (90.00)	100.00 (90.00)	88.33 (70.00)	9.63	9.73	5.93	25.33	24.33	20.33
6	150	100.00 (90.00)	100.00 (90.00)	86.67 (68.61)	9.40	9.50	5.77	24.33	22.67	18.67
7	180	95.00 (73.08)	100.00 (90.00)	55.00 (47.87)	9.20	9.30	2.97	22.33	21.33	6.33
8	210	91.67 (73.26)	96.67 (79.53)	28.33 (32.14)	9.10	8.77	2.13	19.33	18.33	0.67
9	240	73.33 (58.89)	85.00 (67.21)	0.0 (0.00)	6.83	6.97	0.0	17.00	16.33	0.00
10	270	55.00 (47.87)	60.00 (50.77)	0.00 (0.00)	4.60	5.20	0.00	12.00	13.00	0.00
11	300	45.00 (42.13)	46.67 (43.11)	0.00 (0.00)	3.40	3.70	0.00	5.67	6.67	0.00
	S.E $\pm$	**2.94**	**1.64**	**1.20**	**0.15**	**0.17**	**0.07**	**0.26**	**0.26**	**0.33**
	C.D.(P= 0.05)	**8.83**	**4.91**	**3.59**	**0.45**	**0.50**	**0.22**	**0.78**	**0.76**	**0.99**

Figures in parentheses are arcsin values.
DAI = Days after Inoculation *M.a. = Metarhizium anisopliae*
A1= ($M_{30}S_{1/1}C_{1/2}$) * B1= ($M_{30}S_{1/1}H_{1/1}$)

4.5 Field efficacy of *N.rileyi* and *M.anisopliae* 5% WP formulations against *S.litura* on soybean

(Period: June to Oct.2011 Av.Temp.(^{0}C) :Max.-30 ±1, Min.-19 ±1 Av.Humidity (%) :Morn.-88, Even.-58)

4.5.1 Larval population

***N.rileyi* :**

Observations of number of larvae per meter row (larvae/m row) were recorded before spraying and at 3, 7 and 14 DAS and presented in Table 57. The precount population of *S.litura* per meter row was non significant. After first spray at 7 DAS, minimum 6.33 larvae/m row were recorded in formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}\,G_{2/1}H_{1/1}$) 0.03% among the treatments. However, it was at par with formulation A 0.025% (6.60 larvae/m row) and B 0.025% (6.67 larvae/m row).

At 14 DAS, 4.33 larvae/m row were recorded in formulation A and B 0.03% and they maintained their superiority in reducing the larval count among all the treatments. However, it was at par with the dose 0.025% of formulation A (4.67 larvae/m row) and B (4.73 larvae/m row).

At 7 days after second spray, the incidence was much more similar to that of 7 DAT of first spray. The population was in the range of 1.67 to 4.27 larvae/m row at 14 days after second spray as against 10.33 larvae/m row in control. Formulation A 0.025% and 0.03%, B 0.03% were highly promising (1.67to1.73 larvae/m row) and at par treatments to the most effective chemical treatment with quinolphos 0.05% (1.53 larvae/m row). The next promising and at par treatments to formulation A 0.025% and 0.03% was formulation B 0.03% (1.67 larvae/m row) when the *S.litura* population in control was 10.33 larvae/m row (Plate-XIII and Plate-XIV).

These results are in conformity with those reported by Kulkarni and Lingappa (2002). The highest reduction of *S.litura* larvae in soybean was observed in *N.rileyi* 1.2x10^{12}conidia/litre at 14 DAS. The soybean grain yield was not varying between NPV and *N.rileyi* treatment. Lingappa *et al.* (2002) recorded 28 to 62% reduction in larval population by *N.rileyi* of *S.litura* in at days after second application. Navi *et al.* (2006) recorded lowest larval

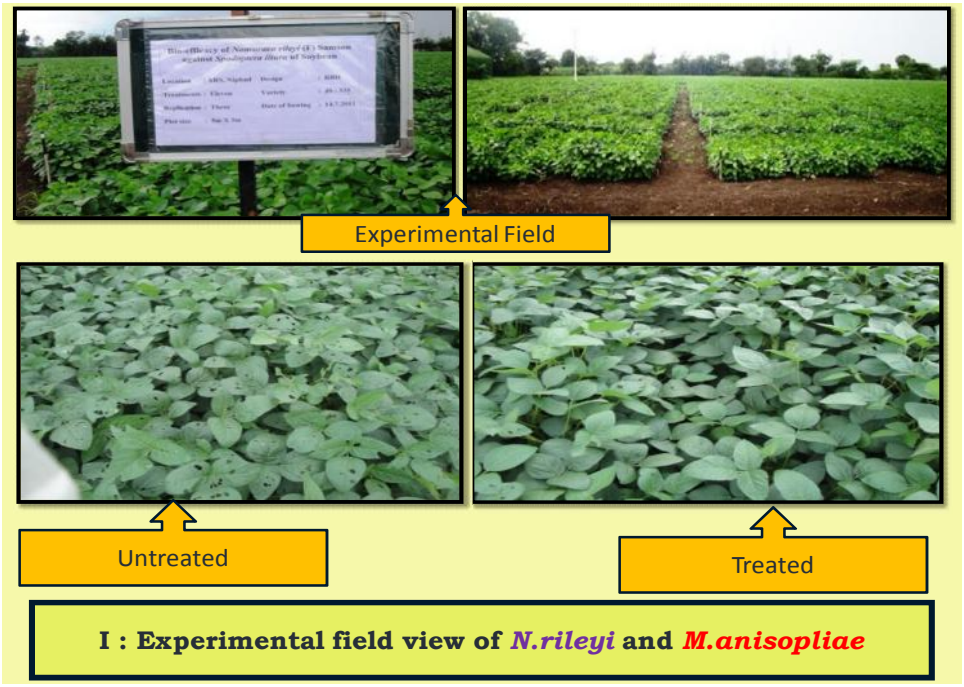
population of *S.litura*, and lowest damage in soybean by spraying of Quinolphos 25EC. Significantly higher yield was obtained in quinolphos where as *N.rileyi* was next. Chaudhari (2009) observed highest field pathogenicity by *N.rileyi* @ 4g and 4ml/liter for high larval reduction of *S.litura* low leaf damage and high yield in soybean.

Table 57. Effect of *N.rileyi* 5% WP formulations against *S.litura* in soybean

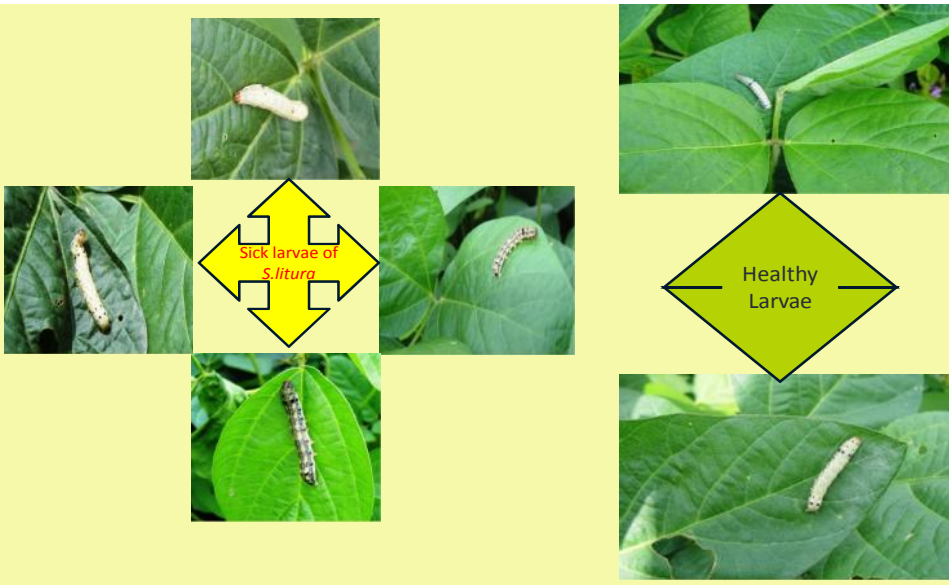
Tr. No	Treatments	Conc. (%)	Dose (g/l)	Pre-count (Larvae/ m row)	Number of larvae per meter row length					
					First Spray			Second Spray		
					3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
T1	*Nr* Form.A 5%WP	0.015	3.0	9.60 (3.18)*	9.20 (3.11)	8.13 (2.94)	6.47 (2.64)	5.13 (2.37)	3.93 (2.73)	3.07 (1.89)
T2	*Nr* Form.A 5%WP	0.02	4.0	9.80 (3.21)	9.47 (3.16)	7.27 (2.79)	5.33 (2.41)	4.20 (2.17)	3.53 (2.00)	2.60 (1.76)
T3	*Nr* Form.A 5%WP	0.025	5.0	9.60 (3.18)	9.20 (3.11)	6.60 (2.66)	4.67 (2.27)	3.67 (2.04)	2.80 (1.82)	1.73 (1.49)
T4	*Nr* Form.A 5%WP	0.03	6.0	9.87 (3.22)	9.33 (3.14)	6.33 (2.61)	4.33 (2.20)	3.33 (1.96)	2.33 (1.68)	1.73 (1.49)
T5	*Nr* Form.B 5%WP	0.015	3.0	9.80 (3.21)	9.40 (3.15)	7.93 (2.90)	5.93 (2.54)	5.00 (2.35)	4.07 (2.14)	3.13 (1.91)
T6	*Nr* Form.B 5%WP	0.02	4.0	9.93 (3.23)	9.53 (3.17)	7.73 (2.87)	5.73 (2.50)	4.80 (2.30)	4.33 (2.20)	2.93 (1.85)
T7	*Nr* Form.B 5%WP	0.025	5.0	10.13 (3.26)	9.93 (3.23)	6.67 (2.68)	4.73 (2.29)	3.80 (2.07)	3.07 (1.89)	2.13 (1.62)
T8	*Nr* Form.B 5%WP	0.03	6.0	10.07 (3.25)	9.67 (3.19)	6.33 (2.61)	4.33 (2.20)	3.33 (1.96)	2.40 (1.70)	1.67 (1.47)
T9	*Nr* Form. (*N.r.*alone) 5%WP	0.02	4.0	10.13 (3.26)	9.80 (3.21)	8.53 (3.00)	6.53 (2.65)	5.53 (2.46)	4.73 (2.29)	4.00 (2.12)
T10	S*l*NPV	-	1.0	10.20 (3.27)	9.93 (3.23)	9.27 (3.13)	7.27 (2.79)	6.27 (2.60)	5.27 (2.40)	4.27 (2.18)
T11	Quinol phos 25 EC	0.05	2.0	9.93 (3.23)	6.53 (2.65)	3.13 (1.91)	5.80 (2.51)	4.67 (2.27)	1.60 (1.45)	1.53 (1.42)
T12	Untreated Control	-	-	10.20 (3.27)	11.00 (3.39)	11.60 (3.48)	12.87 (3.66)	12.60 (3.62)	10.93 (3.38)	10.33 (3.29)
	S.E ±			**0.03**	**0.03**	**0.03**	**0.04**	**0.04**	**0.05**	**0.04**
	C.D. (P=0.05)			**NS**	**0.09**	**0.08**	**0.11**	**0.12**	**0.15**	**0.13**

*Figures in parentheses are arcsin values. DAS = Days after spray *N.r.*= *Nomuraea rileyi*

A = (N_{30} $S_{1/1}$) B = ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)



I : Experimental field view of *N.rileyi* and *M.anisopliae*



II : Sick and Healthy larvae of *S.litura*

PLATE – XIII

Experimental field view, sick and healthy larvae of *S.litura*

***M.anisopliae* :**

Observations of number of larvae per meter row were recorded before spraying and at 3,7 and 14 DAS and presented in Table 58. The precount population was non significant. After first spray at 7 DAS, minimum 6.80 larvae/m row were recorded in formulation B1($M_{30}S_{1/1}H_{1/1}$) 0.018% among the treatments with *M.anisopliae* formulations and *SlNPV*. However, it was at par with formulation A1($M_{30}S_{1/1}C_{1/2}$) 0.03% (6.93 larvae/m row), formulation B1 0.025% (7.07 larvae/m row) and A1 0.025% (7.13 larvae/m row). At 14 DAS, 5.67 larvae/m row were recorded in formulation A1. However, it was at par with quinolphos 0.05% (5.40 larvae/m row).

At 7 days after second spray, the incidence was much more similar to that of 7 DAT of first spray. At 14 DAS, the treatment with chemical insecticide quinolphos 0.05% recorded minimum (1.53 larvea/m row) population. The next promising treatments for the control of *S.litura* were formulation B1 and A1 0.03%, which recorded 3.0 larvae/m low length. However, it was at par with formulation B1 and A1 0.025% (3.27 and 3.47 larvae/m row), respectively, when the *S.litura* population in control was 9.80 larvea/m row (Plate-XIII and Plate-XIV).

These results are in support with those reported by Wikramatileki *et al*. (2000) that the considerable high mortality of cabbage semilooper larvae under field conditions indicates suitability of *M.anisopliae*. *M.anisopliae* at $8.8x10^{13}$ caused 65% mortality of cabbage semiloooper. Zhao *et al. (*2001) reported the control efficiency of *M.anisopliae* 77.48% against diamond back moth (*Plutella xylostella*) and 77.73% against cabbage worm (*Pieris rapae*). Bhagat *et al*. (2003) reported that *M.anisopliae* formulation applied at $5x10^{13}$conidia/ha along with chloropyriphos 20EC at 200g.a.i/ha was effective in controlling the grub population. Nahar *et al*. (2004) reported that the *M.anisopliae* was most efficient against *H.armigera* of pigeonpea. The higher yield was obtained in treatment with *M.anisopliae* (14.04 g/ha) as compared to control (7.31 g/ha). Sreeniwas *et al.*(2006) recorded lowest larval population of *H.armigera* by spraying of *N.rileyi* in chickpea. *N.rileyi* @ 1 g/lit recorded highest net return Rs. 279636 ha and B:C ratio (4.69)

followed by *M.anisopliae* @ 1 g/lit. Shakti Khajuria *et al.* (2007) reported that *B.bassiana* at 5 g/litre was effective against aphid of potato, followed by *M.anisopliae*.

Table 58 : Effect of *M.anisopliae* 5% WP formulations against *S.litura* in soybean

Tr. No.	Treatments	Conc. (%)	Dose (g/l)	Pre-count (Larvae/m row)	Number of larvae per meter row					
					First Spray			Second Spray		
					3 DAS	7 DAS	14 DAS	3 DAS	7 DAS	14 DAS
T1	*M.a* Form. A1 5% WP	0.015	3.0	9.73 (3.20)*	9.80 (3.21)	8.07 (2.93)	6.07 (2.56)	5.67 (2.48)	4.80 (2.30)	4.00 (2.12)
T2	*M.a* Form. A1 5% WP	0.02	4.0	9.93 (3.23)	9.80 (3.21)	7.60 (2.85)	6.20 (2.59)	5.27 (2.40)	4.40 (2.21)	3.93 (2.10)
T3	*M.a* Form. A1 5% WP	0.025	5.0	9.53 (3.17)	9.40 (3.15)	7.13 (2.76)	6.00 (2.55)	4.67 (2.27)	3.73 (2.06)	3.47 (1.99)
T4	*M.a* Form. A1 5% WP	0.03	6.0	9.53 (3.17)	9.40 (3.15)	6.93 (2.73)	5.87 (2.52)	4.07 (2.36)	3.40 (1.97)	3.00 (1.87)
T5	*M.a* Form. B1 5% WP	0.015	3.0	9.73 (3.20)	9.67 (3.19)	8.27 (2.96)	6.13 (2.57)	5.60 (2.47)	4.87 (2.32)	4.20 (2.17)
T6	*M.a* Form. B1 5% WP	0.02	4.0	9.93 (3.23)	9.67 (3.19)	7.53 (2.83)	6.00 (2.55)	5.33 (2.41)	4.60 (2.26)	3.67 (2.04)
T7	*M.a* Form. B1 5% WP	0.025	5.0	9.47 (3.16)	9.47 (3.16)	7.07 (2.75)	5.93 (2.54)	5.00 (2.35)	4.40 (2.21)	3.27 (1.94)
T8	*M.a* Form. B1 5% WP	0.03	6.0	9.60 (3.18)	9.33 (3.13)	6.80 (2.70)	5.67 (2.48)	4.73 (2.29)	4.00 (2.12)	3.00 (1.87)
T9	*M.a.* Form. (*M.a.*alone)	0.02	4.0	10.00 (3.24)	9.80 (3.21)	8.53 (3.00)	7.53 (2.83)	6.47 (2.64)	6.00 (2.55)	5.73 (2.50)
T10	SlNPV	-	1.0	10.00 (3.24)	10.00 (3.24)	8.87 (3.06)	6.20 (2.59)	5.13 (2.37)	4.73 (2.29)	4.00 (2.12)
T11	Quinol phos 25 EC	0.05	2.0	9.60 (3.18)	6.33 (2.61)	3.13 (1.91)	5.40 (2.43)	4.27 (2.18)	1.80 (1.52)	1.53 (1.42)
T12	Untreated Control	-	-	9.67 (3.19)	9.93 (3.23)	10.07 (3.25)	10.07 (3.25)	10.53 (3.32)	9.93 (3.23)	9.80 (3.21)
	S.E ±			**0.02**	**0.02**	**0.02**	**0.04**	**0.03**	**0.03**	**0.05**
	C.D. (P=0.05)			**NS**	**0.06**	**0.06**	**0.13**	**0.08**	**0.09**	**0.14**

*Figures in parentheses arc sin values. DAS = Days after spray *M.a.= Metarhizium anisopliae*
A1 = ($M_{30}S_{1/1}C_{1/2}$) B1 = ($M_{30}S_{1/1}H_{1/1}$)

I :Infected *S.litura* cadaver in field by *N.rileyi*

II :Infected *S.litura* cadaver in field by *M.anisopliae*

PLATE – XIV

Infected cadaver

4.5.2 Yield

***N.rileyi* :**

The yield (q/ha) of soybean from the different treatment plots is presented in Table 59 and depicted in Fig.31. The yield from all the treatments differ significantly and it was superior over control. The yield in the range of 24.80 to 28.66 q/ha was recorded where as in control 21.87 q/ha. The treatment with quinolphos 0.05% recorded maximum yield of 28.66q/ha. It was at par with formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) 0.03% (27.47q/ha) and 0.025% (27.17q/ha). Among the treatments of *N.rileyi* formulation B 0.03% recorded highest yield (27.47 q/ha). However, it was at par with formulation B 0.025% (27.17q/ha), A ($N_{30}S_{1/1}$) 0.03% (26.93 q/ha), 0.025% (26.67q/ha), B 0.02% (26.40q/ha) and A (26.13q/ha).

Table 59. Influence of *N.rileyi* 5% WP formulations on yield of soybean

Tr. No.	Treatments	BAI Conc. (%)	Dose (g/l)	Yield (q/ha)	Per cent increase in yield over control
T1	*Nr* Formulation A 5%WP	0.015	3.0	24.8	13.40
T2	*Nr* Formulation A 5%WP	0.02	4.0	26.13	19.48
T3	*Nr* Formulation A 5%WP	0.025	5.0	26.67	21.95
T4	*Nr* Formulation A 5%WP	0.03	6.0	26.93	23.14
T5	*Nr* Formulation B 5%WP	0.015	3.0	25.33	15.82
T6	*Nr* Formulation B 5%WP	0.02	4.0	26.40	20.71
T7	*Nr* Formulation B 5%WP	0.025	5.0	27.17	24.23
T8	*Nr* Formulation B 5%WP	0.03	6.0	27.47	25.61
T9	*Nr* Formulation (*N.r.*alone) 5%WP	0.02	4.0	23.73	8.50
T10	S*l*NPV	-	1.0	25.10	14.77
T11	Quinolphos 25 EC	0.05	2.0	28.66	31.05
T12	Untreated Control	-	-	21.87	-
	S.E ±			**0.51**	**-**
	C.D.(P=0.05)			**1.53**	**-**

N.r.= Nomurae rileyi A = (N_{30} $S_{1/1}$) B = ($N_{30}T_{1/2}$ $G_{2/1}$ $H_{1/1}$)

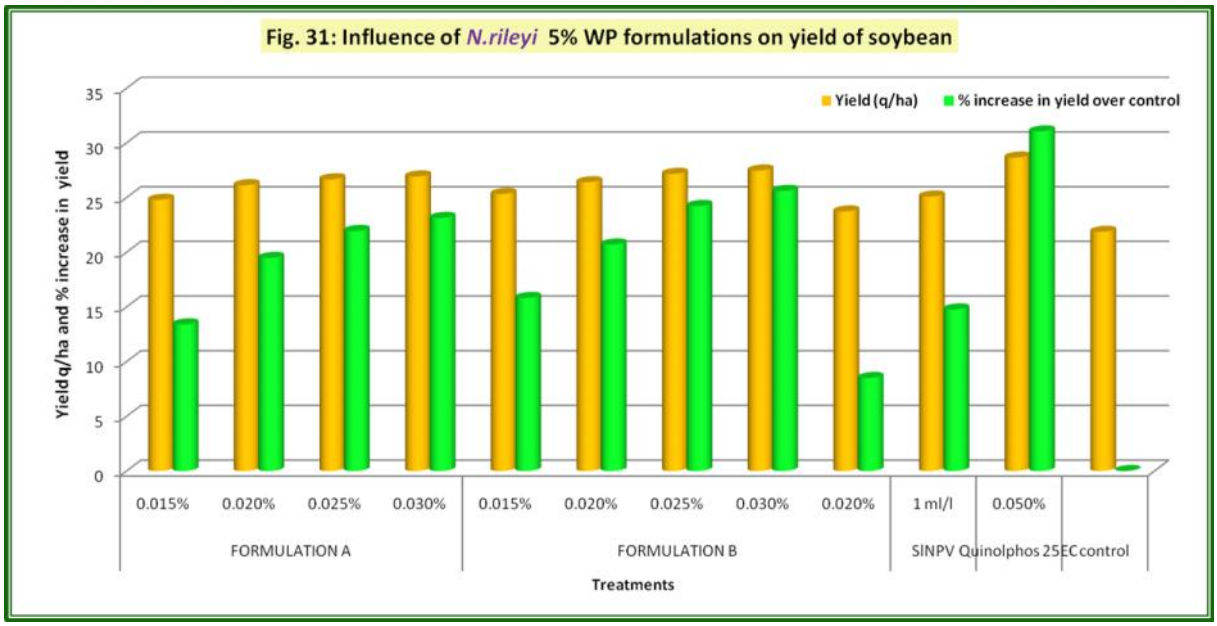


Fig. 31: Influence of N.rileyi 5% WP formulations on yield of soybean
Yield (q/ha)
% increase in yield over control
Yield q/ha and % increase in yield
35
30
25
20
15
10
5
0
0.015%
0.020%
0.025%
0.030%
0.015%
0.020%
0.025%
0.030%
0.020%
1 ml/l
0.050%
FORMULATION A
FORMULATION B
SINPV
Quinolphos 25EC
control
Treatments

***M.anisopliae* :**

The yield (q/ha) of soybean from the different treatment plots is presented in Table 60 and depicted in Fig.32. The yield from all the treatments differ significantly and it was superior over control. The yield in the range of 23.47 to 28.50 q/ha was recorded where as in control 21.25 q/ha. The treatment with quinolphos 0.05% recorded maximum yield of 28.10 q/ha. It was at par with formulation A1($M_{30}S_{1/1}C_{1/2}$) 0.03% (26.67q/ha) and B1($M_{30}S_{1/1}H_{1/1}$) 0.03% (26.50q/ha). Among the treatments of *M.anisopliae* formulation A1 0.03% recorded highest yield (26.67 q/ha). However, it was at par with formulation B1 0.03% (26.50 q/ha) and A1 0.025% (26.16 q/ha), B1 0.025% (25.47q/ha) and A1 0.025% (25.17q/ha).

Table 60. Influence of WP formulations of *M.anisopliae* on yield of soybean

Tr. No.	Treatments	BAI Conc. (%)	Dose (g/l)	Yield (q/ha)	Per cent increase in yield over control
T1	*M.a* Formulation A1 5% WP	0.015	3.0	23.68	11.43
T2	*M.a* Formulation A1 5% WP	0.02	4.0	25.17	18.44
T3	*M.a* Formulation A1 5% WP	0.025	5.0	26.16	23.11
T4	*M.a* Formulation A1 5% WP	0.03	6.0	26.67	25.51
T5	*M.a* Formulation B1 5% WP	0.015	3.0	23.47	10.45
T6	*M.a* Formulation B1 5% WP	0.02	4.0	24.64	15.95
T7	*M.a* Formulation B1 5% WP	0.025	5.0	25.47	19.86
T8	*M.a* Formulation B1 5% WP	0.03	6.0	26.50	24.70
T9	*M.a.* Formulation (*M.a.*alone) 5% WP	0.02	4.0	22.91	7.81
T10	S*l*NPV	-	1.0	25.37	19.38
T11	Quinolphos 25 EC	0.05	2.0	28.10	32.23
T12	Untreated Control	-	-	21.25	-
	S.E +			**0.54**	**-**
	C.D.(P=0.05)			**1.62**	**-**

M.a.= Metarhizium anisopliae A1 = ($M_{30}S_{1/1}C_{1/2}$) B1 = ($M_{30}S_{1/1}H_{1/1}$)

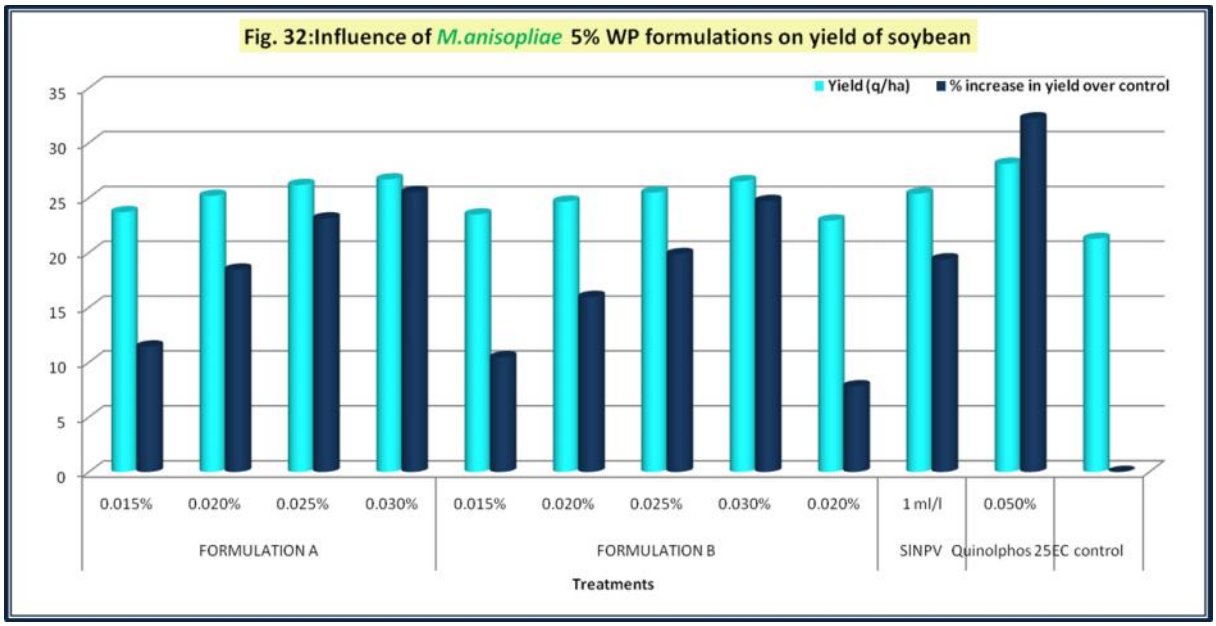


Fig. 32:Influence of M.anisopliae 5% WP formulations on yield of soybean
Yield (q/ha)
% increase in yield over control
35
30
25
20
15
10
5
0
0.015%
0.020%
0.025%
0.030%
0.015%
0.020%
0.025%
0.030%
0.020%
1 ml/l
0.050%
FORMULATION A
FORMULATION B
SINPV
Quinolphos 25EC
control
Treatments

4.6 Phytotoxicity test of the *N.rileyi* and *M.anisopliae* 5%WP formulations on soybean

The observations on phytotoxicity considering symptoms of scorching, leaf injury on tips, wilting, vein clearing etc. for both formulations A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) of *N. rileyi* and formulations A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$) of *M.anisopliae* with respective control which at 0.01 to 0.08 per cent concentrations recorded upto 15 days are presented in Table 61 and 62.

The results revealed that there were no phytotoxicity symptoms in all the treatments and at all the concentrations. Formulation A and A1 recorded no any visible phytotoxicity symptoms. The soybean plant was found completely healthy even upto 15 days after treatment. Formulaiton B and B1 also showed zero per cent scorching of leaves with no other phytotoxicity symptoms with the zero phytotoxicity score.

Table 61. Effect of *N.rileyi* 5%WP formulations on phytotoxicity on soybean

Tr. No.	Treatment *N.rileyi* 5% WP	Conc. (%)	Phytotoxicity score At day after treatment		
			3 DAT	7 DAT	15 DAT
1	Formulation A	0.01	0	0	0
2	Formulation A	0.015	0	0	0
3	Formulation A	0.02	0	0	0
4	Formulation A	0.025	0	0	0
5	Formulation A	0.03	0	0	0
6	Formulation A	0.04	0	0	0
7	Formulation A	0.08	0	0	0
8	Formulation B	0.01	0	0	0
9	Formulation B	0.015	0	0	0
10	Formulation B	0.02	0	0	0
11	Formulation B	0.025	0	0	0
12	Formulation B	0.03	0	0	0
13	Formulation B	0.04	0	0	0
14	Formulation B	0.08	0	0	0
15	Control (*N.r* alone)	0.01	0	0	0
16	Control (*N.r* alone)	0.015	0	0	0
17	Control (*N.r* alone)	0.02	0	0	0
18	Control (*N.r* alone)	0.025	0	0	0
19	Control (*N.r* alone)	0.03	0	0	0
20	Control (*N.r* alone)	0.04	0	0	0
21	Control (*N.r* alone)	0.08	0	0	0

0 = No Phytotoxicity
10 = 91-100 per cent scorching of leaves and dry of plants.
Formulation A = ($\mathbf{N_{30}S_{1/1}}$) Formulation B = ($\mathbf{N_{30}T_{1/2}G_{2/1}H_{1/1}}$)

The control formulation also exhibited similar trend of phytotoxicity symptoms. The present findings are in support with those reported by Mahajan (2003) that both liquid and WP formulaltion of *V.lecanii* at varied concentrations did not show any phytotoxic symptoms on leaves and flowers of ornamental gerbera.

Table 62. Effect of *M.anisopliae* 5% WP formulations on phytotoxicity on soybean

Tr. No.	Treatment *M.anisopliae* 5% WP	Conc. (%)	Phytotoxicity score At day after treatment		
			3 DAT	7 DAT	15 DAT
1	Formulation A1	0.01	0	0	0
2	Formulation A1	0.015	0	0	0
3	Formulation A1	0.02	0	0	0
4	Formulation A1	0.025	0	0	0
5	Formulation A1	0.03	0	0	0
6	Formulation A1	0.04	0	0	0
7	Formulation A1	0.08	0	0	0
8	Formulation B1	0.01	0	0	0
9	Formulation B1	0.015	0	0	0
10	Formulation B1	0.02	0	0	0
11	Formulation B1	0.025	0	0	0
12	Formulation B1	0.03	0	0	0
13	Formulation B1	0.04	0	0	0
14	Formulation B1	0.08	0	0	0
15	Control (*M.a.* alone)	0.01	0	0	0
16	Control (*M.a.* alone)	0.015	0	0	0
17	Control (*M.a.* alone)	0.02	0	0	0
18	Control (*M.a.* alone)	0.025	0	0	0
19	Control (*M.a.* alone)	0.03	0	0	0
20	Control (*M.a.* alone)	0.04	0	0	0
21	Control (*M.a.* alone)	0.08	0	0	0

0 = No Phytotoxicity

10 = 91-100 per cent scorching of leaves and dry of plants.

Formulation A1 = ($\mathbf{M_{30}S_{1/1}C_{1/2}}$) Formulation B1 = ($\mathbf{M_{30}S_{1/1}H_{1/1}}$)

5. SUMMARY AND CONCLUSION

Biological control is an important component of IPM in almost all important crops for the development of sustainable cropping systems.Some pesticide and beneficial organisms have been very effective in the laboratory only to fail at some stage in the field, even after development of product for marketing. Common causes of this demise are poor stability of the product during storage prior to application, too little active material reaching to the target and rapid degradation of the active material. Formulation plays a vital role in helping to solve these problems.

Hence, considering the ecofriendly and biologically management of the pest without disturbing the ecosystem,it was felt necessary to develop a viable and potential WP formulation of *N.rileyi* and *M.anisopliae* by undertaking basic research on this aspect. The summary and conclusions of the research findings of these studies are given in this chapter.

5.1 Development of WP formulation of *N. rileyi* and *M.anisopliae*

5.1.1. Finding most suitable nutrient medium for mass production

Nine media of various nutrient sources comprising Sabouraud's dextrose broth + 1% yeast extract, Sabouraud's maltose broth + 1% yeast extract, potato peptone broth, yeast extract glucose broth, potato maltose broth, malt extract, potato glucose broth and potato dextrose broth + 1% yeast extract were evaluated for their suitability for mass production of *N. rileyi* and *M.anisopliae*.

***N.rileyi* :**

Out of nine media evaluated, the Sabouraud's dextrose broth + 1% yeast extract was the best and most potential medium to produce the mycoagent. It produced maximum biomass (6.10g) and highest colony forming units (8.33 $x10^8$ cfu/ml). The next best media were sabouraud's maltose broth + 1% yeast extract and potato dextrose broth + 1% yeast extract with production of biomass 5.63g and 4.20g and viability 7.33$x10^8$ and 5.67$x10^8$cfu/ml, respectively.

***M.anisopliae* :**

The Sabouraud's dextrose broth + 1% yeast extract was also found most suitable for mass production of *M.anisopliae* It produced maximum(7.20g) biomass and resulted in highest ($12.33x10^8$cfu/ml) viability. The next highly promising media were Sabourauds maltose broth + 1% yeast extract and potato dextrose broth + 1% yeast extract with production of 6.27g and 5.73 g biomass and viability 10.33 x 10^8 and 10.67 x 10^8 cfu/ml, respectively.

5.1.2 Standardization of concentration of inoculum

***N.rileyi* :**

The data on growth of *N.rileyi* showed that the fungal growth increased with increase in concentration of inoculums. Highest medium surface growth (100%) was observed within 7 days in all treatments of 10 to 90% culture of *N. rileyi*. Biomass production was also increased with increase in concentration of inoculums (7.63 to 11.17g). But there was not proportionate increase in biomass development as per test concentrations. However, considering the law of diminishing return, the concentration of 30% (v/v) of the formulation was considered as optimum.

Bioefficacy of the mycoagent against II and III instar larvae recorded at 5, 7 and 10 DAT revealed that the mortality increased with increase in concentration of inoculums and duration of exposure. The treatment with highest concentration $1.8x10^7$cfu/ml was found most promising recorded 90.0% and 86.67% mortality of II and III instar larvae of *S.litura*, respectively at 10 DAT. The treatment of $1.6x10^7$cfu/ml was at par with $1.8x10^7$cfu/ml.

***M.anisopliae* :**

The data on growth of *M.anisopliae* showed that the fungal growth increased with increase in concentration of inoculums. Highest growth

(100%) was observed within 7 days in all treatments of 10 to 90% inoculums of *M.anisopliae* culture. Biomass production was also increased with increase on concentration of inoculums (7.85 to 10.40g). However, considering the law of diminishing return, the concentration of 30% (v/v) of the formulation was considered as optimum.

Bioefficacy of the mycoagent tested against II and III instar larvae recorded at 5, 7 and 10 DAT revealed that the mortality increased with increase in concentration of inoculums and advancement of period after treatment. At 10 DAI, the highest concentration of $1.8x10^7$cfu/ml was most promising.It caused 83.33% and 80.00 % mortality of II and III instar larvae of *S.litura*, respectively. The treatment of $1.6x10^7$cfu/ml was found next to $1.8x10^7$cfu/ml for lethal effect against *S.litura*.

5.1.3 Influence of adjuvants with inoculum on growth and development

Chemical adjuvants

Chemical adjuvants comprising glycerol (1.0 to 5.0%), tween-80 (0.03 to 1.0%) triton-X-100 (0.03 to 1.0%), boric acid (0.5 to 5.0%), carboxymethyl cellulose (0.5 to 1.5%) and magnesium sulphate (0.5 to 5.0%) with optimum concentration of 30% AS culture of *N.rileyi* ($2x10^9$cfu/ml) and *M.anisopliae* ($17.67x10^8$cfu/ml) inoculums were evaluated for growth and development of the mycopathogen for optimization of concentration of chemical adjuvant in formulations.

***N.rileyi* :**

At 10 DAI, the treatment with triton-X-100 1.0 per cent was extremely detrimental to the *N.rileyi* as evidenced from only 10 per cent surface coverage; when rest of the treatments including control without adjuvant showed cent per cent surface coverage. However, the treatment with glycerol 2.0 per cent produced highest (9.97g) fungal biomass and was at par with glycerol 3.0 and 5.0 per cent recording 9.93 and 9.0g fungal biomass,

respectively. In view of uncomparable surface coverage the adjuvants were evaluated on the basis of biomass development.

***M.anisopliae* :**

Glycerol (1.0 to 5.0%) recorded cent per cent growth of fungus. *M.a*+Glycerol 3.0% recorded highest (8.17 g) biomass. However, it was at par with *M.a*+Glycerol 2.0% (7.97g). The glycerol above 3% decreased biomass. Various treatments of *M.a* + Tween-80 recorded 36.67 to 100.0 per cent growth of fungus at 7 DAI. *M.a* + Tween-80 @ 0.50% produced highest 8.83g of biomass. Tween 80 @ 0.5% was considered the most promising and optimum concentration for growth and development of *M.anisolpliae.*

The treatments with lowest concentration of 0.03 per cent triton–X-100 with *M.a.* recorded the highest (6.90g) fungal biomass.

The treatment *M.a.*+boric acid 3.0 per cent registered highest (8.17g) fungal biomass. Carboxymethyl cellulose 0.5 to 1.5 per cent although registered cent per cent growth of fungus at 7 DAI; *M.a.*+CMC 0.5 per cent produced highest (9.00g) fungal biomass at 10 DAI.

The results indicated that the increase in concentration of glycerol, tween-80, triton-X-100 and boric acid increased the biomass of *N.rileyi* and *M.anisopliae* up to certain level and again it decreased. Reverse trend in biomass was observed in treatments with CMC.

Edible oils as adjuvants :

Groundnut, sunflower, soybean, coconut and mustard oil each at 0.25, 0.5, 1.0 and 2.0 per cent and ghee at 0.25, 0.50 and 1.0 per cent with fungal inoculums were tested for optimization of concentration.

***N.rileyi* :**

As fungal inoculums (*N.r.*) with adjuvant groundnut oil 1.0 and 2.0 per cent recorded maximum (85%) surface coverage. It was at par with *N.r.*+GNO 0.5 per cent (80.0%). At 7 DAI, all treatments, *N.r.*+Groundnut oil recorded

cent per cent growth of fungus. *N.r.*+GNO 2.0 per cent produced the highest (11.17g) biomass. It was at par with *N.r.*+GNO 1.0 per cent (11.10g) and *N.r.* +GNO 0.5 per cent (11.00g).

Sunflower oil 2.0 per cent with *N.rileyi* was the most superior for producing fungal biomass (12.50g) at 10 DAI. The biomass in control was 7.40g. The next promising treatment was *N.r.*+SFO 1.0 per cent (12.43g); which was at par *N.r.*+SFO 2.0 per cent.

Among the treatments with soybean oil, coconut oil and mustard oil the treatment *N.r* + SBO of 2.0 per cent, *N.r* + CNO 2.0 per cent and *N.r* + MUO 2.0 per cent produced maximum biomass of 10.17, 9.37 and 9.00g, respectively.

Among ghee concentration at 10 DAI, *N.r.*+GH 1.0 per cent produced highest fungal biomass of 11.80 g; which was at par with *N.r.*+GH 0.5 per cent (11.77g).

***M.anisopliae* :**

Edible oils irrespective of their concentrations showed significantly higher growth of *M.anisopliae* at 3, 7 and 10 DAI. At 7 DAI and 10 DAI, all the treatments showed cent per cent surface coverage.

SFO 2% was the best adjuvant treatment resulting in maximum biomass of 8.40g. However, it was at par to the treatment with SFO 1.0% (8.37g), GNO 1.0% (8.23g), GNO 2.0% (8.23g), GNO 0.5% (8.20g), GH 1.0% (8.20g) and GH 0.5% (8.07g). The next highly promising treatment was SFO 0.5% (8.00g). The biomass in rest of the treatments ranged from 6.40 to 7.53g; when it was 6.30g in control.

All the edible oils showed increase in growth and development of *N.rileyi* and *M.anisopliae* with increase in concentration of oils.

The biomass development in lower concentrations of oil than their higher concentrations, the lower concentrations were considered optimum and more appropriate adjuvant. Accordingly SFO 1.0%, GH 0.5%, GNO

0.5%, SBO 0.5%, MUO 0.5% and CNO 1.0% were most promising adjuvants for growth and development of *N.rileyi* and *M.anisopliae*. These showed 8.97 to 12.43g and 7.20 to 8.37g biomass developed by *N.rileyi* and *M.anisopliae,* respectively.

Other edible substrates

Five different flours comprising wheat, corn, sorghum, bajra and rice each at 1 to 5.0 per cent, three others like honey (0.5 to 2.0%), molasses (1.0 to 4.0%) and milk (0.5 to 2.0%) and one anti fungal as turmeric (0.5 to 1.5%) with optimum concentration of fungal inoculums were evaluated for growth and development of *N.rileyi* and *M.anisopliae* with object to optimize the concentration of adjuvant in formulations.

***N.rileyi* :**

All the adjuvant treatment allowed cent per cent surface coverage at 10 DAI except turmeric 1.5% and molasses 1 to 3%.

Honey 2.0 per cent was the best nutrient source which showed highest (11.77g) biomass. However, it was at par with that of 1.0 per cent (11.67g). Except adverse effect, turmeric, molasses, sorghum flour 1 to 2 per cent and bajra flour (4.67 to 7.20g) rest of the treatments showed more fungal biomass (7.43 to 8.03g) than control (7.30g). However, the increase in biomass was not satisfactory.

***M.anisopliae* :**

Honey 2.0 per cent was the best nutrient source which showed highest (9.20g) biomass. However, it was at par with that of 1.0 per cent (9.10g). Except adverse effect, turmeric 1.5% rest of the treatments showed more fungal biomass (6.33 to 9.20g) than control (6.30g). However, the increase in biomass was not satisfactory.

These results established that honey 0.5, 1.0 and 2.0 per cent was superior nutrient source to *N.rileyi* and *M.anisopliae*. Hence, honey 1.0 per

cent was considered as optimum concentration as edible substrates giving 11.67g and 9.10g biomass of *N.rileyi* and *M.anisopliae.*

5.1.4 Effect of UVC rays on growth and development of inoculums in formulation of *N.rileyi* and *M.anisopliae*

***N.rileyi* :**

N.rileyi liquid culture with various adjuvants were exposed to UVC rays for 10 to 50 minutes and 2, 3 and 5 hours.The per cent surface coverage by the fungus for 10,20, 30, 40, 50 minutes, 2, 3 and 5 hrs of the treatments to UVC was in the range of 48.33 to 100.0, 45.00 to 100.00, 43.33 to 100.0, 33.33 to 100.0, 33.33 to 100.0, 20.0 to 100, 18.33 to 100.0, 16.67 to 100.0 at 10 DAI, respectively. *N.r.*+Indigo 0.5% treatment showed maximum (5.40g) biomass production among all the treatments at 10 minutes UVC rays exposure. It was at par with *N.r.*+sunflower oil 1.0 per cent(5.37g). The sunflower oil 1.0 per cent was best for fungal biomass production at 30, 40, 50 minutes and 2, 3 and 5 hrs. UVC rays exposure.

The fungus culture with adjuvant molasses and tween-80 produced lesser biomass. The surface coverage and biomass produced by *N. rileyi* with or without adjuvants in culture medium after UVC rays exposure for 10 to 50 minutes, 2, 3 and 5 hrs. decreased with increase in exposure period. The adjuvants reacted variably to UVC ray protecting capacity for *N.rileyi*. However, higher concentrations of the adjuvants were better than their lower ones except turmeric and carboxymethyl cellulose.

***M.anisopliae* :**

The per cent surface coverage by the fungus for 50 minutes, 2 hrs, 3hrs and 5hrs of the treatments to UVC 23.33 to 80.00, 16.67 to 75.00, 11.67 to 75.00 and 0.00 to 70.00 at 10 DAI, respectively.

5.1.5 Influence of combination of adjuvants with inoculum on growth and development

The combinations of chemical adjuvants

Fourteen formulations with multiple chemical adjuvants and five with individual adjuvants were evaluated for growth and development of *N.rileyi* and *M.anisopliae*.

***N.rileyi* :**

At 7 DAI, the growth and development of fungus ranged from 10 to 100 per cent in all treatments.Corresponding observations on biomass produced in gram per 40 ml liquid medium showed that *N.r.*+Tw+GLY+CMC proved its superiority, producing 12.17g fungal biomass. Other combination treatments and single adjuvant developed 7.80 to 10.97g biomass. Some combinations of various chemical adjuvants developed more fungal biomass than single ones with the mycoagent. The pH of formulation producing maximum biomass (12.17g) with pH 8.10 was observed in Tween-80 0.5% +Glycerol 2% +CMC 0.5%, when the pH was 8.48 in control which produced biomass of 7.70g/ 40ml medium. These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI.

***M.anisopliae* :**

At 10 DAI, the treatments except *M.a.*+Tw+Tx (10.00%), *M.a.*+Tx+Gl (0%), *M.a.*+Tx+Bo (0%), *M.a.*+Tx+CMC (0%) and *M.a.*+Tw+Tx+Gl (0%) showed cent per cent surface coverage by the mycoagent. *M.a.*+TW+CMC was the best formulations when produced 10.30g fungal biomass. It was followed by *M.a.*+CMC (9.93g), *M.a.*+TW+BA (9.43g) and *M.a.*+TW+GLY (8.90g). *M.a.*+TX+GLY, *M.a.*+TX+BA (0%) and *M.a.*+ TW+TX+GLY did not produced the biomass; whereas, *M.a.*+TW+TX (1.50g) and *M.a.*+TX+CMC (3.40g) produced negligible amount of biomass. The biomass in control was 6.60g.

N.r.+TW+GLY+CMC and *M.a.*+TW+CMC were most promising formulations of *N.rileyi* and *M.anisopliae.*

The combinations of chemicals and oils as adjuvants

Thirty nine formulations with multiple chemical and edible oils as adjuvants and 11 with individual adjuvant were evaluated for growth and development of *N.rileyi* and *M.anisopliae*.

***N. rileyi* :**

At 3 DAI, *N.r.*+GH+BA, *N.r.*+GH+CMC, *N.r.*+SFO recorded signiciantly highest per cent surface growth (86.67%) over remaining treatments (5 to 80%) except *N.r.*+GH (85.0%). *N.r.*+GLY+GH produced highest (12.07g) fungal biomass. It was at par with *N.r.*+TW+GH (11.90), *N.r.*+TW+GLY+SFO+CMC (11.87g) and *N.r.*+GLY+SFO (11.83g).

The least growth and development of fungus was observed TW+CNO and TW+MUO (5% each). Nevertheless, the triton-X-100 alone or with other adjuvants was detrimental to the fungal growth and development. The pH of the fungal culture developed from 51 treatment formulations ranged from 7.10 in *N.r.*+TW+TX+GLY+SFO+CNO to 8.81 in *N.r*+TW+GLY+GH registering biomass of 8.17 and 10.67g, respectively.

***M.anisopliae* :**

At 10 DAI the treatments except triton-X-100 combined with various oils and chemicals recorded cent per cent coverage of surface area by mycoagent. *M.a.*+SFO+CMC produced highest (11.20g) biomass. It was at par with *M.a.*+GNO+BA (11.00g) and *M.a.*+GNO+CMC (10.90g).

The treatments with various combinations of adjuvants with triton-X-100 registered very lesser fungal biomass (1.17 to 3.13g) than control (6.60g).

Thus, among combination of chemical and edible oils as adjuvants, *N.r.*+GLY+GH, *N.r.*+TW+GH, *N.r.*+TW+GLY+SFO+CMC, *N.r.*+GLY+SFO of *N.rileyi* and *M.a.*+SFO+CMC, *M.a.*+GNO+BA and *M.a.*+GNO+CMC of *M.anisopliae* were highly promising for formulations.

Mixtures of oils as adjuvants

Thirteen formulations with multiple adjuvants and six with individual oils were evaluated for growth and development of *N.rileyi* and *M.anisopliae*.

***N. rileyi* :**

N.r.+SFO alone registered significantly higher (12.47g) yield of fungal biomass than rest of the treatments. Among the mixtures of oils, *N.r.*+SFO+GH produced maximum (11.97g) biomass. However, it was at par with *N.r.*+GH alone (11.80g). The pH of the fungal culture developed from 20 treatment formulations ranged from 7.16 in treatment *N.r.*+SFO to 7.90 in *N.r.*+GNO+SBO registering biomass of 12.47 and 9.17g respectively.

***M.anisopliae* :**

At 3 DAI, treatments recorded 10.00 to 65.00 per cent surface coverage; while at 7 DAI it ranged from 62.00 to 100.0 per cent. The maximum fungal biomass of 10.60g was observed in *M.a.*+GNO+GH. The next promising at par treatments were *M.a*+SFO and *M.a.*+SFO+CNO. These produced 8.90 and 8.80g of biomass, respectively. The performance of the oil adjuvants based combinations at10 DAI *N.r.*+SFO+GH yielded highest (11.97g) biomass but at par with *N.r.*+GH (11.80g). Among sole oil adjuvants *N.r.*+SFO produced maximum biomass (12.47g). Hence, *N.r.*+SFO+GH, *N.r.*+SFO and *N.r.*+GH and *M.a.*+GNO+GH were selected as base of *N.rileyi* and *M.anisopliae* formulation.

The combinations of chemicals, edible oils and other edible substrates

Seventeen test formulations with multiple adjuvants and 12 with single adjuvant were evaluated for growth and development of *N.rileyi* and *M.anisopliae*.

***N. rileyi* :**

At 10 DAI, all the treatments showed cent per cent growth. The biomass in the treatments was 7.80 to 12.28 g against 7.50g in control.

N.r.+TW+GLY+HO produced significantly highest (12.28g) biomass. The pH of the fungal culture developed from 30 treatment was 7.16 in *N.r.*+SFO to 8.75 in *N.r*+BA+HO registering biomass of 12.17 and 9.90g, respectively.

***M.anisopliae* :**

At 10 DAI, *M.a.*+SFO+HO yielded highest biomass (11.37g). However, it was at par with *M.a.*+GH+HO (10.60g). The biomass range in the treatments was 1.60g to 11.00g. The least biomass (1.60g) was observed in *M.a.*+TX+CMC+HO

The pH of *M.anisopliae* cultures developed in the treatments varied broadly from 4.15 to 8.89 against biomass range of 1.70g to 11.00g/40ml medium. The treatments with combination of adjuvants helped in increasing fungal biomass at 10 DAI. *N.r.*+TW+GLY+HO (12.28g) produced highest biomass and *N.r.*+HO (11.90g) and *N.r.*+SFO (12.17g) was at par to it of *N.rilelyi* and *M.a.*+SFO+HO (11.37g) and *M.a.*+GH+HO (10.60g) was at par to it of *M.anisopliae* were selected as base of *N.rileyi* and *M.anisopliae* formulation.

Considering the overall performance of the adjuvants for growth and development of *N.rileyi* at 10 DAI, 10 formulations comprising 1*)N.r.*+HO 1% 2*)N.r*+SFO 1% 3*)N.r.*+GH 0.5% 4*)N.r.*+TW 0.5%+GH 0.5% 5*)N.r.*+GLY 2%+SFO 1% 6*)N.r.*+GLY 2%+GH 0.5% 7*)N.r.*+SFO 1%+GH 0.5% 8*)N.r.*+TW 0.5%+GLY 2%+SFO 1%+CMC 0.5% 9*)N.r.*+TW 0.5%+GLY 2%+HO 1% 10*)N.r.*+TW 0.5%+GLY 2%+CMC 0.5% were highly promising. Similarly incase of *M.anisopliae* the high performance of 7 formulations were 1*)M.a.*+TW 0.5%+CMC 0.5% 2*)M.a.*+SFO 1.0%+CMC 0.5% 3*)M.a.*+SFO 1.0%+HO 1.0% 4*)M.a.*+GNO 0.5%+BA 2.0% 5*)M.a.*+GNO 0.5%+CMC 0.5% 6*)M.a.*+GNO 0.5%+GH 0.5% and 7*)M.a.*+GH 0.5%+HO 1.0%. These were the advanced stage formulations of *N. rileyi* and *M.anisopliae* for further study.

On the basis of wet fungal mat volume of both fungi the standardized concentration of bioactive ingredient was 5 per cent.

5.1.6 Potential of advanced test formulations of *N.rileyi* and *M.anisopliae* on growth, development, viability and bioefficacy

The advanced stage formulations were evaluated for their growth, development, viability and bioefficacy against II and III instar larvae of *S. litura*.

***N.rileyi* :**

N.r+SFO was best for growth of *N.rileyi* (88.33% surface coverage) at 3 DAI. It was found at par with *N.r.*+HO (86.67%). *N.r*+TW+GLY+HO and *N.r.*+SFO were the most promising formulations which produced maximum biomass of 12.57g and 12.53g, respectively and maintain its superiority. Maximum ($2.57x10^9$cfu/ml and 109.68) cfu count and viability index were observed in *N.r*+SFO. The next promising formulation for production of cfu count and viability index was *N.r*+TW+GLY+HO ($2.50x10^9$cfu/ml and 104.85).

The per cent kill of of II and III instar larvae of *S.litura* at 10 DAT, was significantly highest (93.33% and 83.33%) in T2- *N.r.*+SFO containing sunflower oil as adjuvant. However, it was at par with *N.r*+TW+GLY+HO (90%) and *N.r*+TW+GLY+CMC (86.67%) for the lethal effect for II instar larvae of *S.litura* and *N.r.*+TW+GLY+HO (80.0%), *N.r.* +TW+GLY+CMC (76.67%) and *N.r.*+HO (73.33%) that for the III instar larvae.

***M.anisopliae* :**

M.a.+GNO+CMC was the best formulation for growth of *M.anisopliae* (88.33% surface coverage) at 3 DAI. The next best were T2- *M.a.*+SFO+CMC (78.33%), *M.a.*+GNO+GH (56.67%) and *M.a.*+GH+HO (51.67%). Least (8.33%) growth was recorded in *M.a.*+TW+CMC. On 10 DAI, *M.a.*+SFO+CMC

maintained its superiority over rest of the treatments by producing 11.43g biomass. However, it was at par with *M.a.*+SFO+HO (11.10g).

Maximum cfu count was observed in *M.a.*+SFO+HO ($2.33x10^9$cfu/ml). However, it was at par with *M.a.*+SFO+CMC ($2.27x10^9$cfu/ml).The viability index was maximum (119.85) in *M.a.*+SFO+CMC and it was followed by *M.a.*+ SFO+HO (119.72). At 10 DAT, all the formulations were significantly superior (56.67 to 73.33%) than fungus culture alone (46.67%) for recording the larval kill. *M.a.*+ SFO+CMC and *M.a.*+SFO+HO (73.33% each) proved significantly superior over the rest of the treatments.

The formulations *M.a.*+SFO+CMC and *M.a.*+SFO+HO were promising among 8 formulations. These included combination of sunflower oil, honey and carboxymethyl cellulose with the mycoagent, *M.anisopliae.*

5.1.7 Potential of WP formulations of *N.rileyi* and *M.anisopliae* on growth, development, viability and bioefficacy

Ten and seven WP formulations of *N.rileyi* and *M.anisopliae*, respectively were most promising advanced stage formulations. These were evaluated for their growth, development, viability and bioefficacy against II and III instar larvae of *S.litura*.

***N.rileyi* :**

At 3 DAI, *N.r.*+SFO covered significantly highest (70.0%) surface of medium. However, it was at par with *N.r.*+TW+GLY+HO (66.67%). *N.r.*+SFO (12.57g) was at par with *N.r.*+TW+GLY+HO (12.50g). The mortality of II (93.33%) and III (86.67%) instar larvae at 10 DAT was significantly highest in formulation *Nr*+SFO. However, it was at par with *N.r.*+TW+GLY+HO (90.0 and 83.33%) and *N.r.*+TW+GLY+CMC (86.67 and 80.00%). It was established from the study that adjuvants substantially contributed to cfu *N.r.*+SFO and *N.r.*+TW+GLY+HO resulted in higher cfu (22.33 to $23.33x10^8$) and viability

index (218.71 to 234.85) than control (14.67x10^8cfu/ml). Both were considered as highly potent WP formulations for growth and biomass production. These were coded as formulation A($N_{30}S_{1/1}$) and B($N_{30}T_{1/2}G_{2/1}H_{1/1}$).

***M.anisopliae* :**

At 3 DAI, *M.a.*+SFO+CMC covered significantly highest (66.67%) surface of medium. The formulation at par with *M.a.*+SFO+HO (63.33%). *M.a.*+SFO+CMC maintained its superiority over rest of the treatments by producing 11.83g biomass. However, *M.a.*+SFO+HO (11.43g) was at par.

The mortality of II instar larvae at 10 DAT was significantly highest (83.33%) in formulation with *M.a.*+SFO+HO. However, it was at par with formulation *M.a.*+SFO+CMC (80.0%). The mortality of III instar larvae at 10 DAT in *M.a.*+ SFO+HO (76.67%) was significantly superior to the rest of the formulations and *M.a.*+ SFO+CMC (73.33%) was on par to it..

It was evident from the study that adjuvants substantially contributed to cfu. *M.a.*+SFO+CMC and *M.a.*+SFO+HO resulted in higher cfu (23.67 to 25.0x10^8) and viability index (238.80 to 245.74) than rest of the formulations. These were considered as highly promising potent WP formulations for highest growth and biomass production. These were coded as formulation A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$)

5.1.8 Effect of UVC rays on growth and development of inoculum in advanced test formulations of *N.rileyi* and *M.anosopliae*

***N.rileyi* :**

The highest UVC range protecting ability after 5 hours exposure period was observed in formulations *N.r*+SFO recording cent percent growth of fungus. The next highly promising formulations for the UVC protect ability were *N.r*+HO (98.33%), *Nr*+GH (98.33%), *Nr*+Tw+Gl+Ho (81.67%).

N.r +SFO produced significantly highest biomass of 5.60, 5.53, 5.37, 5.28, 5.22, 5.13, 5.02, and 4.95 g after 10, 20, 30, 40, 50 minutes, 2, 3 and 5 hours exposure to UVC rays, respectively.

***M.anisopliae* :**

M.a.+SFO+CMC, *M.a.*+SFO+HO, *M.a.*+GNO+CMC gave highest protection against UVC rays after 5 hours exposure as evidenced from 75.0 percent growth of fungus. The formulation *M.a.*+SFO+HO produced significantly highest biomass of 5.33, 5.27, 5.22, 5.13, 5.10, 5.00, 4.90, and 4.70 g after 10, 20, 30, 40, 50 minutes, 2, 3 and 5 hours exposure to UVC rays, respectively.

5.2 Pathogenicity, field bioefficacy, shelf life and phytotoxicity of each of two best WP formulations of *N.rileyi* and *M.anisopliae*

Pathogenicity

***N. rileyi* :**

The LC_{50} values of formulation A($N_{30}S_{1/1}$) on the basis of bioactive ingredient (BAI) were 0.0116% and 0.0157% for II and III instar larvae of *S.litura*, respectively. The LC_{50} values of formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0120% and 0.0176% for II and III instar larvae of *S.litura.*

It indicated that among two larval instar of *S.litura* tested, II instar larvae was most susceptible to the *N.rileyi* WP formulation A and B. The LT_{50} value of formulation A ($N_{30}S_{1/1}$) was 6.34 days and it was the lowest time registered for 50 per cent kill of II instar larvae. In case of III instar larvae of *S.litura,* the LT_{50} for formulation A was 7.12 days.

***M.anisopliae* :**

The LC_{50} values of formulation A1($M_{30}S_{1/1}C_{1/2}$) were 0.0174% and 0.0180% for II and III instar larvae of *S.litura*, respectively against the values of 0.0149% and 0.0163% for II and III instar larvae of *S.litura*, respectively for formulation B1($M_{30}S_{1/1}H_{1/1}$). It indicated that among the two larval

instars, II instar of larvae were most susceptible to formulation A1 and B1 than the III instar. The lowest LT_{50} value of 7.78 days was registered by formulation B1 for II instar larvae of *S.litura*. In case of III instar larvae of *S.litura* the LT_{50} for B1 was 8.68 days.

Bioefficacy of final stage WP formulations of *N.rileyi* and *M.nisopliae* against *S.litura*

***N.rileyi* :**

At 10 DAT, the effectiveness against II instar larvae was highest (92.50%) in formulation A 0.04% and 0.03% and B 0.04%. However, it was at par with formulation B 0.03% (90.0%), A 0.025% (87.50%) and B 0.025% (85.0%). These were followed by formulation A 0.02% (77.50%) and B 0.02% (75.0%). The concentrations 0.01 (52.50%) and 0.015% of both formulations (60.0 to 65.0%) showed at par mortality of the pest.

At 10 DAT, the mortality of III instar larvae was highest (82.50%) in the treatment of formulation A 0.04% and it was on par to formulation B 0.04% (80.0%), A 0.03% (77.50%), B 0.03% (77.50%) and A 0.025% (72.50%) and B 0.025% (70.0%). The next best treatments were formulation A 0.02% (65.0%), A 0.015% (52.50%) and B 0.015% (47.50%) as compared to control (*N.r.*alone) 0.02% (55.0%) and control with water spray (0%).

***M.anisopliae* :**

The mortality of II instar larvae at 10 DAT was higher in all concentrations of formulation B1 than A1. The formulation B1 0.04% registered highest (85.0%) mortality. However, it was at par with formulation B1 0.03% (82.50%), A1 0.04% (82.50%) and A1 0.03% (77.50%). The next promising treatments were formulation A1 and B1 both 0.025% (70.0%). The concentration of 0.01% of both formulations showed up to 50.0 per cent mortality os II instar. The mortality of III instar larvae was highest (82.50%)

in formulation B1 0.04%. It was on par to formulation A1 0.04% (80.0%), B1 0.03% (77.50%) and A1 0.03% (77.50%).

Shelf-life assessment of WP formulations of *N.rileyi* and *M.anisopliae* .

***N.rileyi* :**

The study indicated that the *N.rileyi* 5%WP formulation A, B and control (without adjuvant) could be stored upto 10, 10 and 7 months, respectively considering surface coverage, biomass produced and viability (cfu/g).

***M.anisopliae* :**

The shelf life of formulated *M.anisopliae* was one month more than *N.rileyi*. The shelf life on the basis of surface coverage, biomass produced and viability (cfu/g) the *M.anisopliae* 5%WP formulation A1, B1 and control could be stored upto 10, 10 and 6 months, respectively

Field efficacy of the new WP formulations against *S.litura* in soybean

***N.rileyi*:**

The larval population was in the range of 1.67 to 4.27 larvae/m row at 14 days after second spray as against 10.33 larvae/m row in control. The treatments with formulation A($N_{30}S_{1/1}$) 0.025% and 0.03%, B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) 0.03% were highly promising (1.67to1.73 larvae/m row) and at par treatments to the most effective chemical treatment with quinolphos 0.05% (1.53 larvae/m row). The next promising and at par treatments to formulation A 0.025% and 0.03% was formulation B 0.03% (1.67 larvae/m row) when the *S.litura* population in control was 10.33 larvae/m row. The treatment with quinolphos 0.05% recorded maximum yield of 28.66 q/ha. It was at par with formulation B 0.03% (27.47q/ha) and 0.025% (27.17q/ha). Among the treatments of *N.rileyi* formulation B 0.03% recorded highest yield (27.47 q/ha). However, it was at par with formulation B 0.025% (27.17q/ha), A 0.03% (26.93 q/ha), 0.025% (26.67q/ha), B 0.02% (26.40q/ha) and A 0.02% (26.13q/ha).

***M.anisopliae* :**

At 14 DAS, the treatment with chemical insecticide quinolphos 0.05% recorded minimum (1.53 larvea/m row) population. The next promising treatments for the control of *S.litura* was formulation B1($M_{30}S_{1/1}H_{1/1}$) and A1($M_{30}S_{1/1}C_{1/2}$) 0.03%, which recorded 3.0 larvae/m low length. However, it was at par with formulation B1 and A1 0.025% (3.27 and 3.47 larvae/m row), respectively, when the *S.litura* population in control was 9.80 larvae/m row

The treatment with quinolphos 0.05% recorded maximum yield of 28.10 q/ha. It was at par with formulation A1 0.03% (26.67q/ha) and B1 0.03% (26.50q/ha). Among the treatments of *M.anisopliae* formulation A1 0.03% recorded highest yield (26.67 q/ha). However, it was at par with formulation B1 0.03% (26.50 q/ha) and A1 0.025% (26.16 q/ha), B1 0.025% (25.47q/ha) and A1 0.025% (25.17q/ha).

Phytotoxicity test of the WP formulations *N.rileyi* and *M.anisopliae* on soybean.

The results revealed that there were no phytotoxicity symptoms in all the concentrations of both formulations of *N.rileyi* as well as *M.anisopliae*.

5.3 Conclusions

- Sabouraud's dextrose broth with 1% yeast extract was the best medium for mass production of *N.rileyi* and *M. anisopliae*.
- The concentration of 30% culture at 10 DAI (v/v) was found optimum for formulating aqua suspension of *N.rileyi* and *M.anisopliae*.
- Out of 21 test adjuvants glycerol, tween-80, boric acid, sunflower oil, groundnut oil, ghee and honey were the best adjuvants for the formulations of both fungi.
- Considering growth, development, viability and bioefficacy of an entomopathogenic fungus the highly promising advance stage formulations of *N.rileyi* were 1*)N.r.*+HO 1% 2*)N.r*+SFO 1% 3*)N.r.*+GH 0.5% 4*)N.r.*+TW 0.5%+GH 0.5% 5*)N.r.*+GLY 2%+SFO 1% 6*)N.r.*+GLY 2%+GH 0.5% 7*)N.r.*+SFO 1%+GH 0.5% 8*)N.r.*+TW 0.5%+GLY 2%+SFO 1%+CMC 0.5% 9*)N.r.*+TW 0.5%+GLY 2%+HO 1% 10*)N.r.*+TW 0.5%+GLY 2%+CMC 0.5%
  Similarly, the high performance formulation of *M.anisopliae* were 1*)M.a.*+TW 0.5%+CMC 0.5% 2*)M.a.*+SFO 1.0%+CMC 0.5% 3*)M.a.*+SFO 1.0%+HO 1.0% 4*)M.a.*+GNO 0.5%+BA 2.0% 5*)M.a.*+GNO 0.5%+CMC 0.5% 6*)M.a.*+GNO 0.5%+GH 0.5% and 7*)M.a.*+GH 0.5%+HO 1.0%. These formulations possessed appreciable UVC protecting ability.
- Among 14 adjuvants sunflower oil, groundnut oil, glycerol, carboxy methyl cellulose, boric acid, honey and milk showed UVC rays protecting ability.
- *N.rileyi* 5% WP formulation A($N_{30}S_{1/1)}$ and formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) while, *M.anisopliae 5*% WP formulation A1($M_{30}S_{1/1}C_{1/2)}$ and formulation B1($M_{30}S_{1/1}H_{1/1)}$ were emerged as the best potential formulations **(Plate X and Plate XI)**.
- The LC_{50} values of *N.rileyi* 5% WP formulation A and B were 0.0116 and 0.0120% against the LC_{50} value of 0.0174 and 0.0149% of

M.anisopliae formulations A1 and B1, respectively, for II instar larvae of *S.litura*.

- The LT_{50} value of *N.rileyi* 5% WP formulation was 6.33 to 6.38 days against the value of 7.78 to 8.11 days of *M.anisopliae* 5% WP for II instar larvae of *S.litura*.
- The storability of *N.rileyi* 5% WP formulations A($N_{30}S_{1/1)}$ and formulation B($N_{30}T_{1/2}G_{2/1}H_{1/1}$) was 10 months against the 7 months shelf life of control formulation without adjuvants.
- The shelf life of both *M.anisopliae* 5% WP formulations A1($M_{30}S_{1/1}C_{1/2}$) and B1($M_{30}S_{1/1}H_{1/1}$) was 10 months against the 6 months storability of control formulation without adjuvants.
- On the basis of the studies, *N.rileyi* 5% WP formulation A 0.02% and B 0.025% and *M.anisopliae 5*% WP formulation A1 0.02% and B1 0.025% were the best and these could be recommended for suppression of *S.litura* and harvesting higher yield (25.17 to 27.17q/ha) of soybean.

Future line of work:

The granular formulations of *N.rileyi* and *M.anisopliae* comprising suitable adjuvants are required to develop. *N.rileyi* granules could be utilized against *S.litura* intermittently coming in contact with soil. While, *M.anisopliae* granules could be included in multiple attack of bioagents against the chronic and accelerating the problem of white grubs on sugarcane and other crops.

I : Final stage developed WP formulations of *N.rileyi*

PLATE – X

Final stage developed WP formulations of *N.rileyi*

Formulation A1

Formulation B1

II : Final stage developed WP formulations of *M.anisopliae*

PLATE – XI

Final stage developed WP formulations of *M.anisopliae*

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- **Originals not seen**

APPENDIX I

Influence of *N. rileyi* with adjuvants on growth of *N. rileyi* exposed to UVC rays

Tr. No.	Treatments	Conc. (%)of adj.	Surface coverage (%) on 3 DAI in UVC exposure upto							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*N.r.*+GLY	1.0	13.33 (21.39)	11.67 (20.00)	11.67 (20.00)	11.67 (20.00)	11.67 (20.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T2	*N.r.*+GLY	2.0	23.33 (28.93)	11.67 (20.00)	16.67 (24.12)	11.67 (20.00)	10.00 (18.44)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T3	*N.r.*+GLY	3.0	23.33 (28.93)	11.67 (20.00)	11.67 (20.00)	11.67 (20.00)	10.00 (18.44)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T4	*N.r.*+GLY	5.0	16.67 (24.12)	13.33 (21.39	10.00 (18.44)	10.00 (18.44)	8.33 (16.78)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T5	*N.r.*+TW-80	0.5	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)	5.00 (12.92)	5.00 (12.92)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T6	*N.r.*+TW-80	1.0	13.00 (21.39	10.00 (18.44)	10.00 (18.44)	5.00 (12.92)	5.00 (12.92)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T7	*N.r.*+BA	1.0	73.33 (58.89)	73.33 (58.89)	71.67 (57.86)	71.67 (57.86)	68.33 (55.73)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T8	*N.r.*+BA	2.0	73.33 (58.89)	73.33 (58.89)	71.67 (57.86)	71.67 (57.86)	70.00 (56.79)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T9	*N.r.*+BA	3.0	66.67 (54.76)	65.00 (53.73)	65.00 (53.73)	60.00 (50.77)	60.00 (50.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T10	*N.r.*+CMC	0.5	73.33 (58.89)	70.00 (56.79)	68.33 (55.73)	66.67 (54.76)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T11	*N.r.*+CMC	1.0	80.00 (63.44)	80.00 (63.44)	75.00 (60.00)	65.00 (53.73)	60.00 (50.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T12	*N.r.*+Indigo	0.5	83.33 (65.88)	83.33 (65.88)	83.33 (65.88)	81.67 (64.67)	78.33 (62.24)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T13	*N.r.*+Indigo	1.0	81.67 (64.67)	80.00 (63.44)	78.33 (62.24)	75.00 (60.00)	75.00 (60.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T14	*N.r.*+Turmeric	0.5	71.67 (57.86)	70.00 (56.79)	68.33 (55.73)	68.33 (55.73)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T15	*N.r.*+Turmeric	1.0	66.67 (54.76)	66.67 54.76)	66.67 (54.76)	63.33 (52.77)	61.67 (51.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T16	*N.r.*+Molasses	1.0	68.33 (55.73)	68.33 (55.73)	68.33 (55.73)	66.67 (54.76)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T17	*N.r.*+Molasses	2.0	73.33 (58.89)	70.00 (56.79)	70.00 (56.79)	66.67 (54.76)	61.67 (51.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T18	*N.r.*+HO	0.5	80.00 (63.44)	75.00 (60.00)	73.33 (58.89)	68.33 (55.73)	68.33 (55.73)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T19	*N.r.*+HO	1.0	90.00 (71.56)	80.00 (63.44)	76.67 (61.14)	71.67 (57.86)	68.33 (55.73)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T20	*N.r.*+Milk	1.0	80.00 (63.44)	80.00 (63.44)	80.00 (63.44)	76.67 (61.14)	75.00 (60.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T21	*N.r.*+Milk	2.0	83.33 (65.88)	81.67 (64.67)	81.67 (64.67)	78.33 (62.24)	78.33 (62.24)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T22	*N.r.*+SFO	0.5	85.00 (67.21)	80.00 (63.44)	76.67 (61.14)	75.00 (60.00)	71.67 (57.86)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T23	*N.r.*+SFO	1.0	80.00 (63.44)	80.00 (63.44)	78.33 (62.24)	75.00 (60.00)	75.00 (60.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T24	*N.r.*+ GNO	0.5	78.33 (62.24)	68.33 (55.73)	75.00 (60.00)	73.33 (58.89)	70.00 (56.79)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T25	*N.r.*+ GNO	1.0	76.67 (61.14)	71.67 (57.86)	70.00 (56.79)	68.33 (55.73)	68.33 (55.73)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T26	*N.r.*+GNO	2.0	75.00 (60.00)	73.33 (58.89)	71.67 (57.86)	68.33 (55.73)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T27	*N.r.*+SBO	0.5	68.33 (55.73)	68.33 (55.73)	65.00 (53.73)	65.00 (53.73)	65.00 (53.73)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T28	*N.r.*+SBO	1.0	71.67 (57.86)	68.33 (55.73)	66.67 (54.76)	65.00 (53.73)	63.33 (52.71)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T29	*N.r.*+MUO	0.5	70.00 (56.79)	70.00 (56.79)	70.00 (56.79)	68.33 (55.73)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T30	*N.r.*+MUO	1.0	68.33 (55.73)	68.33 (55.73)	66.67 (54.76)	66.67 (54.76)	61.67 (51.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T31	*N.r.*+GH	0.5	71.67 (57.86)	68.33 (55.73)	68.33 (55.73)	66.67 (54.76)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T32	*N.r.*+GH	1.0	75.00 (60.00)	71.67 (57.86)	71.67 (57.86)	66.67 (54.76)	66.67 (54.76)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T33	Control (*N.r.* Alone)	-	20.00 (26.56)	20.00 (26.56)	18.33 (25.33)	18.33 (25.33)	15.00 (22.79)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T34	Control (*N.r.* Alone) (wt.UVC)	-	28.33 (32.14)	30.00 (33.21)	30.00 (33.21)	33.33 (35.24)	30.00 (33.21)	33.33 (35.24)	30.00 (33.21)	28.33 (32.14)
	S.E +		**1.73**	**0.99**	**1.11**	**1.18**	**1.00**	-	-	-
	C.D(P=0.05)		**4.88**	**2.81**	**3.14**	**3.34**	**2.83**	-	-	-

APPENDIX II

Influence of *N. rileyi* with adjuvants on growth of *N. rileyi* exposed to UVC rays

Tr. No.	Treatments	Conc.(%) of adj.	Surface coverage (%) on 7 DAI in UVC exposure upto							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*N.r.*+GLY	1.0	58.33 (49.78)	56.67 (48.85)	55.00 (47.87)	46.67 (43.11)	38.33 (38.23)	25.00 (30.00)	25.00 (30.00)	21.67 (27.76)
T2	*N.r.*+GLY	2.0	60.00 (50.77)	56.67 (48.85)	53.33 (46.89)	46.67 (43.11)	41.67 (40.22)	28.33 (32.14)	30.00 (33.21)	23.33 (28.86)
T3	*N.r.*+GLY	3.0	60.00 (50.77)	56.67 (48.85)	56.67 (48.85)	38.33 (38.23)	38.33 (38.23)	33.33 (35.24)	30.00 (33.21)	18.33 (25.33)
T4	*N.r.*+GLY	5.0	58.33 (49.78)	58.33 (49.78)	51.67 (45.97)	38.33 (38.23)	36.67 (37.29)	26.67 (31.11)	18.33 (25.33)	15.00 (22.79)
T5	*N.r.*+TW-80	0.5	33.33 (35.24)	30.00 (33.21)	30.00 (33.21)	26.67 (31.11)	8.33 (16.78)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)
T6	*N.r.*+TW-80	1.0	46.67 (43.11)	45.00 (42.13)	43.33 (41.15)	40.00 (39.23)	35.00 (36.27)	25.00 (30.00)	23.33 (28.86)	20.00 (26.56)
T7	*N.r.*+BA	1.0	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)
T8	*N.r.*+BA	2.0	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)
T9	*N.r.*+BA	3.0	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)
T10	*N.r.*+CMC	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)	98.33 (82.51)	95.00 (77.08)
T11	*N.r.*+CMC	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	96.67 (79.53)	95.00 (77.08)	86.67 (68.61)
T12	*N.r.*+Indigo	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)
T13	*N.r.*+Indigo	1.0	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)
T14	*N.r.*+Turmeric	0.5	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	93.33 (75.00)	91.67 (73.26)	86.67 (68.61)
T15	*N.r.*+Turmeric	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	83.33 (65.88)	76.67 (61.14)	71.67 (57.86)
T16	*N.r.*+Molasses	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	98.33 (82.51)	88.33 (70.00)	85.00 (67.21)
T17	*N.r.*+Molasses	2.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	96.67 (79.53)	93.33 (75.00)	86.67 (68.61)
T18	*N.r.*+HO	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)
T19	*N.r.*+HO	1.0	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)
T20	*N.r.*+Milk	1.0	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)
T21	*N.r.*+Milk	2.0	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)
T22	*N.r.*+SFO	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)
T23	*N.r.*+SFO	1.0	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)
T24	*N.r.*+ GNO	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)
T25	*N.r.*+ GNO	1.0	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)
T26	*N.r.*+GNO	2.0	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)
T27	*N.r.*+SBO	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)
T28	*N.r.*+SBO	1.0	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)
T29	*N.r.*+MUO	0.5	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	96.67 (79.53)	93.33 (75.00)	93.33 (75.00)
T30	*N.r.*+MUO	1.0	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	98.33 (82.51)	95.00 (77.08)	95.00 (77.08)
T31	*N.r.*+GH	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)	98.33 (82.51)
T32	*N.r.*+GH	1.0	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)
T33	Control (*N.r.* Alone)	-	60.00 (50.77)	55.00 (47.87)	50.00 (45.00)	48.33 (44.03)	48.33 (44.03)	40.00 (39.23)	36.67 (37.29)	30.00 (33.21)
T34	Control (*N.r.* Alone) (W.UVC)	-	65.00 (53.73)	65.00 (53.73)	61.67 (51.77)	65.00 (53.73)	60.00 (50.77)	60.00 (50.77)	55.00 (47.87)	61.67 (51.77)
	S.E ±		**0.62**	**0.67**	**0.50**	**0.83**	**0.85**	**1.66**	**1.41**	**1.51**
	C.D(P=0.05)		**1.75**	**1.89**	**1.41**	**2.34**	**2.40**	**4.67**	**3.99**	**4.27**

APPENDIX III

Tr. No.	Treatments	Conc. (%)of adjuvant	Surface coverage (%) on 3 DAI in UVC exposure upto							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*M.a.*+GLY	1	20.00 (26.56)	16.67 (24.12)	11.67 (20.00)	10.00 (18.44)	6.67 (15.00)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)
T2	*M.a.*+GLY	2	23.33 (28.86)	13.33 (21.39)	11.33 (19.64)	8.33 (16.78)	8.33 (16.78)	7.00 (15.34)	6.67 (15.00)	6.33 (14.54)
T3	*M.a.*+GLY	3	23.33 (28.86)	16.67 (24.12)	12.67 (20.88)	11.67 (20.00)	8.33 (16.78)	7.00 (15.34)	7.00 (15.34)	6.33 (14.54)
T4	*M.a.*+GLY	5	25.00 (30.00)	13.33 (21.39)	13.33 (21.39)	11.00 (19.37)	10.00 (18.44)	8.00 (16.43)	7.33 (15.68)	7.00 (15.34)
T5	*M.a.*+TW-80	0.5	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)
T6	*M.a.*+TW-80	1	10.00 (18.44)	10.00 (18.44)	8.33 (16.78)	5.00 (12.92)	5.00 (12.92)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
T7	*M.a.*+BA	1	10.00 (18.44)	6.67 (15.00)	6.67 (15.00)	6.67 (15.00)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)
T8	*M.a.*+BA	2	8.33 (16.78)	8.33 (16.78)	8.33 (16.78)	8.33 (16.78)	8.33 (16.78)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)
T9	*M.a.*+BA	3	8.33 (16.78)	8.33 (16.78)	8.33 (16.78)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)	8.00 (16.43)
T10	*M.a.*+CMC	0.5	16.67 (24.12)	13.33 (21.39)	13.33 (21.39)	11.67 (20.00)	11.67 (20.00)	21.67 (27.76)	11.67 (20.00)	11.67 (20.00)
T11	*M.a.*+CMC	1	16.67 (24.12)	13.33 (21.39)	11.67 (20.00)	8.33 (16.78)	11.67 (20.00)	23.33 (28.86)	10.00 (18.44)	6.67 (15.00)
T12	*M.a.*+Indigo	0.5	11.67 (20.00)	13.33 (21.39)	11.67 (20.00)	11.67 (20.00)	11.67 (20.00)	11.67 (20.00)	11.00 (19.37)	11.00 (19.37)
T13	*M.a.*+Indigo	1	13.33 (21.39)	13.33 (21.39)	13.33 (21.39)	11.67 (20.00)	11.67 (20.00)	11.00 (19.37)	11.00 (19.37)	11.00 (19.37)
T14	*M.a.*+Turmeric	0.5	16.67 (24.12)	16.67 (24.12)	8.33 (16.78)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	6.67 (15.00)	6.67 (15.00)
T15	*M.a.*+Turmeric	1	16.67 (24.12)	13.33 (21.39)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	3.33 (10.47)	3.33 (10.47)	1.67 (7.49)
T16	*M.a.*+Molasses	1	11.67 (20.00)	11.67 (20.00)	10.00 (18.44)	8.33 (16.78)	6.67 (15.00)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)
T17	*M.a.*+Molasses	2	11.67 (20.00)	11.67 (20.00)	10.67 (19.09)	8.00 (16.43)	6.67 (15.00)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)
T18	*M.a.*+HO	0.5	13.33 (21.39)	13.33 (21.39)	12.00 (20.27)	11.67 (20.00)	7.33 (15.68)	7.00 (15.34)	10.00 (18.44)	10.00 (18.44)
T19	*M.a.*+HO	1	13.33 (21.39)	13.33 (21.39)	11.67 (20.00)	8.33 (16.78)	11.67 (20.00)	11.00 (19.37)	7.00 (15.34)	7.00 (15.34)
T20	*M.a.*+Milk	1	16.67 (24.12)	16.67 (24.12)	13.33 (21.39)	11.67 (20.00)	11.00 (19.37)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)
T21	*M.a.*+Milk	2	18.33 (25.33)	16.67 (24.120	15.00 (22.79)	11.67 (20.00)	11.33 (19.64)	10.00 (18.44)	10.00 (18.44)	10.00 (18.44)
T22	*M.a.*+SFO	0.5	28.33 (25.33)	28.33 (32.14)	26.67 (31.11)	23.33 (28.86)	23.33 (28.86)	20.00 (26.56)	20.00 (26.56)	15.00 (22.79)
T23	*M.a.*+SFO	1	28.33 (25.33)	28.33 (32.14)	27.00 (31.31)	26.67 (31.11)	25.00 (30.00)	22.33 (28.18)	20.00 (26.56)	20.00 (26.56)
T24	*M.a.*+ GNO	0.5	30.00 (33.21)	31.67 (34.27)	30.00 (33.21)	25.00 (30.00)	23.33 (28.86)	20.00 (26.56)	20.00 (26.56)	18.33 (25.33)
T25	*M.a.*+ GNO	1	31.67 (34.27)	28.33 (32.14)	28.33 (32.14)	18.33 (25.33)	17.67 (24.88)	15.00 (22.79)	15.00 (22.79)	15.00 (22.79)
T26	*M.a.*+GNO	2	33.33 (35.24)	31.67 (34.27)	30.00 (33.21)	18.33 (25.33)	17.67 (24.88)	21.67 (27.06)	18.00 (25.10)	16.67 (24.12)
T27	*M.a.*+SBO	0.5	36.67 (37.29)	30.00 (33.21)	28.33 (32.14)	18.33 (25.33)	18.33 (25.33)	15.00 (22.79)	15.00 (22.79)	10.00 (18.44)
T28	*M.a.*+SBO	1	35.00 (36.27)	33.33 (35.24)	31.33 (34.02)	16.67 (24.12)	15.00 (22.79)	13.33 (21.39)	13.33 (21.39)	10.00 (18.44)
T29	*M.a.*+MUO	0.5	35.00 (36.27)	31.67 (34.27)	31.67 (34.27)	25.00 (30.00)	18.33 (25.33)	11.67 (20.00)	15.00 (21.39)	13.33 (21.39)
T30	*M.a.*+MUO	1	35.67 (36.79)	33.33 (35.24)	33.33 (35.24)	26.67 (31.11)	21.67 (27.76)	15.00 (22.79)	14.33 (22.22)	13.33 (21.39)
T31	*M.a.*+GH	0.5	16.67 (24.12)	16.67 (24.12)	11.67 (20.00)	6.67 (15.00)	6.67 (15.00)	6.33 (14.54)	6.67 (15.00)	6.67 (15.00)
T32	*M.a.*+GH	1	16.67 (24.12)	15.00 (22.79)	11.67 (20.00)	5.00 (12.92)	8.33 (16.78)	8.33 (16.78)	8.33 (16.78)	5.00 (12.92)
T33	Control (*M.a.*alone)	-	15.00 (22.79)	10.00 (18.44)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	5.00 (12.92)	0.00 (0.00)
T34	Control ($_{M.a.alone}$) (W.UVC)	-	31.67 (34.27)	31.67 (34.27)	36.67 (37.29)	30.00 (33.21)	30.00 (33.21)	28.33 (32.14)	28.33 (32.14)	31.67 (34.27)
	S.E ±		**1.53**	**1.34**	**1.33**	**1.28**	**1.19**	**1.03**	**1.25**	**1.29**
	C.D(P=0.05)		**4.31**	**3.77**	**3.75**	**3.61**	**3.36**	**2.91**	**3.53**	**3.64**

APPENDIX IV

Influence of *M.anisopliae* with adjuvants on growth of *M.anisopliae* exposed to UVC rays

Tr. No.	Treatments	Conc.(%) of adj.	Surface coverage (%) on 7 DAI in UVC exposure upto							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*M.a.*+GLY	1	60.00 (50.77)	56.67 (48.85)	53.33 (46.89)	50.00 (45.00)	48.33 (44.03)	45.00 (42.13)	45.00 (42.13)	43.33 (41.15)
T2	*M.a.*+GLY	2	61.67 (51.77)	58.33 (49.79)	56.67 (48.85)	53.33 (46.89)	50.00 (45.00)	46.67 (43.11)	43.33 (41.15)	41.67 (40.22)
T3	*M.a.*+GLY	3	63.33 (52.71)	61.67 (51.77)	56.67 (48.85)	55.00 (47.87)	53.33 (46.89)	50.00 (45.00)	48.33 (44.03)	48.33 (44.03)
T4	*M.a.*+GLY	5	65.00 (53.73)	63.33 (52.71)	61.67 (51.77)	55.00 (47.87)	53.33 (46.89)	50.00 (45.00)	48.33 (44.03)	45.00 (42.13)
T5	*M.a.*+TW-80	0.5	30.00 (33.21)	26.67 (31.11)	25.00 (30.00)	23.33 (28.86)	23.33 (28.86)	21.67 (27.76)	20.00 (26.56)	16.67 (24.12)
T6	*M.a.*+TW-80	1	26.67 (31.11)	25.00 (30.00)	25.00 (30.00)	23.33 (28.86)	23.33 (28.86)	16.67 (24.12)	11.67 (20.00)	10.00 (18.44)
T7	*M.a.*+BA	1	55.00 (47.87)	50.00 (45.00)	48.33 (44.03)	46.67 (43.11)	45.00 42.13)	40.00 (39.23)	40.00 (39.23)	35.00 (36.27)
T8	*M.a.*+BA	2	60.00 (50.77)	53.33 (46.89)	50.00 (45.00)	48.33 (44.03)	46.67 (43.11)	43.33 (41.15)	40.00 (39.23)	36.67 (37.29)
T9	*M.a.*+BA	3	55.00 (47.87)	53.33 (46.89)	50.00 (45.00)	48.33 (44.03)	45.00 (42.13)	40.00 (39.23)	40.00 (39.23)	35.00 (36.27)
T10	*M.a.*+CMC	0.5	60.00 (50.77)	55.00 (47.87)	46.67 (43.11)	43.33 (41.15)	40.00 (39.23)	35.00 (36.27)	35.00 (36.27)	30.00 (33.21)
T11	*M.a.*+CMC	1	60.00 (50.77)	48.33 (44.03)	43.33 (41.15)	41.67 (40.22)	40.00 (39.23)	35.00 (36.27)	33.33 (35.24)	28.33 (32.14)
T12	*M.a.*+Indigo	0.5	55.00 (47.87)	46.67 (43.11)	43.33 (41.15)	43.33 (41.15)	40.00 (39.23)	40.00 (39.23)	38.33 (38.23)	35.00 (36.27)
T13	*M.a.*+Indigo	1	60.00 (50.77)	58.33 (49.79)	55.00 (47.87)	50.00 (45.00)	50.00 (45.00)	46.67 (43.11)	45.00 (42.13)	45.00 (42.13)
T14	*M.a.*+Turmeric	0.5	45.00 (42.13)	40.00 (39.23)	36.67 (37.29)	28.33 (32.14)	26.67 (31.11)	25.00 (30.00)	23.33 (28.86)	21.67 (27.76)
T15	*M.a.*+Turmeric	1	40.00 (39.23)	38.33 (38.23)	28.33 (32.14)	25.00 (30.00)	25.00 (30.00)	25.00 (30.00)	23.33 (28.86)	21.67 (27.76)
T16	*M.a.*+Molasses	1	45.00 (43.13)	38.33 (38.23)	35.00 (36.27)	30.00 (33.21)	30.00 (33.21)	26.67 (31.11)	26.67 (31.11)	25.00 (30.00)
T17	*M.a.*+Molasses	2	46.67 (43.11)	46.67 (43.11)	38.33 (38.23)	36.67 (37.29)	36.67 (37.29)	35.00 (36.27)	35.00 (36.27)	35.00 (36.27)
T18	*M.a.*+HO	0.5	85.00 (67.21)	85.00 (67.21)	80.00 (63.44)	80.00 (63.44)	80.00 (63.44)	75.00 (60.00)	75.00 (60.00)	70.00 (56.79)
T19	*M.a.*+HO	1	90.00 (71.56)	85.00 (67.21)	80.00 (63.44)	80.00 (63.44)	80.00 (63.44)	75.00 (60.00)	70.00 (56.79)	70.00 (56.79)
T20	*M.a.*+Milk	1	70.00 (56.79)	65.00 (53.73)	61.67 (51.77)	56.67 (48.85)	55.00 (47.87)	55.00 (47.87)	50.00 (45.00)	50.00 (45.00)
T21	*M.a.*+Milk	2	70.00 (56.79)	66.67 (54.76)	65.00 (53.73)	61.67 (51.77)	60.00 (50.77)	55.00 (47.87)	50.00 (45.00)	50.00 (45.00)
T22	*M.a.*+SFO	0.5	80.00 (63.44)	78.33 (62.24)	70.00 (56.79)	68.33 (55.73)	66.67 (54.76)	65.00 (53.73)	63.33 (52.71)	60.00 (50.77)
T23	*M.a.*+SFO	1	81.67 (64.67)	80.00 (63.44)	76.67 (61.14)	76.67 (61.14)	75.00 (60.00)	70.00 (56.79)	70.00 (56.79)	65.00 (53.73)
T24	*M.a.*+ GNO	0.5	81.67 (64.67)	81.67 (64.67)	75.00 (60.00)	70.00 (56.79)	70.00 (66.79)	68.33 (55.73)	65.00 (53.73)	60.00 (50.77)
T25	*M.a.*+ GNO	1	83.33 (65.88)	86.67 (68.61)	75.00 (60.00)	73.33 (58.89)	70.00 (66.79)	65.00 (53.73)	63.33 (52.71)	63.33 (52.71)
T26	*M.a.*+GNO	2	88.33 (70.00)	86.67 (68.61)	70.00 (56.79)	71.67 (57.86)	70.00 (66.79)	66.67 (54.76)	65.00 (53.73)	63.33 (52.71)
T27	*M.a.*+SBO	0.5	85.00 (67.21)	81.67 (64.67)	66.67 (54.76)	66.67 (54.76)	65.00 (53.73)	66.67 (54.76)	60.00 (50.77)	55.00 (47.87)
T28	*M.a.*+SBO	1	86.67 (68.61)	83.33 (65.88)	68.33 (55.73)	68.33 (55.73)	65.00 (53.73)	63.33 (52.71)	60.00 (50.77)	50.00 (45.00)
T29	*M.a.*+MUO	0.5	85.00 (67.21)	83.33 (65.88)	71.67 (57.86)	68.33 (55.73)	65.00 (53.73)	65.00 (53.73)	60.00 (50.77)	55.00 (47.87)
T30	*M.a.*+MUO	1	88.33 (70.00)	85.00 (67.21)	75.00 (60.00)	73.33 (58.89)	70.00 (66.79)	68.33 (55.73)	65.00 (53.73)	60.00 (50.77)
T31	*M.a.*+GH	0.5	63.33 (52.71)	61.67 (51.77)	60.00 (50.77)	58.33 (49.79)	58.33 (49.79)	55.00 (47.87)	53.33 (46.89)	53.33 (46.89)
T32	*M.a.*+GH	1	65.00 (53.73)	63.33 (52.71)	61.67 (51.77)	60.00 (50.77)	58.33 (49.79)	58.33 (49.79)	55.00 (47.87)	50.00 (45.00)
T33	Control (*M.a.*alone)	-	40.00 (39.23)	35.00 (36.27)	33.33 (35.24)	31.67 (34.27)	30.00 (33.21)	28.33 (32.14)	26.67 (31.11)	25.00 (30.00)
T34	Control (*M.a.*alone)(W.UVC)	-	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
	S.E ±		**0.98**	**1.11**	**1.10**	**1.01**	**0.83**	**0.83**	**0.78**	**0.93**
	C.D(P=0.05)		**2.76**	**3.13**	**3.11**	**2.86**	**2.34**	**2.34**	**2.21**	**2.64**

APPENDIX V

Mean weekly weather data of Agril. Research Station, Niphad during the year 2011

Month	MW	Temperature(^{o}C)		Humidity(%)		Wind Velo(km/h)	Rain Fall(mm)	Sunshine hrs.
		Max.	Min.	Morn	Even			
June 11	23	34.1	22.3	88	54	6.2	41.8	7.1
	24	31.0	22.3	85	57	9.6	2.7	5.4
	25	31.1	23.1	81	57	14.6	3.0	5.3
	26	30.0	22.8	86	67	13.8	6.1	2.3
July 11	27	30.9	21.9	89	59	7.9	31.4	5.3
	28	26.7	21.8	92	77	9.3	26.1	1.6
	29	28.8	22.5	89	71	10.7	33.1	2.5
	30	29.5	22.2	90	67	7.7	4.0	2.1
	31	28.0	21.9	92	73	7.1	16.0	2.0
August 11	32	28.1	22.4	89	73	11.2	5.7	3.4
	33	27.8	21.4	93	73	6.4	16.9	2.6
	34	28.7	21.8	92	78	5.5	10.0	2.6
	35	27.4	21.9	96	74	4.9	37.2	2.6
September 11	36	27.2	22.0	92	75	7.3	52.3	2.5
	37	28.8	20.5	95	69	4.9	11.0	4.6
	38	28.5	19.9	93	59	4.8	0.7	5.5
	39	30.8	19.8	92	46	4.4	0.0	7.3
October 11	40	30.5	17.6	95	49	2.7	29.3	5.0
	41	33.3	19.7	88	41	4.0	16.3	7.4
	42	32.7	18.5	90	38	2.4	73.9	6.8
	43	21.9	12.9	80	51	4.3	0.0	8.4
	44	31.4	14.3	71	39	3.4	0.0	9.2
November 11	45	31.7	12.6	77	48	2.6	0.0	9.9
	46	31.8	11.6	80	40	3.3	0.0	9.9
	47	30.2	10.2	76	39	4.2	0.0	10.0
	48	30.1	13.7	84	37	3.3	0.0	7.5

APPENDIX VI

Mean weekly weather data of University MPKV, Rahuri during the year 2010 to 2012

Month	MW	Temperature(ºC)		Humidity(%)		Wind Velo(km/h)	Rain Fall(mm)	Sunshine hrs.
		Max.	Min.	Morn	Even			
Jan.10	1	28.0	11.7	90	46	2.7	0.0	8.6
	2	28.6	11.9	91	39	2.9	0.0	7.7
	3	28.1	9.8	91	38	2.2	0.0	8.9
	4	28.1	8.1	90	34	2.1	0.0	9.6
	5	29.7	11.9	89	37	2.3	0.0	8.4
Feb.10	6	29.9	14.3	89	44	2.6	0.0	7.1
	7	31.2	13.9	91	41	2.9	0.0	8.6
	8	32.8	13.6	89	33	3.6	0.0	9.9
	9	33.6	13.9	88	31	2.6	0.0	10.0
March 10	10	34.3	14.5	88	32	3.5	0.0	9.4
	11	35.4	16.2	89	29	3.6	20.2	9.0
	12	37.8	17.2	88	23	3.5	0.0	9.7
	13	37.7	17.3	87	22	4.6	0.0	9.3
April 10	14	38.1	18.2	86	21	4.9	0.0	9.8
	15	39.5	21.0	90	19	4.8	0.0	7.7
	16	40.5	22.3	87	17	7.6	0.0	10.3
	17	40.3	21.2	86	16	6.5	0.0	10.6
	18	37.2	21.2	87	27	6.4	6.4	7.4
May 10	19	40.6	20.3	87	22	7.1	0.0	10.3
	20	41.2	22.1	88	23	7.7	0.0	10.3
	21	41.2	24.6	89	26	13.8	0.0	9.7
	22	37.5	23.8	91	36	10.4	3.8	10.1
June 10	23	36.1	23.0	93	38	10.8	25.2	9.7
	24	32.4	22.7	93	60	8.5	75.9	2.7
	25	33.4	22.9	93	54	7.9	15.2	4.6
	26	33.3	23.0	94	57	6.6	132.8	6.5
July 10	27	30.5	22.1	93	67	10.7	35.4	3.2
	28	31.6	22.4	93	68	7.9	84.9	5.2
	29	30.9	22.3	93	64	12.4	18.7	4.5
	30	29.5	22.3	94	65	12.6	11.4	1.7
	31	30.0	21.3	93	66	12.8	18.2	1.9
August 10	32	29.8	21.1	93.0	63.0	9.0	26.6	5.4
	33	30.1	21.9	93.0	67.0	4.6	20.2	4.5
	34	29.1	21.1	92.0	70.0	3.0	11.9	2.1
	35	28.7	21.6	94.0	75.0	7.0	138.3	2.2
September 10	36	29.0	21.5	92.0	68.0	8.1	46.3	2.8
	37	31.0	20.6	91.0	53.0	5.8	0.0	7.8
	38	30.8	21.6	94.0	62.0	4.0	105.8	6.4
	39	30.8	21.5	94.0	64.0	3.0	103.4	7.4
October 10	40	30.6	20.8	92.0	56.0	2.1	1.5	8.1
	41	32.0	19.1	94.0	42.0	2.8	1.0	7.4
	42	31.2	21.7	92.0	56.0	4.2	8.6	5.1
	43	30.7	19.8	92.0	47.0	2.8	3.2	6.6
	44	29.9	17.7	91.0	44.0	4.0	0.0	8.2
November 10	45	29.1	19.5	92.0	44.0	4.6	3.8	4.3
	46	30.5	19.9	92.0	66.0	2.7	58.5	5.9
	47	29.8	20.1	91.0	59.0	5.0	1.0	6.4
	48	30.3	16.2	90.0	51.0	2.9	0.0	7.7
December 10	49	28.4	13.8	90	44	3.5	0	7.8
	50	28.0	9.7	91	38	3.0	0	5.0
	51	27.5	5.3	88	30	2.7	0	4.5
	52	28.9	10	91	34	3.3	0	7.5

APPENDIX VI Contd……

Month	MW	Temperature(°C)		Humidity(%)		Wind Velo(km/h)	Rain Fall(mm)	Sunshine hrs.
		Max.	Min.	Morn	Even			
Jan.11	1	25.1	9.2	92.0	50.0	2.9	0.0	5.1
	2	28.4	5.1	90.0	30.0	2.3	0.0	10.0
	3	29.8	8.1	89.0	30.0	2.4	0.0	10.0
	4	30.5	9.6	88.0	29.0	2.7	0.0	10.0
	5	31.2	10.1	90.0	31.0	3.3	0.0	10.0
Feb.11	6	31.8	10.0	91.0	28.0	2.6	0.0	10.1
	7	30.9	11.5	90.0	29.0	3.8	0.0	9.8
	8	30.2	11.6	91.0	32.0	4.1	0.0	9.6
	9	32.4	14.2	91.0	32.0	3.8	0.0	8.7
March 11	10	34.7	14.2	91.0	27.0	3.2	0.0	9.8
	11	34.2	11.9	89.0	26.0	3.7	0.0	10.6
	12	36.4	14.7	90.0	26.0	4.3	0.0	10.0
	13	36.4	15.2	88.0	22.0	4.9	0.0	10.3
April 11	14	35.6	15.4	89.0	23.0	4.6	0.0	10.0
	15	36.8	20.5	93.0	25.0	4.4	8.5	9.1
	16	37.7	21.5	92.0	27.0	5.3	0.0	10.2
	17	38.2	21.5	91.0	28.0	5.0	0.0	10.2
	18	38.8	22.0	90.0	28.0	7.8	0.0	10.7
May 11	19	38.0	21.3	91.0	28.0	7.7	0.0	10.7
	20	39.1	22.7	91.0	29.0	7.9	0.0	8.5
	21	37.1	23.1	90.0	32.0	10.4	0.0	9.1
	22	37.7	23.8	91.0	35.0	10.7	9.0	9.7
June 11	23	34.2	22.7	93.0	55.0	8.8	14.1	6.9
	24	32.1	22.2	93.0	55.0	14.7	16.1	5.4
	25	33.3	23.5	93.0	53.0	18.2	1.1	5.4
	26	31.2	22.6	94.0	59.0	15.8	3.0	2.4
July 11	27	32.1	21.7	95.0	61.0	9.0	94.9	4.8
	28	29.2	21.8	92.0	65.0	12.1	13.5	3.5
	29	30.4	22.7	92.0	63.0	13.5	2.4	1.9
	30	30.4	22.1	94.0	61.0	8.4	44.2	2.2
	31	30.3	22.0	93.0	63.0	10.8	4.0	3.0
August 11	32	30.7	22.3	93.0	57.0	16.5	1.3	3.6
	33	30.9	21.8	91.0	58.0	12.3	6.3	4.8
	34	30.0	21.7	94.0	69.0	4.1	54.0	3.8
	35	28.7	24.4	94.0	74.0	6.9	50.3	2.2
September 11	36	29.3	21.7	93.0	70.0	12.5	27.2	5.3
	37	31.1	20.8	91.0	57.0	6.7	15.6	5.4
	38	30.4	19.6	92.0	54.0	5.4	5.2	7.8
	39	30.9	20.2	91.0	53.0	2.8	29.1	6.8
October 11	40	30.0	20.3	91.0	57.0	1.8	1.0	5.7
	41	32.6	20.1	90.0	44.0	3.5	3.5	7.4
	42	32.6	20.7	93.0	44.0	2.4	17.2	6.3
	43	32.1	15.8	90.0	33.0	3.6	0.0	10.0
	44	31.9	14.3	91.0	39.0	3.1	0.0	9.8
November 11	45	31.8	13.2	90.0	33.0	2.6	0.0	10.0
	46	31.6	12.3	90.0	31.0	3.3	0.0	9.7
	47	30.4	8.9	90.0	30.0	3.2	0.0	9.2
	48	30.8	14.4	90.0	38.0	3.4	0.0	8.0
December 11	49	31.7	10.6	91.0	31.0	2.4	0.0	9.6
	50	29.9	8.7	87.0	35.0	1.8	0.0	8.8
	51	29.4	8.3	86.0	32.0	2.0	0.0	8.3
	52	29.5	7.0	87.0	25.0	1.7	0.0	9.6

APPENDIX VI Contd…...

Month	MW	Temperature(^{o}C)		Humidity(%)		Wind Velo(km/h)	Rain Fall(mm)	Sunshine hrs.
		Max.	Min.	Morn	Even			
Jan.12	1	31.5	12.0	90.0	28.0	2.9	0.0	8.9
	2	26.5	6.3	90.0	29.0	1.7	0.0	8.8
	3	28.6	5.8	89.0	27.0	2.6	0.0	9.2
	4	28.9	10.9	86.0	31.0	1.4	0.0	9.5
	5	29.3	9.9	88.0	32.0	1.2	0.0	9.3
Feb.12	6	29.7	8.6	89.0	26.0	2.0	0.0	9.0
	7	31.7	10.7	89.0	27.0	1.6	0.0	8.9
	8	34.1	12.4	88.0	22.0	1.0	0.0	8.7
	9	34.0	10.0	88.0	21.0	2.3	0.0	9.0
March 12	10	32.7	9.2	88.0	22.0	2.8	0.0	9.9
	11	35.0	12.4	87.0	19.0	1.8	0.0	10.0
	12	37.4	13.6	87.0	18.0	2.0	0.0	10.2
	13	37.6	16.4	89.0	16.0	2.5	0.0	10.1
April 12	14	38.8	20.1	88.0	17.0	3.3	0.0	10.5
	15	38.6	20.0	91.0	21.0	2.8	0.0	10.3
	16	37.4	21.6	91.0	28.0	2.1	4.2	9.3
	17	37.3	14.1	88.0	29.0	1.9	0.0	10.0
	18	39.2	20.9	89.0	21.0	6.0	0.0	10.3

VITA

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A candidate for the degree
of
DOCTOR OF PHILOSOPHY (AGRICULTURE)
in
AGRICULTURAL ENTOMOLOGY
2012

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